Biomarkers, menopausal hormone therapy and risk of venous thrombosis: The Women’s Health Initiative

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Abstract

**Background:** Oral menopausal hormone therapy causes venous thrombosis but whether biomarkers of thrombosis risk can identify women at risk is unknown.

**Methods:** We completed a nested case control study in the two Women’s Health Initiative hormone trials; 27 347 women aged 50-79 were randomized to hormone therapy (conjugated equine estrogen with or without medroxyprogesterone acetate) or placebo. With 4 years follow-up, biomarkers were measured using stored baseline samples prior to starting treatment, and one-year later, in 215 women who developed thrombosis and 867 controls.

**Results:** Overall, lower protein C and free protein S, and higher D-dimer, prothrombin fragment 1.2 and plasmin-antiplasmin complex were associated with risk of future thrombosis with odds ratios ranging from 1.9 to 3.2. Compared to women with normal biomarkers assigned to placebo, the risk of thrombosis with hormone therapy was increased among women with abnormal biomarkers, especially elevated D-dimer, elevated plasmin-antiplasmin, and low free protein S; the largest association was for D-dimer: odds ratio 6.0 (95% CI 3.6-9.8). Differences in associations by
hormone use were not significant on the multiplicative scale. Considering a multi-marker score of eight biomarkers, women with three or more abnormal biomarkers had 15.5-fold increased odds of VT (95% CI 6.8-35.1). One-year changes in biomarkers were not robustly associated with subsequent thrombosis risk.

**Conclusion:** Abnormal levels of biomarkers of thrombosis risk identified women at increased risk of future venous thrombosis with oral menopausal hormone therapy. Findings support the potential for clinical use of D-dimer testing in advance of hormone therapy prescription.

**Keywords**
blood coagulation, D-dimer, menopausal hormone therapy, risk assessment, risk factor, venous thrombosis, venous thromboembolism

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**1 | INTRODUCTION**

Oral menopausal hormone therapy (HT) increases the risk of venous thrombosis (VT). As this treatment provides effective relief of menopausal symptoms and VT is the most common adverse vascular outcome of HT, knowledge of susceptibility factors might assist women and their physicians in decision-making on risks and benefits of HT use.

In perimenopausal women, the annual rate of VT is 1-2 per 1000, which rises to 0.5-1% over 5 years of HT use. In the Women’s Health Initiative (WHI) trials women who were older, obese, or had factor V Leiden were at higher risk of VT with HT. For example, conjugated equine estrogens plus medroxyprogesterone acetate (E+P) doubled the risk of VT overall but in women with factor V Leiden the risk was increased 6.7-fold, predicting a cumulative incidence of 3.3-6.7% over 5 years of HT use. Other hemostatic disorders associated with VT risk might predict susceptibility for HT-related VT, as might HT-induced changes in hemostasis or inflammation factors. We hypothesized that levels of hemostasis factors and C-reactive protein (CRP), an inflammation marker, would be associated with risk of VT with HT in the WHI trials, and that changes in some of these factors while on treatment would be associated with increased risk of VT.

We conducted a case-control study nested in the two WHI hormone trials. We measured biomarkers of thrombosis risk (factors VIIc, VIIIc, and IXc, von Willebrand factor, fibrinogen, protein C, protein S, antithrombin, prothrombin, D-dimer, and CRP) and others that are altered by HT but have no or uncertain associations with VT (plasminogen activator inhibitor-1 [PAI-1], prothrombin fragment 1.2, plasmin antiplasmin complex [PAP]). Associations of one-year HT-induced changes in some of these biomarkers with risk of VT were also studied.

**2 | METHODS**

The study design was a nested case control study embedded in two randomized controlled trials of hormone use versus placebo (clinicaltrials.gov identifier NCT 00000611; Women’s Health Initiative).

**2.1 | Subjects**

Detailed descriptions and results of the WHI hormone trials, including Consolidated Standards of Reporting Trials diagrams, were previously published. Eligible postmenopausal women aged 50-79 years were enrolled in 1993-1998. Exclusion criteria related to safety concerns with HT. Methods were approved at each site by institutional review committees, and participants provided written informed consent.

The WHI hormone trials included 16 608 women with an intact uterus who were randomly assigned in double-blind fashion to receive E + P or identical placebo, and 10 739 women without a uterus who were randomized to E or placebo. Treatment included one daily tablet containing 0.625 mg conjugated equine estrogen with or without 2.5 mg medroxyprogesterone acetate or identical placebo. At baseline and one year later, blood was drawn and stored at -70 °C.
Race/ethnicity was self-reported using a list and categorized as black or white/other. Body mass index (BMI) was measured to define overweight (BMI 25-30 kg/m$^2$) and obesity (BMI >30 kg/m$^2$).

### 2.2 Events ascertainment

Participants were queried every 6 months for possible VT. Hospital discharge summaries were reviewed at each clinical center for all overnight hospitalizations except selected elective procedures. Outpatient-treated VT events were ascertained starting in 1999 by investigating self-reports of participants. Validation of potential VT events was done as previously described. Validated deep vein thrombosis (DVT) was based on a physician diagnosis and positive findings on doppler or duplex ultrasound, or rarely venogram, plethysmography, isotope scan, or at autopsy. Validated pulmonary embolism (PE) was based on a discharge summary diagnosis of PE and positive findings on ventilation-perfusion lung scan, pulmonary angiogram, computed tomography, or at autopsy.

### 2.3 Nested case control study

Among all trial participants, excluding baseline warfarin users, a nested case control study of biomarkers in relation to VT, stroke, and myocardial infarction occurring between randomization and February 28, 2001 was conducted. One control was selected for each case with matching on age, randomization date and prevalent vascular disease (myocardial infarction, stroke, or VT). In this study we utilized data from the 215 VT cases and all selected controls (867 total controls).

### 2.4 Laboratory analysis

Baseline and follow-up blood samples were analyzed in cases and controls using the following methods: fibrinogen (clot-rate assay, STA-R instrument, Diagnostica Stago, Parsippany, NJ, USA), factor VIII and IX activity (clotting time on mixing with factor VIII or IX deficient plasma using STA-Deficient VIII or IX;
TABLE 2  Odds ratio (95% CI) of VT by categories of baseline biomarkers

| Biomarker                  | Odds Ratio<sup>b</sup> | (95% CI)  |
|----------------------------|------------------------|-----------|
| Procoagulant factors       |                        |           |
| Prothrombin >P90, ug/mL    | 0.6                    | (0.4, 1.2) |
| Factor VIIIc >P75, %       | 1.3                    | (0.9, 1.9) |
| Factor IXc >P90, %         | 0.9                    | (0.5, 1.5) |
| von Willebrand factor >P75, % | 1.3              | (0.9, 1.9) |
| Fibrinogen >P90, mg/dL     | 0.7                    | (0.4, 1.2) |
| D-dimer >P75, ug/mL        | 2.8                    | (2.0, 4.0) |
| Fragment 1.2 > P90, nmol/L | 1.9                    | (1.2, 3.1) |
| Anticoagulant factors      |                        |           |
| Protein C <P5, %           | 1.8                    | (0.9, 3.8) |
| Total protein S <P5, %     | 1.9                    | (0.9, 4.1) |
| Free protein S <P5, %      | 3.2                    | (1.6, 6.2) |
| Antithrombin <P5, %        | 0.9                    | (0.3, 3.2) |
| Fibrinolytic factors       |                        |           |
| PAI-1 > P90, ng/ml         | 0.9                    | (0.5, 1.7) |
| PAP >P90, nmol/L           | 2.4                    | (1.5, 3.8) |
| Inflammation factor        |                        |           |
| C-reactive protein >P75, mg/L | 1.2              | (0.8, 1.7) |
| Number of abnormal biomarkers |                  |           |
| 0-1                       | 1.0                    | (ref)     |
| 2-3                       | 2.9                    | (2.0, 4.3) |
| 4+                        | 7.8                    | (1.7, 35.1) |

BMI, body mass index; CI, confidence interval; P, percentile; PAI-1, plasminogen activator inhibitor-1; PAP, plasmin antiplasmin complex; VT, venous thrombosis.

<sup>a</sup>Cutoff values: prothrombin >137 ug/mL, factor VIIIc >150%, factor IXc >172%, vWF >140%, fibrinogen >4.17 g/L, D-dimer >0.54 mg/L, F1 + 2 > 1.76 nM, protein C < 84%, total protein S < 83%, free protein S < 75%, antithrombin < 67%, TAFI > 7.53, PAI-1 > 57.7 ng/ml, PAP >7.5 ng/mL, CRP >10%, fragment 1.2 > 7.2 nmol/L (>3 SD above the mean), PAI-1 > 70 ng/mL.

<sup>b</sup>Adjusted for age, race, BMI, treatment assignment, self-reported VT, and hysterectomy at screening.

2.5  **Statistical analysis**

Data from both trials were combined for primary analysis. Separate analyses by trial were completed secondarily. For baseline biomarkers in cases and controls, skewed distributions were log-transformed to achieve a normal distribution and geometric means were reported. Hormone therapy use was based on intention-to-treat.

Logistic regression, adjusting for age, race, BMI, treatment assignment, pre-baseline self-reported VT, and hysterectomy status, was used to determine odds ratios of VT for abnormal levels of each biomarker compared to normal levels. Cutoff levels for most biomarkers were defined a priori based on the literature with values shown in the footnote to Table 1. For the following biomarkers, since there is no evidence on VT to suggest cutoffs for abnormal values a priori, we selected the following cutoffs: fragment 1.2 and PAI-1 > 90th percentile, and for antithrombin, protein C and free and total protein S values less than the 5th percentile. Assessments for linear association were also made using each biomarker or its log transformed distribution treated as a continuous variable. We determined the association of each woman’s number of abnormal biomarkers with VT risk, including previously published data for factor V Leiden.

The additive risk of VT with abnormal biomarkers and HT was assessed by cross-classifying women by treatment assignment and whether they had an abnormal level of each biomarker. Odds ratios were determined by logistic regression adjusted for age, race, and BMI, with women having normal levels of each biomarker assigned to placebo comprising the reference group. Multiplicative interaction terms between HT assignment and each biomarker were also evaluated.

Evaluation of the association of one-year change in biomarkers with VT risk required exclusion of 83 women with VT between the two phlebotomies. We calculated change in each biomarker by subtracting the baseline from one-year values. Change was divided into quartiles with the lowest quartile including those that decreased the most and the top quartile those that increased the most. Logistic regression was used to analyze the association of quartiles of change in biomarkers, and the change values as continuous variables, with subsequent VT.

To examine change in biomarker levels in HT compared to placebo recipients, linear regression was used comparing treatment groups, adjusting for age, race, BMI, pre-baseline VT, and hysterectomy status.

3  **RESULTS**

With mean follow up of 4.1 years, 215 women had VT, 69 in the E trial and 146 in the larger E+P trial. There were 359 and 508 controls selected in each trial. Among cases, 59% in the E trial and 54% in the E+P trial had DVT without PE, with the remainder having PE. There were 132 women with VT after the one-year follow up phlebotomy.
3.1 | Baseline characteristics

Table 2 shows baseline characteristics by case-control status. Few women had pre-baseline self-reported VT. Cases had higher mean BMI, higher prevalence of pre-baseline VT, and higher mean baseline levels of factor VIII, von Willebrand factor, D-dimer, fragment 1.2, and CRP than controls and slightly lower protein C, antithrombin and free protein S. These differences were similar considering the trials separately, but in the E+P trial, cases had similar free protein S levels as controls.

3.2 | Associations of biomarkers with VT

Table 1 shows the odds ratios of VT for abnormal biomarkers, adjusted for age, race, BMI, pre-baseline VT, treatment assignment, and hysterecy status. High levels of D-dimer, fragment 1.2 and PAP, and low free protein S were significantly associated with increased risk of VT with adjusted odds ratios between 1.9 and 2.8. Four factors were only associated with risk when considered as continuous variables (all \(P < 0.05\)). Specifically, for these the adjusted odds ratios per 1 SD higher value were: factor VIII (1.2; 95% CI 1.03-1.4), von Willebrand factor (1.3; 95% CI 1.1-1.5), total protein S (0.8; 95% CI 0.7-0.98), and antithrombin (0.8; 95% CI 0.7-0.98). Considering factor V Leiden and binary terms for abnormal D-dimer, F1-2, protein C, total protein S, free S, antithrombin, PAP, women with increasing numbers of abnormal biomarkers had a higher risk of VT.

When the activation markers D-dimer, F1.2 and PAP were included together in the same model, the odds ratios of VT for each of these were 2.7 (95% CI 1.9-4.0), 1.6 (95% CI 1.0-2.6) and 2.1 (95% CI 1.3-3.5), respectively.

None of the above results differed materially comparing the two trials (data not shown).

3.3 | Joint associations of HT and abnormal biomarkers with VT

To evaluate whether abnormal biomarkers were susceptibility factors for HT-related VT, women were cross-classified by treatment assignment and whether they had an abnormal biomarker and odds ratios for VT calculated for exposed groups compared to women randomized to placebo with a normal biomarker (Table 3). In general, HT in the absence of an abnormal biomarker was associated with a 2.2-5 fold increased risk of VT, while women with abnormal biomarkers assigned to placebo had a 1.0- to 6.5-fold increased risk. Women with the combination of HT plus an abnormal biomarker had consistent elevated risks for VT (OR 2.4-6.0) with the largest odds ratio seen for the combination of HT and high D-dimer at 6.0 (95% CI 3.6-9.8). The odds ratios associated with the combination of HT plus elevated factor VIII or von Willebrand factor, or lower total protein S or antithrombin were approximately additive, while the odds ratios for the combination of HT and elevated fragment 1.2, PAP, CRP, free protein S, and low protein C were less than additive. There were no material differences between the two trials in these results (data not shown). Despite the elevated risk of HT plus abnormal biomarkers, tests for multiplicative interaction between HT assignment and each biomarker as a continuous or binary variable revealed no statistically significant multiplicative interactions (all \(P > 0.05\)).

To evaluate a multi-marker score considering eight biomarkers associated with VT risk (factor V Leiden and binary terms for abnormal D-dimer, F1-2, protein C, total protein S, free protein S, antithrombin, and PAP), women were classified as having 0-1, 2, or 3+ abnormal factors. In the figure, compared to women with 0-1 abnormal factors assigned to placebo, the odds ratio of VT with 2 or 3+ abnormal factors rose progressively such that women assigned to HT who had 3+ abnormal factors had 15.5-fold increased odds of VT (95% CI 6.8-35.1) adjusting for age, race, BMI, pre-baseline VT, and hysterectomy status.

3.4 | Change in biomarkers

The one-year changes in biomarkers in each trial by case-control status, excluding women who had VT in that year, are shown in Supplemental Table A. In the E trial all factors changed similarly in cases and controls (all \(P > 0.15\)) except von Willebrand factor, which rose more among cases than controls (11 vs 1%, \(P = 0.05\)). In the E+P trial factor VIIIc and fragment 1.2 rose more in cases than in controls (factor VIII 10 vs. 0%, \(P = 0.02\), fragment 1.2, 0.32 vs. 0.09 nmol/L, \(P = 0.04\); for all other factors \(P > 0.15\)).

One-year changes in biomarkers with treatment compared to placebo were generally similar for E and E+P (Supplemental Table B). In the combined trials, fibrinogen, PAI-1 and antithrombin declined with HT compared to placebo while PAP and CRP increased and the other factors did not change.

Table 4 shows associations of quartiles of change in biomarkers with odds of VT after the second blood collection. Compared to women in the first quartile of change in each biomarker, women in the top quartile of change in prothrombin, factor VIII, von Willebrand factor, fragment 1.2, PAP, and CRP were at increased risk of subsequent VT. While the 95% confidence intervals for these odds ratios all included 1.0, PAP and CRP change in the top compared to bottom quartile were associated with a 1.9-fold increased risk. Considering the biomarkers as continuous variables, only larger increases in factor VIII were associated with subsequent VT; the odds ratio of VT for a 32% greater one-year increase of factor VIII (1 SD increment) was \(1.3 (95\% \text{ CI } 1.1-1.6)\). Interpretation of results did not differ materially considering the trials separately (data not shown).

4 | DISCUSSION

4.1 | Main findings

In this study some thrombosis biomarkers were susceptibility factors for HT-associated VT, especially higher baseline D-dimer, which was associated with 6-fold increased odds of VT with HT. This risk increase is comparable to that of the combination of factor V Leiden...
In the presence of three or more of eight VT risk factors in combination with HT, the odds ratio of VT was substantially higher at 15.5. One-year change in biomarkers with HT was not robustly associated with subsequent VT risk, although modest associations were seen for factor VIII, PAP, and C-reactive protein. New findings regarding risk factors for VT include associations of higher levels of prothrombin fragment 1.2 and PAP with VT risk (although prothrombin fragment 1.2 has been reported in relation to VT risk in cancer patients).15

### Table 3 (Continued)

| Factor | Odds Ratio (95% CI) |
|--------|---------------------|
| Factor VIII<sub>c P75, %</sub> |               |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 1.9 (1.3, 2.7) |
| Elevated, Placebo | 1.0 (0.5, 1.9) |
| Elevated, HT | 2.7 (1.7, 4.5) |
| von Willebrand factor >P75, % |          |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 1.9 (1.3, 2.7) |
| Elevated, Placebo | 1.0 (0.5, 2.1) |
| Elevated, HT | 2.7 (1.7, 4.5) |
| D-dimer >P75, mg/mL |          |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.1 (1.4, 3.3) |
| Elevated, Placebo | 2.8 (1.6, 4.9) |
| Elevated, HT | 6.0 (3.6, 9.8) |
| Fragment 1.2 < P90, nmol/L |         |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.4 (1.6, 3.5) |
| Elevated, Placebo | 2.7 (1.3, 5.9) |
| Elevated, HT | 3.9 (2.1, 7.2) |
| PAP >P90, nmol/L |          |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.5 (1.7, 3.6) |
| Elevated, Placebo | 3.3 (1.6, 6.7) |
| Elevated, HT | 4.9 (2.6, 9.3) |
| Protein C <P5, % |          |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.4 (1.6, 3.7) |
| Reduced, Placebo | 3.3 (1.1, 10.1) |
| Reduced, HT | 3.2 (1.2, 8.3) |
| Total protein S <P5, % |        |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.3 (1.5, 3.5) |
| Reduced, Placebo | 2.0 (0.7, 5.9) |
| Reduced, HT | 4.3 (1.5, 12.3) |
| Free protein S <P5, % |       |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.6 (1.7, 4.0) |
| Reduced, Placebo | 6.5 (2.3, 17.8) |
| Reduced, HT | 5.1 (2.0, 12.6) |
| Antithrombin <P5, % |         |
| Normal, Placebo | 1.0 (ref) |
| Normal, HT | 2.1 (1.5, 2.9) |

BMI, body mass index; CI, confidence interval; HT, hormone therapy; P, percentile; PAP, plasmin antithrombin complex; VT, venous thrombosis. Women were cross-classified for their level of each hemostatic factor (cutoffs provided in Table 2 footnote) and treatment assignment, and each group was compared using logistic regression models to those randomized to placebo and who had normal levels of each factor. Models were adjusted for age, race, BMI, treatment assignment, self-reported VT, and hysterectomy status at screening. P-values for multiplicative interaction between treatment assignment and abnormal biomarkers were all >.05.

### 4.2 Relation to other work

Venous thrombosis is a common serious vascular complication of menopausal HT and limited studies suggest the risk is higher in women who are older, obese or have factor V Leiden, prothrombin 20210A or non-O blood group.1–2,16–19 In contrast to literature for oral contraceptives, despite many studies on effects of HT on hemostasis factors,7 we are aware of no other prospective studies on biomarkers related to VT risk (or their changes on treatment) as predisposing factors for HT-related VT.

### 4.3 Potential implications of the findings

Among the biomarkers considered here, D-dimer was most strongly related to VT. Women with D-dimer >0.54 mg/L (top quartile) had nearly a three-fold higher risk of future VT than women in the lowest quartile. While much clinical interest has focused on D-dimer in predicting recurrent VT,20–22 our findings confirm prior publications on D-dimer and risk of first VT in men and women.23–25 Further, women with elevated D-dimer randomized to HT were at six-fold increased risk compared to women with lower D-dimer on placebo. In the WHI trials, baseline D-dimer was also associated with increased risk of
stroke and coronary heart disease and, among a variety of biomarkers, only the change in D-dimer with HT predicted stroke risk (but not coronary risk) during follow-up.\textsuperscript{26,27}

Considering possible clinical application of D-dimer testing, the threshold value defining VT risk in this study is similar to the threshold used to rule out acute VT with this assay (0.50 mg/L), and that which has been proposed for clinical use in determining a group at low risk of recurrent VT after completing a course of anticoagulation for first unprovoked VT.\textsuperscript{20,21} Based on our definition of elevated D-dimer, 25% of women considering HT could be identified as having an increased risk based on D-dimer, with an estimated five-year cumulative incidence of VT of 6% with HT (assuming an annual rate without treatment and with normal D-dimer of 2 per 1000). If HT were withheld from women with elevated D-dimer, their five-year cumulative incidence of VT would be reduced to 3%. The number needed to test to prevent one VT over five years of treatment would then be 33 (1/0.03). Free protein S and PAP had similar odds ratios for VT as D-dimer in combination with HT use, but these point estimates were not precise (wide CIs) and the threshold defining abnormal values would only identify 5-10% of women at risk so the number needed to screen would be much higher. Similarly, considering a multi-marker approach (Figure 1) among women with three or more abnormal biomarkers there was an incremental increase in the odds ratio of VT to 15.5, but only 3% of non-cases had three or more abnormal biomarkers. The estimates were not precise (wide CIs) and the threshold defining abnormal values would only identify 5-10% of women at risk so the number needed to screen would be much higher. Similarly, considering a multi-marker approach (Figure 1) among women with three or more abnormal biomarkers there was an incremental increase in the odds ratio of VT to 15.5, but only 3% of non-cases had three or more abnormal biomarkers.

We are unaware of previous studies in healthy people demonstrating associations of higher levels of PAP and prothrombin fragment 1.2 with risk of future VT. In the Longitudinal Investigation of Thromboembolism Etiology, elevated PAP was not associated with VT risk.\textsuperscript{28} Plasminogen activator inhibitor-1; PAP, plasmin antiplasmin complex; Q, quartile; VT, venous thrombosis.

\textsuperscript{a}P value from a logistic regression model modeling VT by continuous 1-year difference in biomarker level. All models adjusted for age, race, BMI, treatment assignment, self-reported VT, and hysterectomy status at screening.
4.4 | Study limitations

Limitations of this study require consideration. Participants were older than women who would currently be considering starting HT. There was some nonadherence to assigned treatment in both placebo and HT groups, although this was less early in the trial when most of our cases occurred. If anything, the impact of nonadherence would most likely bias our findings to the null, making our estimates of interaction of biomarkers with HT underestimates and thus conservative. We had limited power to analyze data by HT type, however most associations were similar by study. Studies suggest a lower risk of VT with estradiol or transdermal treatment than oral conjugated equine estrogens and we could not address this. To conserve power, we did not exclude women with pre-baseline self-reported VT, but we did adjust for this. Use of ELISAs for proteins C and S would miss functional deficiencies that might have clinical relevance but would be rare. Due to concern for type I error, we did not study nonlinear associations of biomarkers with VT nor did we explore other thresholds (besides our a priori defined ones) to define abnormal values of biomarkers. Assessment of change in hemostatic factors in relation to VT risk was limited because we necessarily excluded VT cases occurring in the first year of follow-up, between the two blood collections. It would have been preferable if the second phlebotomy had been done four to six weeks after randomization to increase the opportunity to relate changes in biomarkers to VT risk. Finally, we did not measure change in protein S. Given our findings for risk of VT with HT plus low baseline protein S, this might be explored in future studies.

4.5 | Study strengths

The key strength of the study was the evaluation of participants from a rigorously conducted randomized controlled trial, eliminating selective prescribing of HT. In addition, we used baseline blood samples prior to HT use or VT for measurement of biomarkers. We are not aware of an existing or planned study with similar design that could be used for replication or which might overcome the limitations mentioned above.

5 | CONCLUSIONS

The WHI clinical trials provided a unique opportunity to examine associations of biomarkers of interest with VT, determine susceptibility factors for HT-associated VT, and determine if changes in biomarkers with HT are related to the incidence of VT. Findings here support potential for clinical use of D-dimer testing in advance of HT prescription to identify women at increased risk of VT. Further study of a multi-marker score in selected high-risk populations might be useful.

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RELATIONSHIP DISCLOSURE

None of the authors have any disclosures relevant to this paper.
AUTHOR CONTRIBUTIONS

M. Cushman: concept and design, analysis and interpretation of data, critical writing, final approval. J. C. Larson: analysis and/or interpretation of data, critical writing, final approval. F. R. Rosendaal: concept and design, analysis and/or interpretation of data, critical writing, final approval. S. R. Heckbert: analysis and/or interpretation of data, critical writing, final approval. J. D. Curb: concept and design, analysis and/or interpretation of data, critical writing, final approval. L. S. Phillips: analysis and/or interpretation of data, critical writing, final approval. C. B. Eaton: analysis and/or interpretation of data, critical writing, final approval. R. S. Stafford: analysis and/or interpretation of data, critical writing, final approval.

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REFERENCES

1. Cushman M, Kuller LH, Prentice R, et al. Estrogen plus progestin and risk of venous thrombosis. JAMA. 2004;292:1573–80.
2. Curb JD, Prentice RL, Bray PF, et al. Venous thrombosis and conjugated equine estrogen in women without a uterus. Arch Intern Med. 2006;166:772–80.
3. Cushman M. Epidemiology and risk factors for venous thrombosis. Semin Hematol. 2007;44:62–9.
4. Koh KK, Horne MK 3rd, Cannon RO 3rd. Effects of hormone replacement therapy on coagulation, fibrinolysis, and thrombosis risk in postmenopausal women. Thromb Haemost. 1999;82:626–33.
5. Cushman M, Legault C, Barrett-Connor E, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) study. Circulation. 1999;100:717–22.
6. van Baal WM, Emeiss JJ, van der Mooren MJ, Kessel H, Kenemans P, Stehouwer CD. Impaired procoagulant-anticoagulant balance during hormone replacement therapy? A randomised, placebo-controlled 12-week study Thromb Haemost. 2000;83:29–34.
7. Cosman F, Baz-Hecht M, Cushman M, et al. Short-term effects of estrogen, tamoxifen and raloxifene on hemostasis: a randomized-controlled study and review of the literature. Thromb Res. 2005;116:1–13.
8. The Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials. 1998;19:61–109.
9. Curb JD, McTiernan A, Heckbert SR, et al. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. Ann Epidemiol. 2003;13:512–8.
10. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288:321–33.
11. Anderson GL, Limacher M, Assaf AR, et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: The Women's Health Initiative randomized controlled trial. JAMA. 2004;291:1701–12.
12. Cushman M, Lemaitre RN, Kuller LH, et al. Fibrinolytic activation markers predict myocardial infarction in the elderly: the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 1999;19:493–8.
13. Smiles AM, Jenny NS, Tang Z, Arnold A, Cushman M, Tracy RP. No association of plasma prothrombin concentration or the G20210A mutation with incident cardiovascular disease: results from the cardiovascular health study. Thromb Haemost. 2002;87:614–21.
14. Macy EM, Meilahn EN, Declerck PJ, Tracy RP. Sample preparation for plasma measurement of plasminogen activator inhibitor-1 antigen in large population studies. Arch Path Lab Med. 1993;117:67–70.
15. Ay C, Vormittag R, Dunkler D, et al. D-dimer and prothrombin fragment 1 + 2 predict venous thromboembolism in patients with cancer: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol. 2009;27:4124–9.
16. Harrington DM, Vittinghoff E, Howard TD, et al. Factor V Leiden, hormone replacement therapy, and risk of venous thromboembolic events in women with coronary disease. Arterioscler Thromb Vasc Biol. 2002;22:1012–7.
17. Straczek C, Oger E, Yon de Jonage-Canonic MB, et al. Prothrombotic mutations, hormone therapy, and venous thromboembolism among postmenopausal women: impact of the route of estrogen administration. Circulation. 2005;112:3495–500.
18. Smith NL, Heckbert SR, Lemaitre RN, et al. Conjugated equine estrogen, esterified estrogen, prothrombotic variants, and the risk of venous thrombosis in postmenopausal women. Arterioscler Thromb Vasc Biol. 2006;26:2807–12.
19. Canonico M, Olle V, Carcaillon L, Tubert-Bitter P, Scarabin PY. Synergism between non-O blood group and oral estrogen in the risk of venous thromboembolism among postmenopausal women: The ESTHER study. Thromb Haemost. 2008;99:246–8.
20. Verhovsek M, Douketis JD, Yi Q, et al. Systematic review: D-dimer to predict recurrent disease after stopping anticoagulant therapy for unprovoked venous thromboembolism. Ann Intern Med. 2008;149:W94.
21. Douketis J, Tosetto A, Marcucci M, et al. Patient-level meta-analysis: effect of measurement timing, threshold, and patient age on ability of D-dimer testing to assess recurrence risk after unprovoked venous thromboembolism. Ann Intern Med. 2010;153:523–31.
22. Eichinger S, Heinze G, Kyrle PA. D-dimer levels over time and the risk of recurrent venous thromboembolism: an update of the Vienna prediction model. J Am Heart Assoc. 2014;3:e000467.
23. Andreescu ACM, Cushman M, Rosendaal FR. D-dimer as a risk factor for deep vein thrombosis: the Leiden Thrombophilia Study. Thromb Haemost. 2002;87:47–51.
24. Cushman M, Folsom AR, Wang L, et al. Fibrin fragment D-dimer and the risk of future venous thrombosis. Blood. 2003;101:1243–8.
25. Folsom AR, Alonso A, George KM, Roetker NS, Tang W, Cushman M. Prospective study of plasma D-dimer and incident venous thromboembolism: the Atherosclerosis Risk in Communities (ARIC) Study. Thromb Res. 2015;136:781–5.
26. Kooperberg C, Cushman M, Hsia J, et al. Can biomarkers identify women at increased stroke risk? The Women's Health Initiative Hormone Trials. PLoS Clin Trials. 2007;2:e28.
27. Rossouw JE, Cushman M, Greenland P, et al. Inflammatory, lipid, thrombotic, and genetic markers of coronary heart disease risk in the Women's Health Initiative trials of hormone therapy. Arch Intern Med. 2008;168:2245–53.
28. Folsom AR, Cushman M, Heckbert SR, Rosamond WD, Aleksic N. Prospective study of fibrinolytic markers and venous thromboembolism. J Clin Epidemiol. 2003;56:598–603.
29. Cushman M, Meilahn EN, Psaty BM, Kuller LH, Dobs AS, Tracy RP. Hormone replacement therapy, inflammation, and hemostasis in elderly women. Arterioscler Thromb Vasc Biol. 1999;19:893–9.
30. Cushman M, O'Meara ES, Folsom AR, Heckbert SR. Coagulation factors IX through XIII and the risk of future venous thrombosis: the Longitudinal Investigation of Thromboembolism Etiology. Blood. 2009;114:2878–83.
31. van Hylckama Vlieg A, van der Linden I, Bertina R, Rosendaal F. High levels of factor IX increase the risk of venous thrombosis. Blood. 2000;95:3678–82.
32. Bezemer ID, Arellano AR, Tong CH, et al. F9 Malmo, factor IX and deep vein thrombosis. Haematologica. 2009;94:693–9.
33. Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. Blood. 2010;116:113–21.
34. Smith NL, Blondon M, Wiggins KL, et al. Lower risk of cardiovascular events in postmenopausal women taking oral estradiol compared with oral conjugated equine estrogens. JAMA Intern Med. 2014;174:25–31.
35. Blondon M, van Hylckama Vlieg A, et al. Differential associations of oral estradiol and conjugated equine estrogen with hemostatic biomarkers. J Thromb Haemost. 2014;12:879–86.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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