The Endogenous Hormonal Milieu Associated with Thrombosis in Postmenopausal Women

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Abstract

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Although the use of exogenous hormones, such as hormone therapy (HT) and oral contraceptives (OC), is known to be positively associated with the risk of venous thrombosis (VT), the endogenous hormonal environment associated with thrombotic biomarkers and endpoints is incompletely characterized. This dissertation aimed to elucidate further the relation between endogenous sex hormones as well as events associated with changes in these hormones (vasomotor symptoms [VMS] and hysterectomy and oophorectomy) and thrombotic risk in postmenopausal women.

In the setting of the Seattle Heart and Vascular Health (HVH) study, we evaluated the cross-sectional association between 9 measures of endogenous hormone levels and 8 hemostatic factor levels using separate multiple linear regression analyses among 131 postmenopausal women. Also in the setting of the HVH study, we used multiple logistic regression to estimate VT risk associated with prior hysterectomy/oophorectomy status and current HT use status, compared with no prior hysterectomy and no current HT use, among a population of postmenopausal cases of incident VT (n=1,358) and their matched controls (n=4,112). In the

setting of the Women's Health Initiative (WHI), we evaluated the cross-sectional association of 9 measures of hemostatic factor levels with VMS presence and severity among 2,149 postmenopausal women using multiple linear regression. We also evaluated the association between VMS presence, severity, menopausal status at VMS onset, and VMS duration and the risk of VT among postmenopausal participants in the WHI-HT trials (n=24,508) and the WHI-Observational Study (WHI-OS) (n=87,783) using Cox proportional hazards models.

For our first aim, after accounting for multiple comparisons, we found no statistically significant evidence of any hormone-hemostatic factor associations (P-min p=0.10). In analyses without correction for multiple comparisons, there was some suggestion that higher levels of estradiol (pg/mL), estrone (pg/mL), sex hormone binding globulin (nmol/L), total testosterone (ng/dL), and dehydroepiandrosterone (DHEA) (ng/dL) may be associated with lower total protein S levels (%) and that higher estrone levels may be associated with lower antithrombin levels (%) (all p-values ≤0.04). There was also some suggestion that higher levels of dehydroepiandrosterone-sulfate (ug/mL) and DHEA (ng/dL) may be associated with lower thrombin generation peak values (nM), lower TG endogenous thrombin potential (nMxMin), and lower nAPCsr levels, and that higher DHEA levels may be associated with lower Factor VII activity levels (%) (all p-values≤0.027). Addressing our second aim, prior hysterectomy with BSO without current HT use was associated with a 30% greater risk of VT than no prior hysterectomy and no current HT use (OR=1.3 [95% CI: 1.0-1.5]; p=0.02) but we found no evidence that prior hysterectomy with BSO with current HT use was associated with VT risk (OR=1.0 [95% CI: 0.78-1.3]). There was no evidence that prior hysterectomy-alone was associated with VT risk with current HT non-use (OR=1.0 [95% CI 0.80-1.3]) or with current HT use (OR=0.76 [95% CI 0.53-1.1]), compared with no prior hysterectomy and no current HT use. For our third aim, after correcting for multiple comparisons (p=0.064), we found no evidence that VMS presence or severity was associated with levels of hemostatic factors among postmenopausal women. There was some suggestion,

however, that VMS presence may be associated with a -0.34 adjusted difference in nAPCsr (ratio) compared with no VMS (SE=0.13; p=0.009). For our final aim, we found no evidence of an association between VMS presence, severity, timing or duration and the risk of VT.

Results from this dissertation work suggest that endogenous and exogenous hormones differently impact thrombotic risk. Although the use of exogenous HT is positively associated with the risk of VT, we found little evidence that endogenous hormone levels were associated with hemostatic factors, we found evidence that hysterectomy with BSO and no current HT use (both associated with lower E2 levels) were associated with a slightly greater risk of incident VT than no hysterectomy and no current HT use, and although VMS are hypothesized to be hormonally-related, we found no evidence that VMS are associated with hemostatic factor levels or with the risk of incident VT.

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INTRODUCTION

The risk of a venous thrombosis (VT) event is greater among postmenopausal women than pre- or perimenopausal women;[1] however, it is poorly understood whether the increased risk of cardiovascular events in postmenopause is due primarily to chronological aging or to the endogenous hormonal profile present in postmenopause as compared with pre- and perimenopause. The decrease in endogenous estradiol (E2), sex hormone binding globulin (SHBG), and total testosterone (T) levels[2-5], yet increase in free T[4] and free androgen index (FAI)[5] occurring during the menopausal transition had historically been proposed to contribute to the increased risk of cardiovascular disease following midlife. However, exogenous oral contraceptive (OC) and hormone therapy (HT) use, which increases E2 and SHBG levels[6,7], have been associated with an increased risk of arterial and venous thrombotic events in women[8-12], suggesting that thrombotic risk associated with hormones may differ between exogenous and endogenous hormonal origin.

Although much research has evaluated the association between exogenous hormone use and the risk of VT, the endogenous hormonal milieu associated with the risk of VT and changes in hemostatic factors is poorly understood. This dissertation aims to evaluate the association between levels of endogenous hormones as well as events associated with changes in these hormones (VMS and hysterectomy and oophorectomy) and thrombotic risk in postmenopausal women (Figure 1).

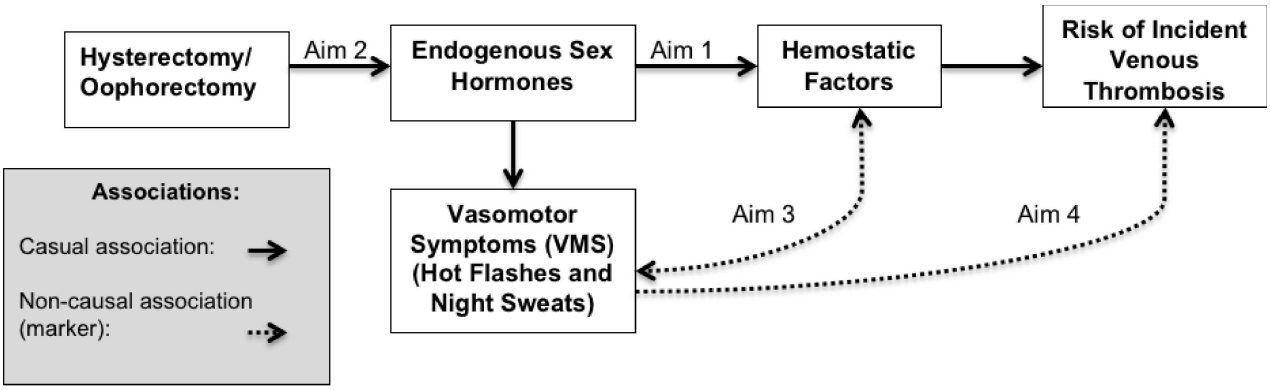

Figure 1. Conceptual model. The goal of this set of studies was to further characterize the endogenous hormonal milieu associated with thrombosis in postmenopausal women. To address this goal, we evaluated the relationship between endogenous sex hormones as well as events associated with changes in endogenous hormones (vasomotor symptoms (VMS) and hysterectomy oophorectomy) with hemostatic factors and venous thrombosis (VT) disease endpoints.

Endogenous Hormones and Hemostatic Factors

Investigators of a study of pre- and perimenopausal women reported associations between levels of endogenous hormone and hemostatic factor levels. In Study of Women's Health Across the Nation (SWAN) pre- and perimenopausal participants, the hormonal profile associated with ovarian aging during the menopausal transition (decreasing E2 and SHBG and increasing follicle stimulating hormone (FSH), T and dehydroepiandrosterone-sulfate [DHEAS][4,13]) was also associated with several hemostatic factors in the direction of greater thrombotic risk.[14,15] Whether endogenous hormone levels are also associated with hemostatic factor levels in postmenopause is incompletely understood. In chapter 1, we evaluate the cross-sectional association between endogenous hormone, SHBG, and prohormone levels and hemostatic factor levels among postmenopausal women.

Hysterectomy, Oophorectomy and Venous Thrombosis

Hysterectomy with and without bilateral salpingo-oophorectomy (BSO) is associated with decreases in endogenous levels of E2, SHBG, and total T,[16-18] and women who undergo hysterectomy with BSO in natural pre- or perimenopause experience a shift into

postmenopause at the time of BSO. Early age at menopause has been associated with a greater risk of cardiovascular disease[19], but the risk of VT associated with young age at menopause is inconsistent[20-22], with results of one study suggesting a decreased VT risk associated with early age at menopause,[22] another suggesting greater VT risk,[20] and yet another suggesting no association.[21] Studies of hysterectomy with and without BSO in relation to VT risk have not provided statistically convincing evidence of an association[20,21,23,24], but these studies have included relatively small numbers of VT cases with a prior BSO (ranging from 13-132 women). In chapter 2, we aim to evaluate the relation between hysterectomy with and without bilateral salpingo-oophorectomy (BSO) and the risk of incident VT.

Vasomotor Symptoms, Hemostatic Factors, and Venous Thrombosis

VMS are common during the menopausal transition and are experienced by most women during pre-, peri- or postmenopause.[25,26] Recent scientific literature has proposed that VMS may be a marker of underlying vascular change, and therefore, that VMS and qualities of VMS, such as when they begin in relation to the menopausal transition, may be associated with changes in levels of cardiovascular biomarkers and in the risk of cardiovascular events.[27,28] VMS frequency has been positively associated with levels of factor VIIc and tissue plasminogen activator antigen (tPA-ag) in a study of pre- and perimenopausal women, but whether VMS are also associated with hemostatic factors in postmenopause, and whether VMS may be a marker of increased VT event risk is unknown. In chapter 3, we evaluate the cross-sectional association of VMS presence and severity with hemostatic factor levels among postmenopausal women. In chapter 4, we evaluate the association of VMS presence, VMS severity, menopausal status at VMS onset, and VMS duration with the risk of VT among postmenopausal women.

Summary

This dissertation evaluates endogenous hormone levels in relation to hemostatic factor levels, and 2 exposures associated with changes in endogenous hormone levels, hysterectomy/oophorectomy and VMS, in relation to thrombotic risk. All women experience changes in endogenous hormones during the menopausal transition, the majority experience VMS[25], and over one-third of US women have a hysterectomy or oophorectomy by age 60[29,30]; however, the risk of VT associated with these hormonally-driven exposures in relation to thrombotic risk is insufficiently understood. An understanding of whether an endogenous hormonal environment is associated with hemostatic factor levels and with the risk of VT may be helpful in identifying women at an increased risk of VT. Earlier identification of higher-risk groups may help to reduce the morbidity and mortality associated with physiologic changes occurring during the menopausal transition.

CHAPTER 1: The association of endogenous hormone, SHBG, and prohormone levels with hemostatic factor levels in postmenopausal women

Abstract

Background: The relation between endogenous hormone levels and hemostatic factor levels is incompletely characterized. We aimed to evaluate the cross-sectional association between endogenous hormone, sex hormone binding globulin (SHBG), and prohormone levels and hemostatic factor levels among postmenopausal women.

Methods: This Heart and Vascular Health study analysis included 131 postmenopausal women (mean=18.8 years since final menstrual period; SD=10.8 years) not using hormone therapy (HT) and with previously measured thrombin generation (TG), normalized activated protein C sensitivity ratio (nAPCsr), factor VII activity (FVIIc), antithrombin activity (ATc), and total protein S antigen levels. Estradiol (E2), estrone (E1), total testosterone (T), dehydroepiandrosterone-sulfate (DHEAS), DHEA, and androstenedione levels were measured using liquid chromatography-tandem mass spectrometry and SHBG levels using immunoassay. Percent free T and free T index were calculated. Using multiple linear regression, we estimated the adjusted mean difference in each hemostatic factor level associated with a 1-unit higher hormone level, for each endogenous hormone separately. In addition to nominal p-values, a multiple comparisons-adjusted p-value was estimated using the P-min procedure.

Results: In analyses without correction for multiple comparisons, we found some evidence that 1-unit higher levels of E2 (pg/mL), E1 (pg/mL), SHBG (nmol/L), total T (ng/dL), and DHEA (ng/dL) may be associated with lower total protein S levels (%) (E2 adjusted average difference [β]=-0.86%, SE=0.27, p=0.002; E1 β=-0.23%, SE=0.056, p<0.001; SHBG β=-0.040%, SE=0.019, p=0.040; total T β=-0.25%, SE=0.12, p=0.040; DHEA β=-0.062%, SE=0.022, p=0.005) and that higher E1 levels may be associated with lower ATc (%) (β=-0.10%, SE=0.04, p=0.012). We also found some evidence that 1-unit higher levels of DHEAS (ug/mL) and DHEA (ng/dL) may be associated with lower TG peak values (nM) (DHEAS β=-39.7 nM, SE=8.9,

p<0.001; DHEA β=-0.17 nM, SE=0.066, p=0.011), lower TG endogenous thrombin potential (nMxMin) (DHEAS β=-143.1 nMxMin, SE=32.3, p<0.001, DHEA β=-0.66 nMxMin, SE=0.26, p=0.013), lower nAPCsr values (ratio) (DHEAS β=-0.89, SE=0.26, p=0.001; DHEA β=-0.0032, SE=0.0014, p=0.027), and that higher DHEA levels may be associated with lower FVIIc (%) (β=-0.081%, SE=0.031, p=0.010). However, after accounting for multiple comparisons, we found no statistically significant evidence of any hormone-hemostatic factor associations (P-min p=0.10). We found no evidence that other measured hormones were associated with hemostatic factor levels.

Conclusions: In postmenopausal women at an average of 19 years post-menopause, we did not find statistically significant evidence that levels of endogenous sex hormones were associated with hemostatic factor levels.

Introduction

Exogenous oral contraception and hormone therapy (HT) use is associated with a greater risk of cardiovascular disease, including a 1.5- to 3-fold greater risk of venous thrombosis (VT).[8-11,31-33] Pathways contributing to the change in risk of VT have been demonstrated for several hemostatic measures, including endogenous thrombin potential (ETP), total protein S, prothrombin fragments 1+2, plasminogen activator inhibitor-1 (PAI-1), normalized activated protein C sensitivity ratio (nAPCsr), and antithrombin, levels of which are associated with use of these exogenous estrogens and progestogens.[34-37] Although a substantial body of research has formed regarding the strong positive association between use of exogenous hormones and hemostatic factor levels and VT event risk,[8-11,31,32] the relation between endogenous hormone levels and thrombotic risk is poorly characterized but may provide insights into underlying risk of disease.

In this cross-sectional analysis among postmenopausal women not using HT at the time of phlebotomy, we aimed to contribute to an improved understanding of the relation between postmenopausal endogenous hormone levels and hemostatic factor levels, a marker for thrombotic risk. Given the greater risk of VT associated with exogenous HT use, we hypothesized that estrogen levels (estradiol [E2] and estrone [E1]) would be positively associated with measures of thrombin generation (TG), nAPCsr values, factor VII activity (FVIIc), and negatively associated with levels of the anticoagulant factors, antithrombin activity (ATc) and total protein S antigen. We hypothesized similar directional differences in hemostatic factors associated with higher total testosterone (T), free T index (FTI), and percent free T, since higher levels of free and total T have been positively associated with other cardiovascular biomarkers and disease risk among midlife and postmenopausal women.[14,38,39] Lower levels of sex hormone binding globulin (SHBG) are associated with higher levels of circulating androgens; therefore, we hypothesized that lower SHBG levels would be associated with similar directional differences in hemostatic factors. Since the prohormones, dehydroepiandrosterone-

sulfate (DHEAS), dehydroepiandrosterone (DHEA), and androstenedione are converted into androgens, and to a lesser extent, estrogens[40], we hypothesized similar directional differences in hemostatic factor levels associated with higher levels of DHEAS, DHEA, and androstenedione.

Methods

Setting and Study Design

This cross-sectional study was set within the Heart and Vascular Health (HVH) study, a large, population-based, case-control study set in Group Health Cooperative (GHC), an integrated health care system in Western Washington State. The HVH study was originally designed to evaluate risk factors for cardiovascular disease, including VT, myocardial infarction (MI), stroke, and atrial fibrillation. The index date for HVH study cases was defined as the date of their incident VT, MI, stroke, or atrial fibrillation event and for controls was defined as a random date in the year for which they were chosen as a control. The GHC Human Subjects Review Committee approved this study.

Cross-sectional Study Participants

HVH study participants eligible for this study were female controls 18-89 years of age who were of perimenopausal or postmenopausal status at their index date, which was between 2003 and 2010 (n=2,543). Controls had not experienced an incident VT event prior to their index date. Of these women, 63% (n=1,590) consented to blood draw and provided a blood specimen. Eighty-nine percent of these women were not currently using oral estrogen-alone or estrogen plus progesterone HT at the time of blood draw (n=1,415). Hemostatic factor levels were measured for a random subset (n=142) of women not currently using oral HT at the time of blood draw. Current HT use at the time of phlebotomy was determined using GHC computerized pharmacy records, which included prescription fill dates, medication quantity, and dosing instructions or

the anticipated days' supply, assuming 80% compliance. Assuming 80% compliance, prior HT use ended 86 months prior to phlebotomy on average, with the most recent HT use ending 2.3 months prior. For this analysis, we further excluded women who were using transdermal estrogens (n=4), who were using oral progesterone-alone (n=1), or who had not yet reached postmenopausal status at the time of blood draw (n=6). These exclusions resulted in an eligible population of 131 women who were postmenopausal at the time of phlebotomy.

Measures

Covariates

Trained abstractors reviewed complete GHC medical record data available prior to the index date and recorded demographic and medical history characteristics. Race/ethnicity, education, and current smoking status at the index date was determined using self-reported data collected during a telephone interview and was augmented with data collected via medical record review. Body mass index (BMI) was determined using data from the medical record and augmented using self-reported height and weight data from the telephone interview. Risk factors for cardiovascular events were collected during medical record review, including diabetes mellitus, treated hypertension, prevalent cardiovascular disease (defined as a history of angina, claudication, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, carotid endarterectomy, or peripheral vascular disease), and the most recent systolic and diastolic blood pressure and cholesterol measures prior to the index date were collected during medical record review. Hysterectomy and oophorectomy status were abstracted from the medical record and classified into one of four categories: no hysterectomy or oophorectomy, hysterectomy-alone, hysterectomy with bilateral salpingo-oophorectomy, or unknown, if either hysterectomy or oophorectomy status was unknown. A woman's age at final menstrual period (FMP) was collected from information noted in the medical record. A woman was considered to be postmenopausal if there was a notation of the cessation of menses in the medical record or

after the initiation of hormone therapy. For women with a prior hysterectomy, a woman was considered to be postmenopausal at the start of menopausal symptoms. All women ≥55 years of age were considered to be postmenopausal.

Blood Collection, Processing, and Storage

Venous blood specimens were collected after the index date (27.8 months after the index date on average; range=4.7-54.4 months). Specimens were collected into separate tubes of 3.2% sodium citrate and ethylenediaminetetraacetic acid (EDTA), centrifuged at 4℃ for 10 minutes at 1300 g and stored at -80℃ within 6 hours of collec tion.

Hemostatic Factor Measurements

Stored tubes of citrated plasma were shipped on dry ice from Seattle, Washington to Leiden University Medical Center (LUMC), Leiden, the Netherlands, for measurement of 5 hemostatic parameters: TG, nAPCsr, FVIIc, ATc, and total protein S antigen. Prior to shipment, samples were stored an average of 4.5 years (SD=2.0), and hemostatic factor measurements were made in October 2010.

Methods of hemostatic factor level measurement in this population have been previously published and will be described in brief.[37,41] The TG assay measured TG lag-time, time-to-peak, peak thrombin concentration value, and the area under the curve, which approximates endogenous thrombin potential (ETP).[42] A fluorogenic assay (Diagnostica Stago, Asnieres, France) was used to measure these 4 TG parameters (coefficient of variation (CV) for ETP from normal pooled plasma: 19.8%).[37] The ETP-based nAPCsr was estimated using the normalized ratio of the area under the TG curve without added APC to the area under the TG curve with APC added. ETP in the absence of APC had been measured as part of the TG assay; ETP in the presence of APC was measured using a fluorogenic assay (Thrombinoscope TM; Synapse BV). FVIIc and ATc were measured using the STA-R analyser (Diagnostica Stago) (CVs: 9.2%

and 3.0%, respectively). Total protein S antigen levels were measured using an enzyme-linked immunosorbent assay (Diagnostica Stago) (CV using a commercial quality control: 4.2%).[37]

Endogenous Hormone Measurements

Stored EDTA plasma samples were transported locally on dry ice from storage to the Department of Veterans Affairs in Seattle, where endogenous hormone, SHBG, and prohormone levels were measured in February 2014. On average, samples had been stored for 6.8 years (SD=2.0) prior to hormone measurement.

Estrogens (E1 and E2) were measured simultaneously using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (inter-assay CVs: 12.1% and 5.6%, respectively). The lower limit of detection (LOD) for E1 was 1.96 pg/mL and for E2 was 0.98 pg/mL; 0 women were below the LOD for E1 and 2 for E2. A separate LC-MS/MS assay measured total T, DHEA, and androstenedione. Inter-assay CVs were 7.1%, 14.3%, and 7.8%, respectively. The LOD for total T was 1.0 ng/dL (0 values<LOD), 15.6 ng/dL for DHEA (7<LOD), and 1.0 ng/dL for androstenedione (0<LOD). DHEAS was measured using a separate LC-MS/MS assay (no inter-assay CV data available, LOD: 0.04 ug/ml; 1<LOD) since its values were greater in magnitude than those of DHEA and other androgens and proandrogens. SHBG was measured using Quantikine SHBG Immunoassay, which is a 4.5 hour solid-phase ELISA. In analyses, E2 (n=2), DHEA (n=7), and DHEAS (n=1) values that were below the LOD were replaced with the LOD. The FTI was calculated by dividing total T nmol/L by SHBG nmol/L and multiplying by 100. We calculated free T using the Vermeulen method,[43] and estimated percent free T by dividing this free T estimate by total T and multiplying by 100.

Statistical Analysis

Demographic and medical history characteristics were reported for all participants. We reported unadjusted medians and interquartile ranges (IQRs) for hormone and hemostatic factor levels.

Pearson correlation coefficients were computed between each endogenous hormone and each hemostatic factor, among the endogenous hormone levels, and among the hemostatic factor levels.

Multiple linear regression with robust standard errors was used to model the association between each individual continuous hormone and continuous hemostatic factor level, adjusting for potential confounders determined *a priori*: linear age, linear BMI (kg/m^2), current vs. never/past smoking status and current diabetes status (current diabetes vs. no current diabetes). Linear regression models estimated the adjusted average difference in the hemostatic factor level associated with a 1-unit difference in the endogenous hormone level. For improved interpretation, adjusted average differences estimated using these models were used to calculate the percent difference in SD units of the hemostatic factor level associated with a 1-SD difference in the endogenous hormone level $((\beta * SD_{hormone})/SD_{hemostatic\ factor})*100$. Although not tested statistically, a visual inspection of scatterplots did not lead us to suspect that associations deviated from linearity.

For each hormone-hemostatic factor analysis, we report nominal p-values, which were compared to an alpha level of 0.05 to determine nominal statistical significance. However, to reduce the possibility of Type I error in our study, which was increased due the large number of comparisons made between hormones and hemostatic factors, and because hormones were correlated with one another and hemostatic factors were correlated with one another, we computed a multiple comparisons corrected p-value using the P-min procedure.[44]

Levels of the endogenous hormones, follicle stimulating hormone (FSH) and E2 continue to fluctuate until about 2 years after the FMP[45]. To restrict our population of women to those experiencing minimal hormonal fluctuations related to recency of the menopausal transition, we conducted sensitivity analyses restricted to women ≥55 years of age at the time of blood draw (n=117). Results of this analysis are presented in the appendix.

Data management and all analyses other than the estimation of the multiple comparisons corrected p-value were completed using Stata 13.1.[46] Permutation and the estimation of the multiple comparison corrected p-value was completed using R, Version 2.13.0.[47]

Results

Table 1.1 presents demographic and health characteristics for all participants. On average, participants were 67 years of age at the time of blood draw, had experienced their FMP 19 years earlier, had a BMI in the obese range (mean BMI=30 kg/m^2), were predominantly of White race (93%) and had not previously had a hysterectomy or oophorectomy (63%). Most women had used oral estrogen HT at some point prior to phlebotomy (67%) but, on average, had not used any oral or transdermal estrogen or progestogen HT for 86 months prior to blood draw. Table 1.A1 in the appendix presents unadjusted median levels of endogenous sex hormone and hemostatic factor levels and unadjusted Pearson correlation coefficients.

Table 1.2 presents results from adjusted multiple linear regression models of the cross-sectional association between endogenous hormone and hemostatic factor levels, with nominal p-values that are not adjusted for multiple comparisons. For example, the adjusted average difference in TG peak value was 0.23 (SD=0.50) nM per 1 pg/mL higher E2. Expressed as a % of SD, the TG peak value was higher by 2.9% of the TG peak value SD for each 1-SD higher E2 level (i.e. per 6.9 pg/mL higher E2).

At a level of 0.05 that did not account for multiple comparisons, higher levels of E2, E1, and SHBG were associated with lower total protein S antigen levels and higher E1 levels with lower ATc levels. Higher total T levels were associated with lower total protein S antigen levels. Higher DHEAS levels were associated with lower TG peak values, lower TG ETP values, and lower nAPCsr values. Higher DHEA levels were associated with lower TG peak values, lower TG ETP values, lower nAPCsr values, lower FVIIc levels, and lower total protein S antigen

levels. However, none of these associations was significant after accounting for multiple comparisons (multiple comparisons adjusted p=0.10.). We found no evidence of an association between percent free T, FTI, or androstenedione levels and any hemostatic factor levels.

In sensitivity analyses restricted to women ≥55 years of age at phlebotomy (appendix Table 1.A2), directionality of associations between hormone levels and hemostatic factor levels remained the same. In these sensitivity analyses, in which women are more likely to be >2 years after their FMP and more likely to have stable E2 levels than in our primary analyses,[45] there was some suggestion that higher total T levels may be associated with lower ATc levels and that higher DHEAS levels may be associated with less TG time-to-peak, but these associations were not statistically significant after accounting for multiple comparisons.

Discussion

In this cross-sectional study among postmenopausal women not currently using HT, after accounting for multiple comparisons, we found no statistically significant evidence of associations between endogenous hormone levels and hemostatic factor levels.

Estradiol, Estrone, and SHBG

In our study of postmenopausal women not using HT at the time of phlebotomy, there was some evidence that higher levels of E2 may be associated with lower total protein S antigen levels, that higher levels of E1 may be associated with lower ATc and total protein S antigen levels, and that higher SHBG levels may be associated with lower total protein S antigen levels; these associations were not statistically significant after accounting for multiple comparisons. Other studies have reported mixed findings relating estrogens and SHBG levels to hemostatic factors, but these studies have included women of different ages and menopausal stages. Levels of E1[48] and E2[49] have been positively associated with fibrinogen levels among postmenopausal women in the Atherosclerosis Risk in Communities (ARIC) Study and the French Three-City

cohort study of women >65 years of age. In the Study of Women's Health Across the Nation (SWAN), which included women aged 42-52 years at baseline, lower E2 levels were associated with higher levels of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA), but not with fibrinogen or FVII activity.[15] Previous studies have reported no evidence of an association between SHBG concentrations and fibrinogen levels[14,48,49], but lower SHBG concentrations were associated with higher levels of PAI-1, tPA, and FVII activity among SWAN participants.[14] Measures of the hemostatic factors PAI-1, tPA, and fibrinogen were not available for our study population.

Total T, Percent Free T, and FTI

We found some evidence that higher total T levels may be associated with lower total protein S antigen levels, but we found no other evidence that total T levels, percent free T or FTI were associated with hemostatic factor levels. In the SWAN study of women at midlife and in the Three-City study of women >65 years of age[49] there was no evidence of an association between the free androgen index (FAI, calculated as 100*T ng/dL/28.84*SHBG nM)[14] or total T levels[14,49] and fibrinogen levels. In contrast, in a cross-sectional analysis among postmenopausal women, fibrinogen was positively associated with higher levels of total T.[48] In SWAN, higher FAI values were associated with higher levels of PAI-1, tPA, and FVII activity; total T was positively associated with concentrations of PAI-1 and tPA but there was no evidence of an association with FVII activity.[14]

DHEAS, DHEA, and Androstenedione

In our study population of postmenopausal women, there was some evidence that higher levels of DHEAS and DHEA may be associated with lower TG peak values, lower ETP values, and lower nAPCsr values and that higher levels of DHEA may also be associated with lower levels of FVIIc and total protein S antigen; these associations were not statistically significant after

accounting for multiple comparisons. All suggestive associations between DHEAS and DHEA levels and hemostatic factor levels were in the direction opposite than what we had hypothesized, other than the association with total protein S.

The relation between measures of TG and nAPCsr with levels of these proandrogens has not been evaluated in other populations of postmenopausal women, and additional study is warranted. In a study of men (average age 54.5-58.1 years), higher levels of plasma DHEA were positively associated with levels of the APC-protein C inhibitor complex, a marker of ongoing protein C activation.[50] Other studies of midlife and postmenopausal women have reported that higher DHEAS concentrations are associated with higher values of PAI-1,[14] tPA,[14] and fibrinogen.[14,48]

The relation between endogenous DHEAS and DHEA levels and cardiovascular biomarkers and events, including VT, is unclear. We hypothesized that higher levels of these proandrogens would be associated with differences in hemostatic factor levels in the direction of greater thrombotic risk due to their downstream metabolism to endogenous estrogens and androgens[51]. However, given that DHEAS and DHEA levels decrease with age, other investigators have previously hypothesized that the increased risk of cardiovascular disease with advancing age may be associated with declining levels of serum DHEAS and DHEA[52], contrary to our study's original hypothesis.

Biologic Plausibility

We found no statistically significant evidence that endogenous hormone levels were associated with hemostatic factor levels. However, estrogens and androgens, and the downstream estrogenic and androgenic metabolites of the proandrogens, DHEAS and DHEA, may plausibly interact with functional androgen and estrogen receptors on vascular endothelial and smooth muscle cells.[53,54] Activated endothelial cells may express tissue factor (TF), which, in conjunction with activated factor VII, initiates the extrinsic pathway of the coagulation

cascade.[55,56] Higher TF levels have previously been associated with higher levels of total T, DHEAS, and androstenedione in a study of women with Polycystic Ovary Syndrome[57], further suggesting that androgens may be associated with hemostasis either via endothelial expression of TF or by another unknown mechanism. Although we did not measure TF in our study, there was some evidence that DHEAS and DHEA levels may be related to several global coagulation measures prior to correction for multiple comparisons: TG peak, which estimates an individual's peak thrombin concentration value, ETP, which represents an individual's endogenous thrombin potential, and nAPCsr, which is a measure of activated protein C resistance.

Whether a relationship between endogenous hormone and hemostatic factor levels would translate into an association with clinical cardiovascular endpoints, including VT risk, is unclear. In a recent study that included postmenopausal women, there was no evidence that endogenous levels of E2 and total T were associated with VT risk,[58] but investigators did not evaluate the relation between E1, SHBG, or adrenal proandrogens with VT risk. In addition, in a study of men aged 50 to 84 years of age, E2, SHBG, and total T levels were not associated with VT risk.[59] Whether levels of endogenous hormones and prohormones at higher concentrations among postmenopausal women than E2, total T, and SHBG, such as E1, DHEAS, and DHEA, are associated with VT risk is unknown.

Limitations and Strengths

Due to the cross-sectional nature of this study, the temporality and directionality of hormonal-hemostasis relationships cannot be determined. Residual confounding associated with unrecognized or unmeasured confounders may also remain. In addition, our study's relatively small sample size of 131 postmenopausal women limits power, and additional studies in populations of larger sample size are warranted. Also a potential limitation, it is unclear whether long-term storage of citrated plasma samples used to measure hemostatic factor levels (mean storage time=4.5 years) and of EDTA plasma samples used to measure endogenous hormone

levels (mean storage time=6.8 years) may have altered our hemostatic factor and hormone level measures. Many hemostatic factor measurements appear to be relatively stable in plasma samples stored for up to 2 years[60,61], but whether longer-term sample storage may bias our results is unclear.[37] Long-term storage of plasma samples may be associated with a decline in the concentration of some hormones, however, rank order of hormone concentrations appears to be relatively stable over at least 3 years of storage.[62] As long as declining hormone or hemostatic measures over storage time are non-differential, this should bias estimates towards the null. Also a limitation, our study measured hormone levels at only one point in time for each of our participants. A study of the short-term reliability of endogenous hormones in postmenopausal women suggested that E1 and T are relatively stable over 4 weeks, but that E2 and androstenedione levels are less stable.[63] In studies of hormone reliability among postmenopausal women over a longer period of 2 to 3 years, most hormone levels remained relatively stable, with some suggestion that E2 and androstenedione levels may be less stable.[63,64]

A strength of our study was the inclusion of measures of TG, which is a global marker for thrombotic risk; this is in contrast to the use of only individual markers of fibrinolysis and coagulation. Also a strength, our study utilized sensitive LC-MS/MS methods of hormone measurement, which enabled us to capture generally low levels of endogenous hormones present for postmenopausal women.

Conclusions

In this study of postmenopausal women not currently using HT, we did not find statistically significant evidence that levels of endogenous sex hormones were associated with hemostatic factor levels. There was some suggestion that higher levels of E2, E1, SHBG, total T, and DHEA may be associated with lower total protein S antigen levels, that higher E1 levels may be associated with lower ATc levels, and that higher levels of DHEAS and DHEA may be

associated with lower TG peak values, lower TG ETP values, and lower nAPCsr values, but these associations were not statistically significant after correcting for multiple comparisons. A larger study would strengthen our understanding of the potentially complex relation between endogenous hormone levels and thrombotic risk.

Table 1.1. Demographic and medical history characteristics of eligible participants.

	All Participants
	n=131
Age at Blood Draw, mean (SD), y	67.1 (9.7)
Years since FMP at Blood Draw, mean (SD), y	18.8 (10.8)
White race/ethnicity, No. (%)	122 (93.1)
Education >High School, No. (%)	97 (74.1)
Diabetes, No. (%)	18 (13.7)
Current Smoking, No. (%)	12 (9.2)
Hypertensive, No. (%)	74 (56.5)
BMI, mean (SD), kg/m^2	29.9 (7.7)
History of any CVD, No. (%)	11 (8.4)
Systolic Blood Pressure, mean (SD), mmHg	132.3 (17.3)
Total cholesterol, mean (SD), mg/dL	208.4 (40.8)
Hysterectomy/Oophorectomy, No. (%)	
No Surgery	83 (63.4)
Hysterectomy-alone	20 (15.3)
Hysterectomy with BSO	22 (16.7)
Unknown	6 94.6)
Ever estrogen HT use, No. (%)	88 (67.2)
Months Since Any HT Use at Blood Draw, mean (SD)	86.4 (67.5) n=96**

*SD = Standard deviation; y = Year; FMP=Final menstrual period; BMI = Body mass index; CVD = Cardiovascular disease; HT = Hormone therapy; BSO = Bilateral salpingo oophorectomy

**96 women used any HT (estrogen or progestogen) prior to blood draw.

Table 1.2. Linear regression-modeled cross-sectional associations between estrogen, SHBG, testosterone, and proandrogen levels and hemostatic factor levels, adjusted for covariates.

Estrogens and SHBG

		Estradiol (pg/mL) (n=131)			Estrone (pg/mL) (n=131)			SHBG (nmol/L) (n=131)		
	mean (SD)	6.5 (6.9)			27.0 (24.2)			143.8 (89.2)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	0.23 (0.50)	0.64	2.9%	0.012 (0.13)	0.93	0.53%	0.032 (0.043)	0.47	5.1%
ETP (nMxMin)	1214.9 (268.8)	-0.27 (0.27)	0.92	-0.69%	-0.35 (0.60)	0.56	-3.1%	-0.019 (0.21)	0.93	-0.63%
Lag-time (min)	2.3 (0.63)	-0.0058 (0.0065)	0.38	-6.2%	-0.00086 (0.0016)	0.59	-3.3%	-0.00043 (0.00046)	0.35	-6.1%
Time-to-peak (min)	4.4 (0.87)	-0.0070 (0.011)	0.51	-5.5%	-0.0012 (0.0024)	0.60	-3.5%	-0.00098 (0.00067)	0.15	-10.0%
nAPCsr	1.6 (1.2)	-0.0090 (0.012)	0.44	-5.2%	0.017 (0.13)	0.90	1.1%	0.00006 (0.00088)	0.95	0.45%
Factor VII (%)	131.1 (36.0)	0.086 (0.46)	0.85	1.6%	-0.26 (0.18)	0.14	-6.8%	-0.0022 (0.038)	0.95	-0.55
Antithrombin (%)	105.3 (16.5)	-0.30 (0.20)	0.14	-12.7%	-0.10 (0.04)	0.012	-15.3%	0.0012 (0.015)	0.94	0.65%
Total Protein S (%)	109.5 (18.9)	-0.86 (0.27)	0.002	-31.2%	-0.23 (0.056)	<0.001	-29.5%	-0.040 (0.019)	0.040	-19.0%

Testosterone

		Total T (ng/dL) (n=131)			Free T (%) (n=131)			Free T Index (n=131)		
	mean (SD)	15.3 (10.6)			0.77 (0.39)			0.49 (0.37)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	0.095 (0.42)	0.82	1.8%	-6.7 (12.7)	0.60	-4.7%	-6.7 (14.7)	0.65	-4.6%
ETP (nMxMin)	1214.9 (268.8)	1.5 (2.8)	0.61	5.6%	25.3 (62.6)	0.69	3.7%	30.1 (77.5)	0.70	4.20%
Lag-time (min)	2.3 (0.63)	-0.00093 (0.0036)	0.80	-1.6%	0.047 (0.15)	0.75	2.9%	-0.021 (0.11)	0.85	-1.3%
Time-to-peak (min)	4.4 (0.87)	0.00010 (0.0064)	0.99	0.12%	0.21 (0.23)	0.35	9.5%	0.13 (0.18)	0.46	5.8%
nAPCsr	1.6 (1.2)	0.00045 (0.0090)	0.96	0.40%	-0.20 (0.25)	0.42	-6.4%	-0.17 (0.23)	0.45	-5.3%
Factor VII (%)	131.1 (36.0)	-0.087 (0.34)	0.80	-2.6%	5.1 (8.3)	0.54	5.5%	3.1 (6.5)	0.63	3.3%
Antithrombin (%)	105.3 (16.5)	-0.23 (0.13)	0.082	-14.8%	-1.1 (4.1)	0.79	-2.6%	-8.7 (4.6)	0.062	-19.8%
Total Protein S (%)	109.5 (18.9)	-0.25 (0.12)	0.040	-14.3%	4.2 (4.5)	0.35	8.7%	-3.9 (4.1)	0.34	-7.8%

Proandrogens

		DHEAS (ug/mL) (n=131)			DHEA (ng/dL) (n=131)			Androstenedione (ng/dL) (n=131)		
	mean (SD)	0.52 (0.46)			110.2 (82.5)			35.8 (19.1)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	-39.7 (8.9)	<0.001	-33.2%	-0.17 (0.066)	0.011	-25.3%	-0.29 (0.29)	0.31	-10.3%
ETP (nMxMin)	1214.9 (268.8)	-143.1 (32.3)	<0.001	-24.4%	-0.66 (0.26)	0.013	-20.3%	-1.1 (2.2)	0.36	-8.2%
Lag-time (min)	2.3 (0.63)	-0.062 (0.083)	0.46	-4.4%	-0.000043 (0.00046)	0.93	-0.56%	0.0026 (0.0028)	0.35	7.8%
Time-to-peak (min)	4.4 (0.87)	0.20 (0.15)	0.19	10.5%	0.00085 (0.00095)	0.37	8.0%	0.0044 (0.0040)	0.27	9.7%
nAPCsr	1.6 (1.2)	-0.89 (0.26)	0.001	-34.0%	-0.0032 (0.0014)	0.027	-21.8%	-0.0056 (0.0049)	0.26	-8.9%
Factor VII (%)	131.1 (36.0)	-1.2 (4.8)	0.80	-1.5%	-0.081 (0.031)	0.010	-18.6%	-0.028 (0.18)	0.88	-1.5%
Antithrombin (%)	105.3 (16.5)	3.2 (3.2)	0.33	8.8%	0.022 (0.024)	0.36	11.1%	0.069 (0.10)	0.50	8.0%
Total Protein S (%)	109.5 (18.9)	-6.1 (3.4)	0.078	-14.7%	-0.062 (0.022)	0.005	-27.2%	-0.13 (0.10)	0.22	-12.9%

*Adjusted for linear age, linear BMI, current smoking, treated diabetes.

**Adjusted average % Difference in 1 SD of hemostatic factor, associated with 1-SD difference in endogenous hormone.

ETP=Endogenous thrombin potential; nAPCsr=Normalized activated protein C sensitivity ratio; SHBG=Sex hormone binding globulin; T=Testosterone; DHEAS=Dehydroepiandrosterone-sulfate; DHEA=Dehydroepiandrosterone; FTI=Free testosterone index

Table 1.A1. Correlations between endogenous hormone levels and hemostatic factor levels.

											Thrombin Generation							
			Estradiol (pg/mL)	Estrone (pg/mL)	SHBG (nmol/L)	Total T (ng/dL)	Free T (%)	FTI	DHEAS (ug/mL)	DHEA (ng/dL)	Androstene-dione (ng/dL)	Peak Value (nM)	ETP (nMxMin)	Lag-Time	Time to peak (min)	nAPCsr	FVII (%)	Anti-thrombin (%)	Total Protein S (%)
		n	131	131	131	131	131	131	131	131	131	131	131	131	131	130	126	125	130
	n	Median (IQR)	4.4 (5.4)	20.9 (17.4)	131.5 (109.4)	12.3 (10.1)	0.65 (0.52)	0.37 (0.38)	0.41 (0.45)	92.7 (79.0)	32.4 (21.4)	263.6 (71.5)	1170.0 (321.0)	2.0 (0.50)	4.3 (1.3)	1.5 (1.0)	125.9 (36.9)	105.6 (14.6)	106.3 (22.1)
		Pearson Correlation Coefficient																	
Estradiol (pg/mL)	131	4.4 (5.4)	-	0.78	0.045	0.17	0.099	0.28	0.087	0.12	0.17	0.099	0.075	-0.012	-0.013	-0.047	0.054	-0.095	-0.11
Estrone (pg/mL)	131	20.9 (17.4)	-	-	0.20	0.16	0.010	0.21	0.060	0.17	0.20	0.085	0.061	-0.017	-0.019	-0.049	0.033	-0.14	-0.18
SHBG (nmol/L)	131	131.5 (109.4)	-	-	-	0.16	-0.83	-0.51	-0.015	-0.088	-0.055	0.014	-0.045	-0.069	-0.11	0.0025	-0.034	-0.0064	-0.24
Total T (ng/dL)	131	12.3 (10.1)	-	-	-	-	-0.20	0.55	-0.005	0.062	0.33	0.018	0.051	0.015	0.010	-0.036	-0.017	-0.15	-0.14
Free T (%)	131	0.65 (0.52)	-	-	-	-	-	0.61	0.19	0.11	0.041	0.0095	0.085	0.030	0.092	-0.040	0.078	-0.029	0.15
FTI	131	0.37 (0.38)	-	-	-	-	-	-	0.23	0.20	0.32	-0.0047	0.088	0.015	0.080	-0.057	0.054	-0.17	-0.0096
DHEAS (ug/mL)	131	0.41 (0.45)	-	-	-	-	-	-	-	0.64	0.34	-0.34	-0.24	-0.018	0.15	-0.25	-0.055	0.13	-0.11
DHEA (ng/dL)	131	92.7 (79.0)	-	-	-	-	-	-	-	-	0.71	-0.23	-0.14	0.043	0.15	-0.21	-0.19	0.16	-0.16
Androstenedione (ng/dL)	131	32.4 (21.4)	-	-	-	-	-	-	-	-	-	-0.12	-0.075	0.12	0.15	-0.14	-0.048	0.10	-0.081
Thrombin Generation																			
Peak Value (nM)	131	263.6 (71.5)	-	-	-	-	-	-	-	-	-	-	0.78	0.11	-0.13	0.21	-0.020	-0.049	0.088
ETP (nMxMin)	131	1170.0 (321.0)	-	-	-	-	-	-	-	-	-	-	-	0.29	0.87	-0.13	0.0007	-0.0037	0.078
Lag-Time	131	2.0 (0.50)	-	-	-	-	-	-	-	-	-	-	-	-	0.28	0.12	-0.0060	0.0034	0.075
Time to peak (min)	131	4.3 (1.3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.24	0.036	0.10	0.084
nAPCsr	130	1.5 (1.0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.086	-0.043	-0.11
Factor VII (%)	126	125.9 (36.9)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.11	0.085
Antithrombin (%)	125	105.6 (14.6)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.29
Total Protein S (%)	130	106.3 (22.1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*SHBG=Sex hormone binding globulin; T=Testosterone; FTI=Free Testosterone Index; DHEAS=Dehydroepiandrosterone-sulfate; DHEA=Dehydroepiandrosterone; ETP=Endogenous thrombin potential; nAPCsr=Normalized activated protein C sensitivity ratio; FVII=Factor VII

Table 1.A2. Linear regression modeled cross-sectional associations between estrogen, SHBG, testosterone, and proandrogen levels and hemostatic factor levels, adjusted for covariates, among women >=55 years of age at blood draw.

Estrogens and SHBG

		Estradiol (pg/mL) (n=117)			Estrone (pg/mL) (n=117)			SHBG (nmol/L) (n=117)		
	mean (SD)	6.3 (6.9)			26.1 (23.0)			142.7 (90.6)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	0.49 (0.49)	0.32	6.1%	-0.024 (0.16)	0.88	-1.0%	0.031 (0.046)	0.51	5.1%
ETP (nMxMin)	1214.9 (268.8)	0.50 (3.1)	0.88	1.3%	-0.31 (0.76)	0.68	-2.7%	-0.0066 (0.23)	0.98	-0.22%
Lag-time (min)	2.3 (0.63)	-0.0094 (0.0054)	0.084	-10.3%	-000084 (0.0021)	0.69	-3.1%	-0.00046 (0.00048)	0.34	-6.6%
Time-to-peak (min)	4.4 (0.87)	-0.011 (0.0099)	0.27	-8.8%	-0.00042 (0.0029)	0.89	-1.1%	-0.0010 (0.00069)	0.15	-10.7%
nAPCsr	1.6 (1.2)	-0.0030 (0.013)	0.81	-1.7%	-0.0032 (0.0030)	0.28	-6.2%	-0.00040 (0.00084)	0.64	-3.0%
Factor VII (%)	131.1 (36.0)	0.13 (0.51)	0.79	2.6%	0.040 (0.15)	0.78	2.6%	-0.0070 (0.041)	0.87	-1.8%
Antithrombin (%)	105.3 (16.5)	-0.24 (0.26)	0.36	-10.0%	-0.11 (0.051)	0.029	-15.8%	-0.0058 (0.015)	0.70	-3.2%
Total Protein S (%)	109.5 (18.9)	-0.98 (0.33)	0.004	-35.8%	-0.27 (0.068)	<0.001	-32.9%	-0.039 (0.022)	0.073	-18.6%

Testosterone

		Total T (ng/dL) (n=117)			Free T (%) (n=117)			Free T Index (n=117)		
	mean (SD)	15.3 (11.0)			0.78 (0.39)			0.49 (0.39)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	-0.019 (0.45)	0.97	-0.38%	-6.3 (14.0)	0.65	-4.5%	-9.8 (16.1)	0.54	-7.1%
ETP (nMxMin)	1214.9 (268.8)	1.2 (3.1)	0.71	4.8%	25.0 (70.2)	0.72	3.6%	22.0 (86.4)	0.80	3.2%
Lag-time (min)	2.3 (0.63)	0.00029 (0.0037)	0.94	0.50%	0.049 (0.16)	0.76	3.0%	0.00081 (0.12)	0.99	0.050%
Time-to-peak (min)	4.4 (0.87)	0.0018 (0.0067)	0.79	2.2%	0.22 (0.25)	0.37	10.0%	0.19 (0.19)	0.32	8.3%
nAPCsr	1.6 (1.2)	-0.0039 (0.0086)	0.65	-3.6%	-0.081 (0.25)	0.75	-2.6%	-0.19 (0.24)	0.44	-6.1%
Factor VII (%)	131.1 (36.0)	-0.037 (0.36)	0.92	-1.1%	4.2 (8.9)	0.64	4.6%	3.3 (6.9)	0.63	3.6%
Antithrombin (%)	105.3 (16.5)	-0.27 (0.13)	0.042	-17.9%	0.35 (4.4)	0.94	0.82%	-7.4 (5.0)	0.14	-17.4%
Total Protein S (%)	109.5 (18.9)	-0.24 (0.13)	0.065	-14.2%	0.34 (5.0)	0.50	6.9%	-4.0 (4.4)	0.37	-8.2%

Proandrogens

		DHEAS (ug/mL) (n=117)			DHEA (ng/dL) (n=117)			Androstenedione (ng/dL) (n=117)		
	mean (SD)	0.47 (0.41)			101.3 (75.8)			34.8 (19.0)		
		Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**	Adjusted Average Difference (SE)*	p-value	Adj. % Diff. in SD**
Thrombin Generation										
Peak Value (nM)	261.1 (54.8)	-48.6 (7.3)	<0.001	-36.3%	-0.21 (0.071)	0.004	-28.5%	-0.33 (0.31)	0.30	-11.3%
ETP (nMxMin)	1214.9 (268.8)	-160.0 (33.9)	<0.001	-24.4%	-0.68 (0.31)	0.032	-19.1%	-0.86 (1.4)	0.54	-6.1%
Lag-time (min)	2.3 (0.63)	-0.068 (0.074)	0.36	-4.4%	0.000073 (0.00053)	0.89	0.88%	0.0034 (0.0030)	0.26	10.4%
Time-to-peak (min)	4.4 (0.87)	0.29 (0.14)	0.039	13.8%	0.0015 (0.0010)	0.15	13.0%	0.0061 (0.0042)	0.15	13.3%
nAPCsr	1.6 (1.2)	-0.91 (0.35)	0.011	-31.2%	-0.0039 (0.0017)	0.025	-24.4%	-0.0069 (0.0051)	0.18	-10.9%
Factor VII (%)	131.1 (36.0)	0.81 (5.5)	0.88	0.92%	-0.093 (0.040)	0.022	-19.7%	-0.049 (0.20)	0.81	-2.6%
Antithrombin (%)	105.3 (16.5)	2.4 (3.5)	0.49	6.0%	0.014 (0.028)	0.63	6.3%	0.039 (0.11)	0.72	4.5%
Total Protein S (%)	109.5 (18.9)	-9.6 (4.8)	0.050	-20.8%	-0.080 (0.027)	0.004	-32.1%	-0.14 (0.11)	0.23	-13.9%

*Adjusted for linear age, linear BMI, current smoking, treated diabetes.

**Adjusted average % Difference in 1 SD of hemostatic factor, associated with 1-SD difference in endogenous hormone.

ETP=Endogenous thrombin potential; nAPCsr=Normalized activated protein C sensitivity ratio; SHBG=Sex hormone binding globulin; T=Testosterone; DHEAS=Dehydroepiandrosterone-sulfate; DHEA=Dehydroepiandrosterone; FTI=Free testosterone index

CHAPTER 2: Hysterectomy and oophorectomy status and incident venous and arterial thrombosis risk among postmenopausal women: a population-based case-control study

Abstract

Background: Prior hysterectomy and bilateral salpingo-oophorectomy (BSO) are associated with decreased ovarian hormone levels. The risk of venous thrombosis (VT) associated with hysterectomy with and without BSO is incompletely characterized.

Methods: In a population-based case-control study, we identified postmenopausal cases of incident VT (n=1,358), myocardial infarction (MI) (n=1,184), and ischemic stroke (n=704) occurring between 1995 and 2010, and their controls matched by age, hypertension status, and calendar year of identification (n=4,112); eligible women had no prior reproductive cancer. The index date was defined as the incident event date for cases and for controls as a randomly chosen date within the year for which they had been sampled as controls. In primary analyses, we used multiple logistic regression to estimate VT risk associated with combined prior hysterectomy/oophorectomy status and current hormone therapy (HT) use status at the index date, compared with no prior hysterectomy and no current HT use. Secondary analyses separately evaluated the associations with risk of MI and stroke.

Results: Prior hysterectomy with BSO and no current HT use was associated with a 30% greater risk of VT than no prior hysterectomy and no current HT use (OR=1.3 [95% CI: 1.0-1.5]; p=0.02) but we found no evidence that prior hysterectomy with BSO with current HT use was associated with VT risk (OR=1.0 [95% CI: 0.78-1.3]). There was no evidence that prior hysterectomy-alone was associated with VT risk with current HT non-use (OR=1.0 [95% CI 0.80-1.3]) or with current HT use (OR=0.76 [95% CI 0.53-1.1]), compared with no prior hysterectomy and no current HT use.

Conclusions: Prior hysterectomy with BSO without current HT use was associated with a modestly greater risk of VT than no prior hysterectomy and no current HT use.

Introduction

Between 2000 and 2004, an estimated 5.4 hysterectomies were performed per 1000 women per year in the United States, at an average age of 46 years.[30] Bilateral salpingo-oophorectomy (BSO) is completed at the same time in more than 50% of all hysterectomies,[30] and removal of the ovaries in premenopause is associated with decreases in ovarian androgen and estrogen levels that persist into postmenopause.[16,17,65] Hysterectomy-alone is associated with smaller reductions in hormone levels[16,18,66]; these decreases in hormone levels are thought to result from reduced ovarian blood supply and subsequently impaired ovarian function.[67] The hormonal decreases associated with hysterectomy with and without concurrent BSO have been hypothesized to increase thrombotic event risk, and the association of early natural menopause with cardiovascular disease risk[19] has led to the hypothesis that hysterectomy and BSO prior to natural menopause may increase the risk of venous and arterial thrombosis compared with hysterectomy-alone.

Hysterectomy with and without BSO have been inconsistently associated with the risk of arterial thrombotic events.[23,68-70] Few studies have evaluated their associations with the risk of venous thrombosis (VT).[20,21,23,24] Due to differences in reference groups between studies and small numbers of VT cases with a prior BSO in some studies (ranging from 13-132 women), the association of hysterectomy with and without BSO and VT risk remains incompletely understood. In 2 studies evaluating VT risk associated with BSO, there has been no evidence that hysterectomy with BSO is associated with greater pulmonary embolism (PE) risk compared with hysterectomy-alone,[23] or that prior BSO is associated with greater VT risk than no prior BSO (with or without hysterectomy).[20] Investigators of a third study found no statistically significant evidence that BSO with or without hysterectomy was associated with VT risk compared with no hysterectomy or oophorectomy, but it is unclear whether this association was null or the study was underpowered (OR_{adj}=1.7 [95% CI 0.9-3.1]).[24]

In this population-based, case-control study among postmenopausal women, we evaluated the risk of incident VT associated with combined prior hysterectomy/oophorectomy status and current HT use status at the event date. As a secondary aim restricted to women with a prior hysterectomy, we evaluated the risk of VT associated with BSO compared with hysterectomy-alone, on average and separately by natural menopausal status, age at hysterectomy, and HT use at the event date. Also as secondary aims, we evaluated the risk of incident myocardial infarction (MI) and ischemic stroke associated with these exposures.

Methods

Setting

Our study setting was the Heart and Vascular Health (HVH) study, a large, population-based, case-control study set in Group Health Cooperative (GHC), an integrated health care system in Western Washington State. The study was approved by the GHC Human Subjects Review Committee.

Cases and Controls

Cases

All newly diagnosed first VT, MI, and ischemic stroke events occurring among postmenopausal GHC members were identified between 1995 and 2010. VT events were either deep vein thrombosis (DVT) or PE events. VT cases included postmenopausal women 18-89 years of age at the time of the event; MI and ischemic stroke cases included postmenopausal women 30-79 years of age. Event ascertainment involved 2 steps: screening and validation, and has been described in a prior publication[41]. Potential VT, MI, and ischemic stroke events were identified using *International Classification of Diseases, Ninth Revision (ICD-9)* hospital discharge diagnosis codes and *ICD-10* death record codes (Appendix Table 2.A1). Cases of DVT were also identified and screened using *ICD-9* codes from urgent care clinics or a prescription for a

low molecular weight heparin for non-hospitalized DVT treatment. All screened cases occurring among GHC members were validated through review of the inpatient and outpatient medical record by trained medical record abstractors using specified criteria[41] adapted from the Leiden Thrombophilia Study[71] for VT and the Cardiovascular Health Study[72,73] for MI and ischemic stroke. Validated VT events had been diagnosed by an imaging modality (95% with positive imaging) or were physician-diagnosed based on symptoms in the absence of imaging. Fatal PE events based on *ICD-9* or *ICD-10* diagnostic or death record codes were also validated as eligible VT events.[41] Eligible MI events were validated based on symptoms, electrocardiogram results, cardiac enzyme levels, and physician diagnosis and treatment.[74] Eligible ischemic stroke events were validated based on the rapid onset of a neurologic deficit lasting for ≥24 hours or evidence of infarction from brain imaging, at surgery, or at autopsy.[75] VT, MI, and stroke cases had no previous event of any type (VT, MI, or stroke) before their index date. The index date was defined as the incident event date for cases.

Controls

Controls were postmenopausal women selected at random from among Group Health members. For controls, the index date was a randomly chosen date within the calendar year for which they were serving as a control. Control subjects were frequency-matched to MI cases, the largest case group in the HVH study, on age (by decade), hypertension status, and calendar year of the index date. Controls had no previous VT, MI, or stroke events before their index date.

Prior Reproductive Cancer Exclusion

We excluded cases and controls with ovarian, endometrial, cervical, vaginal, vulvar, and other/unspecified female genital cancers prior to the index date (n=390/8,982; 4.3%), in an effort

to restrict our population to women who underwent hysterectomy with or without oophorectomy for benign conditions, resulting in a population of 8,592 postmenopausal women.

Measures

HVH study data were collected by trained abstractors from medical records, laboratory and pharmacy data, and from a telephone interview with surviving individuals who gave consent. GHC medical records contain a record of all inpatient and outpatient care received at GHC facilities or paid for by GHC insurance at non-GHC facilities. Women averaged 23 years of follow-up documented in the medical record between the first GHC visit and the index date.

Menopausal, Hysterectomy, and Oophorectomy Status

Menopausal status at the index date was based on information from the GHC medical record; women were considered to be postmenopausal following the cessation of menses, receipt of BSO, or, in women who had a hysterectomy without BSO, at the onset of menopausal symptoms occurring naturally. All women ≥55 years of age were considered to be postmenopausal. Hysterectomy and oophorectomy status, surgical dates, and the number of ovaries removed were determined by review of GHC medical records. If a woman had a hysterectomy or oophorectomy prior to GHC enrollment, this was also recorded by abstractors if noted in the medical record. Of eligible women, 45% of hysterectomies occurred during GHC enrollment (n=1,108) and 55% occurred prior to GHC enrollment (n=1,344). The amount of follow-up time between GHC enrollment and the index date was similar for women with a prior hysterectomy (mean=23.1 years; standard deviation [SD]=13.1) and women without a prior hysterectomy (mean=23.0 years; SD=13.2 years). We also identified the original source of medical record information regarding hysterectomy (patient-reported, included in a physician's note, from an operative pathology report, or unknown) and whether hysterectomy occurred

during natural pre/perimenopause, postmenopause, or whether a concurrent oophorectomy induced a transition into postmenopause.

Hormone Therapy

Current HT use at the index date was determined using computerized pharmacy records from GHC, including prescription fill dates, quantity of medication prescribed, and dosing instructions or the anticipated days' supply. We classified a woman as a current HT user at the index date if her most recent HT prescription prior to the index date would last until the index date, assuming that she took her medications 80% of the time. In this study population, 91% of women surveyed by telephone interview reported that they filled all or most all prescriptions at a GHC pharmacy, suggesting that our ascertainment of HT use is nearly complete.

Reproductive Cancer

For cases and controls with an index date on or after January 1, 1999, cancer diagnosis date and type was abstracted from GHC medical records, but was not abstracted for women with an index date between January 1, 1995 and December 31, 1998. For these women, we requested cancer diagnosis date and type information from the GHC cancer registry, which is comprised of cancer diagnosis data from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute.[76]

Covariates

Demographic and health characteristics prior to the index date were collected via review of the GHC medical record for cases and controls. Race, education, and smoking status were determined using information self-reported during the telephone interview and augmented with data from the medical record when missing. Body mass index (BMI) was determined using height and weight information contained in the medical record and was augmented with

information reported during the telephone interview when missing. From the medical record we also collected information regarding risk factors for cardiovascular events, including hypertension status, prevalent cardiovascular disease other than MI or stroke (defined as a history of angina, claudication, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, carotid endarterectomy, or peripheral vascular disease), prevalent diabetes mellitus and the most recent total cholesterol level prior to the index date. Data were missing for BMI in 1.1%, race in 0.4%, education in 25.7%, smoking in 0.3%, physical activity in 5.3%, and total cholesterol level in 4.7% of women.

Statistical Analyses

Data Management

We classified women's hysterectomy and oophorectomy status at the index date into 3 categories: no hysterectomy or oophorectomy, hysterectomy without the removal of any ovaries (hysterectomy-alone), and hysterectomy with BSO. We excluded women with an unknown hysterectomy or oophorectomy status (n=487/8,592, 5.7%), an oophorectomy without a hysterectomy (n=128/8,592, 1.5%), a hysterectomy with the removal of only 1 ovary (n=278/8,592, 3.2%) or an unknown number of ovaries removed (n=14/8,592, 0.16%), a hysterectomy with an unknown date of hysterectomy (n=164/8,592, 2.0%), or a hysterectomy and BSO that were performed on different dates (n=148/8,592, 1.7%).

Women were also excluded from analyses if HT use at the index date was not as expected based on hysterectomy status: current estrogen (E) plus progestogen (P) use with prior hysterectomy (n=21/8,592, 0.24%), current E-alone use without prior hysterectomy (with an intact uterus) (n=99/8592, 1.2%), or current P-alone use (n=60/8592, 0.70%). Collectively, these exclusions resulted in a group of 7,358 women eligible for analyses.

Analyses among All Eligible Women

Multiple logistic regression analyses were conducted separately for each cardiovascular endpoint (VT, MI, and ischemic stroke) and relative risks were estimated by comparing 5 mutually exclusive hysterectomy, BSO, and current HT use groups to 1 reference group defined as no prior hysterectomy and no current HT. Groups compared to this reference category were: 1) no prior hysterectomy and current E+P HT use; 2) prior hysterectomy-alone and no current HT use; 3) prior hysterectomy-alone and current E-alone HT use; 4) prior hysterectomy with BSO and no current HT use; and 5) prior hysterectomy with BSO and current E-alone HT use. Models were adjusted for case-control design variables (age at the index date [linear], treated hypertension status [yes vs. no], and grouped index year [categorical]) and for potential confounding variables determined *a priori*: race (White vs. non-White), BMI (linear), education (greater than high school education vs. high school or less), and tobacco smoking status (current vs. former/never). We estimated adjusted odds ratios (ORs) with 95% confidence intervals (CIs). Missing data for BMI, race, education, and smoking status were imputed using a chained equations approach[77] in Stata 13.1[46] with 10 imputed datasets. Case-control status (categorical), combined hysterectomy/oophorectomy and HT use (categorical), age (linear), age in decade (categorical), hypertension (yes vs. no), index year (categorical), and decade of birth (linear) were included as predictors in the imputation model. We tested for heterogeneity in risk among the 6 hysterectomy/oophorectomy and HT use status categories using a likelihood ratio test. To test for multiplicative statistical interaction between hysterectomy-BSO status and HT use at the index date, we used a likelihood ratio test.

Analyses among Women with a Prior Hysterectomy

To address our secondary aims among women with a prior hysterectomy, we excluded women without a prior hysterectomy and directly evaluated the risk of VT, MI, and ischemic stroke associated with hysterectomy with BSO compared with hysterectomy without BSO. In these analyses among women with a prior hysterectomy, we adjusted for current HT use, since all HT

used by women with a prior hysterectomy was consistently E-alone. We were interested in estimating the relative risk of thrombotic events associated with hysterectomy with BSO compared with hysterectomy-alone, above and beyond the well-characterized risk associated with HT use compared with non-use; therefore, although likely on the causal pathway between BSO status and thrombotic event risk, we included HT use as a covariate. Adjustment variables included age (linear), treated hypertension status (yes vs. no), grouped index year (categorical), race-ethnicity (White vs. non-White), BMI (linear), education (greater than high school vs. high school or less), tobacco smoking status (current vs. former/never), and current HT use at the index date (any vs. none).

As secondary analyses among women with a prior hysterectomy, we also evaluated the risk of VT, MI, and ischemic stroke associated with hysterectomy with BSO among subgroups of women by menopausal status at the time of hysterectomy (naturally pre/perimenopausal vs. postmenopausal), and separately, by age category at the time of hysterectomy (<45 years, ≥45-54 years, ≥55 years), using logistic regression models. Of 2,542 eligible women with a prior hysterectomy, menopausal status at the time of hysterectomy was missing for 4.3% (n=107); these women were excluded from subgroup analyses by natural menopausal status at hysterectomy. In addition, we evaluated the risk of VT, MI, and ischemic stroke associated with hysterectomy with BSO among subgroups of current HT users and non-users at the index date. To evaluate whether relative risks associated with BSO differed, separately, by natural menopausal status at hysterectomy, age at the time of hysterectomy, or current HT use at the index date, we used likelihood ratio tests.

Sensitivity Analyses

Sensitivity analyses excluded women known to have been diagnosed with cancer of any type prior to the index date, and separately, restricted the population to women with index dates on or after January 1, 1999 to only include women who had GHC cancer data available.

Results

Between January 1, 1995 and December 31, 2010, we identified 1,358 postmenopausal women with incident VT, 1,184 with incident MI, 704 with incident ischemic stroke, and 4,112 eligible controls without a history of reproductive cancer and eligible for analyses (N=7,358). Characteristics of case and control subjects are provided in Table 2.1. On average, women were 69 years of age at the index date. Compared with controls, cases had a higher mean BMI, a larger proportion were current smokers, and a larger proportion had diabetes mellitus. Among women with a prior hysterectomy, the interval between the hysterectomy and the index date was on average 25 years (SD=11.9; range=0-62.4 years). The majority of hysterectomy information in the medical record was from a doctor's note or pathology report (78.6%). Characteristics of controls by hysterectomy and oophorectomy status and current HT use are presented in Appendix Table 2.A2.

Hysterectomy with and without BSO, By Categories of HT Use at the Index Date

In Table 2.2, we present results from a logistic regression model that includes all eligible women and, compared with one reference category of no prior hysterectomy and no current HT use, evaluates VT risk associated with prior hysterectomy/oophorectomy and current HT use status. There was no evidence that hysterectomy-alone with current HT non-use was associated with VT risk (OR=1.0 [95% CI 0.80-1.3]) and little evidence that hysterectomy-alone with current HT use was associated with VT risk (OR=0.76 [95% CI 0.53-1.1]), compared to the referent category of no prior hysterectomy and no current HT use. Prior hysterectomy with BSO and no current HT use was associated with a 30% greater risk of VT than no hysterectomy and no current HT use (OR=1.3 [95% CI 1.0-1.5]). We did not find evidence that prior hysterectomy with BSO and current HT use was associated with VT risk, compared with no prior hysterectomy and no current HT use. Current E+P use among women without a hysterectomy was associated

with a 50% greater risk of VT (OR=1.5 [95% CI 1.2-1.9]). There was evidence of heterogeneity in risk among the 6 hysterectomy/oophorectomy and current HT use categories (p=0.003). In a sensitivity analysis restricted to women without a cancer diagnosis of any type prior to the index date and a separate analysis restricted to index years ≥1999, when cancer information was abstracted from the GHC medical record for all subjects, findings were similar to those in the primary analyses (Appendix Tables 2.A5 and 2.A6).

Analyses addressing secondary aims separately evaluated the risk of MI and ischemic stroke associated with prior hysterectomy/oophorectomy and current HT use status. We found no evidence of an altered risk of MI or ischemic stroke in women who had undergone hysterectomy-alone or hysterectomy with BSO, whether or not women were currently using HT. Results of these secondary analyses are presented as tables in the appendix (Table 2.A3).

Hysterectomy with BSO Compared with Hysterectomy-Alone

Analyses addressing our secondary aims, presented in table 2.3, are restricted to women with a prior hysterectomy and evaluate VT risk associated with hysterectomy with BSO compared with hysterectomy-alone, on average, and among subgroups of menopausal status at hysterectomy, age at hysterectomy, and current HT use at the index date. Among women with a prior hysterectomy, receipt of BSO may be associated with a 30% greater risk of VT (OR=1.3 [95% CI 0.99-1.6]), but this association was not statistically significant (p=0.057). We found no evidence that the association between hysterectomy with BSO and VT risk differed, separately, by menopausal status (p=0.46), age at hysterectomy (p=0.62), or by current HT use at the index date (p=0.97).

Secondary analyses evaluating MI and ischemic stroke risk among women with a prior hysterectomy are presented in the appendix (Table 2.A4). Among women with a hysterectomy, we found no evidence of an association between receipt of BSO and the risk of MI or ischemic

stroke either overall or in subgroup analyses by menopausal status (MI p=0.14; ischemic stroke p=0.99) or by current HT use (MI p=0.79; ischemic stroke p=0.65).

Discussion

In this case-control study of postmenopausal women without a history of reproductive cancer, women who had previously undergone a hysterectomy with BSO and were not currently using HT had a modestly greater risk of VT than women with no prior hysterectomy who were not currently using HT. There was no such greater risk associated with a prior hysterectomy-alone, with or without current HT use, or with a prior hysterectomy with BSO and current HT use. In secondary analyses restricted to women with a prior hysterectomy, hysterectomy with concurrent BSO may be associated with somewhat greater VT risk than hysterectomy with ovarian conservation. We did not find evidence of an association between hysterectomy with or without BSO and the risk of MI or ischemic stroke.

Venous Thrombosis

Hysterectomy with and without BSO Compared with No Hysterectomy

Among women not currently using HT at the index date, we estimated a 30% greater risk of VT associated with hysterectomy with BSO compared with no hysterectomy or BSO. Investigators of a study set in the General Practice Research Database in the United Kingdom that estimated VT risk compared to that associated with no hysterectomy or oophorectomy reported some suggestion of a greater VT risk associated with BSO with or without hysterectomy, adjusting for HT use, but this finding was not significant (OR_{adj}=1.7 [95% CI 0.9-3.1]).[24] Given their study's small number of VT cases with BSO (n=13), it is unclear whether the study was underpowered to detect an association were one to exist, or if this was a true null association. As in our study, they reported no evidence that hysterectomy without BSO was associated with greater risk than no hysterectomy or oophorectomy (OR_{adj}=0.9 [95% CI 0.6-1.4]).[24]

Investigators of an analysis set in the Women's Health Initiative HT (WHI-HT) trial evaluated VT risk associated with BSO, compared to that associated with no BSO (with or without hysterectomy), adjusting for HT arms, hormone use at baseline, hormone use duration, and other possible confounders.[20] Among WHI-HT trial enrollees, there was no evidence of an association between prior BSO and risk compared with no BSO (HR_{adj}=1.0 [95% CI 0.7-1.3]). Our findings may differ from those reported by WHI investigators because of differences in reference and exposure groups and in the consideration of HT use. The WHI analysis reference group included a mixture of women with and without hysterectomy and the analysis was adjusted for HT use rather than separated in exposure groups by E+P and E-alone use. When we conducted a relatively similar analysis in our population, evaluating the risk of VT associated with BSO compared with no BSO (with or without hysterectomy), adjusting for current HT use (any vs. none) and our other covariates, our results were similar to those in WHI (HVH study OR=1.0 [95% CI 0.86-1.3]).

Hysterectomy with BSO Compared with Hysterectomy-Alone

In secondary analyses among women with a prior hysterectomy, concurrent BSO may be associated with a modest, 30% greater risk of VT than hysterectomy-alone, adjusting for current E-alone HT use; however, this risk estimate was not statistically significant. In a Nurses' Health Study (NHS) analysis restricted to women with a prior hysterectomy, there was no evidence of an association between hysterectomy with BSO compared with hysterectomy-alone and the risk of PE (HR_{adj}=1.1 [95% CI 0.85-1.5]).[23] Elective BSO may be performed at the time of hysterectomy to reduce the risk of ovarian cancer, benign neoplasms, endometriosis, and pelvic pain[78], and concomitant BSO accompanies over half of all hysterectomies[30]. Modest in magnitude, the suggestion in our study that hysterectomy with BSO may be associated with a greater VT risk than hysterectomy-alone requires replication. An improved understanding of possible VT risk differences associated with BSO vs. ovarian conservation at the time of

hysterectomy may be helpful for medical practitioners and women in weighing the risks and benefits of elective BSO at the time of hysterectomy.

We had hypothesized that hysterectomy with BSO might be associated with greater VT risk than hysterectomy-alone among women who had their hysterectomy in pre/perimenopause, but that there may not be evidence of such greater risk among women who received their BSO in postmenopause. We anticipated similar interactions by age at hysterectomy, expecting to see hysterectomy with BSO associated with greater VT risk among women younger at the age at hysterectomy rather than older. In our study and the NHS analysis, there was no evidence that this association differed by subgroups of age at hysterectomy, but point estimates within the subgroup of women <45 years of age at hysterectomy were similar between the NHS (HR_{adj}=1.3 [95% CI 0.87-2.0])[23] and our analysis. We also found no evidence of interaction by menopausal status at hysterectomy, and risk estimates associated with BSO were similar between women who were pre/perimenopausal and postmenopausal at the time of hysterectomy. In contrast to the fluctuation of ovarian hormone levels over several years during the natural menopausal transition, BSO prior to natural menopause initiates an abrupt decrease in estradiol (E2), sex hormone binding globulin (SHBG) and testosterone (T).[16-18] Furthermore, the ovaries continue to produce androgens into postmenopause,[79] and lower levels of total T, bioavailable T, and androstenedione persist into postmenopause among women with a prior oophorectomy as compared to women with intact ovaries and a uterus.[16] The risk of VT associated with young age at menopause is inconsistent.[20-22] Even though BSO prior to natural menopause is associated with abrupt hormonal decreases, in our study this did not translate into a greater risk of VT associated with BSO in pre/perimenopause as compared to in postmenopause.

Myocardial Infarction and Ischemic Stroke

Hysterectomy with and without BSO Compared with No Hysterectomy

In secondary analyses, we did not find evidence of a greater risk of MI and ischemic stroke associated with hysterectomy with or without BSO compared with no hysterectomy, among current HT users or non-users. Investigators of a WHI-observational study analysis that included women both with and without a hysterectomy reported that hysterectomy-alone and hysterectomy with BSO were both associated with a slightly greater risk of cardiovascular disease than no hysterectomy/oophorectomy, adjusting for demographics and body measures (HR=1.2 [95% CI 1.0-1.3]) but after adjustment for CVD risk factors, estimates shifted slightly towards the null (HR=1.1 [95% CI 0.99-1.2]).[69]

Hysterectomy with BSO Compared with Hysterectomy-Alone

Among women with a prior hysterectomy, our investigative group also found no evidence that MI or ischemic stroke was associated with hysterectomy with BSO compared with hysterectomy-alone, either on average or within subgroups of women by menopausal status or age at hysterectomy or by current HT use. Hysterectomy with BSO has been inconsistently associated with arterial thrombotic event risk in prior studies. Compared with hysterectomy-alone, hysterectomy with BSO has been associated with greater coronary heart disease risk (HR_{adj}=1.2 [95% CI 1.0-1.4)] but not with stroke risk in the NHS.[23] In the WHI-HT trials, there was no evidence of an association of hysterectomy with BSO and the risk of total fatal and nonfatal CVD, stroke, or total CVD within any age at hysterectomy subgroup.[70]

Limitations and Strengths

Our study has several limitations. There is the potential for misclassification of hysterectomy and oophorectomy status if this information is inaccurately reported in the medical record. However, the majority of hysterectomies in our study were reported via a doctor's note, operative report, or pathology report in the medical record (78.6% overall) and only 21.3% were from patient self-report or an unknown source. In an attempt to exclude misclassified

hysterectomies and oophorectomies, we also excluded all women who were identified as having had a hysterectomy or oophorectomy but who were missing dates for either procedure (3.1% missing). There is also potential for misclassification of menopausal status at the time of hysterectomy. However, analyses within subgroups of women by age at hysterectomy also estimated no evidence of a difference in the risk of VT associated with hysterectomy with BSO compared with hysterectomy-alone.

Our analyses only considered current HT use at the time of the index date. GHC pharmacy records available for this study include information from March 1, 1977 forward and we were therefore unable to account for lifetime duration of HT use. In a 2011 WHI analysis, however, VT risk associated with HT use dissipated following HT cessation.[80] Therefore, our consideration only of HT use at the index date likely includes the period of HT use most relevant to VT risk.

In this observational study, we did not randomize women to hysterectomy/oophorectomy or HT use status and the potential for residual confounding by indication or by other factors remains. Our study did not collect information regarding the indication for hysterectomy or oophorectomy or family history of cancer but we excluded all women with any history of reproductive cancer prior to index in an effort to restrict our population to women who underwent these procedures for benign medical condition. There is the potential that some women in our study proactively had a hysterectomy with or without oophorectomy due to undiagnosed cancers or a family history of cancer. However, in the United States, cancer is the primary indication for less than 10% of hysterectomies.[30] A 2002-2004 National Hospital Discharge Survey Study reported that among women aged 15-29 at hysterectomy, the most common indications for hysterectomy were categorized into an “other” category that included cervical dysplasia and menstrual disturbances.[30] Endometriosis was the most common indication for women aged 30-34, uterine leiomyoma for women aged 35-54, and uterine prolapse and cancer for women aged ≥55 years at hysterectomy.[30]

Our study also included several strengths. The population-based design reduces the likelihood of selection bias. Furthermore, since all women eligible for our study were members of a single integrated health care system there is the potential for reduced residual confounding by health care delivery factors, provider, socioeconomic factors, and access to health care. Additional strengths include the verification of MI, ischemic stroke, and VT outcome events, and the use of GHC pharmacy data to classify current HT use at the index date.

Conclusions

In this population-based, case-control study of incident VT, we found that among women not currently using HT, women with a prior hysterectomy with BSO had a modestly greater risk of VT than women with no hysterectomy. Among women with a prior hysterectomy, there may be an association with VT of hysterectomy with BSO compared with hysterectomy with ovarian conservation; we found no significant evidence of a difference in this association by menopausal status or age at hysterectomy, or by current HT use at index. These findings suggest that changes in ovarian hormones following hysterectomy with BSO may potentially contribute to the pathogenesis of VT. Additional research regarding the relation between endogenous hormones and VT risk may contribute to an improved understanding of VT risk associated with hysterectomy with BSO.

Table 2.1. Characteristics of VT, MI, and ischemic stroke cases and controls at the index date (n=7,358).				
	Controls	**VT Cases**	**MI Cases**	**Ischemic Stroke Cases**
	n=4,112	**n=1,358**	**n=1,184**	**n=704**
Age, mean (SD), years	69.3 (9.8)	70.0 (11.4)	68.8 (8.3)	70.4 (7.8)
White race/ethnicity, No.(%)	3645 (88.7)	1210 (91.2)	1079 (91.2)	616 (87.5)
Greater than High School Education, No (%)	2077 (64.9)	601 (62.4)	483 (56.7)	259 (57.6)
BMI, mean (SD), (kg/m2)	28.1 (6.4)	29.8 (8.2)	29.6 (7.2)	29.3 (6.9)
Current Smoker, No (%)	347 (8.5)	122 (9.1)	240 (20.3)	99 (14.1)
Prevalent CVD, No. (%)	341 (8.3)	128 (9.4)	239 (20.2)	108 (15.3)
Diabetes Mellitus, No. (%)	362 (8.8)	136 (10.0)	318 (26.9)	194 (27.6)
Treated Hypertension, No. (%)	2100 (51.1)	618 (45.5)	695 (58.7)	484 (68.8)
Total Cholesterol Level, mean (SD), mg/dL	223.9 (50.4)	221.8 (46.1)	232.8 (50.1)	227.6 (48.6)
Hysterectomy and Oophorectomy Status, No. (%)				
Intact Ovaries and Uterus	2767 (67.3)	912 (67.2)	785 (66.3)	442 (62.8)
Hysterectomy-Alone	548 (13.3)	156 (11.5)	170 (14.4)	111 (15.8)
Hysterectomy with BSO	797 (19.4)	290 (21.4)	229 (19.3)	151 (21.5)
Characteristics among Women with Hysterectomy	**n=1345**	**n=446**	**n=399**	**n=262**
Age at Hysterectomy, No. (%)				
<45 Years	726 (54.0)	206 (46.2)	220 (55.2)	147 (56.1)
45-54 Years	441 (32.8)	151 (33.8)	131 (32.8)	82 (31.3)
>=55 Years	178 (13.2)	89 (20.0)	48 (12.0)	33 (12.6)
Years since Hysterectomy, mean (SD)	25.2 (12.0)	23.2 (13.3)	24.9 (10.5)	26.5 (10.3)
Medical Record Source of Hysterectomy Information, No. (%)				
Patient	248 (18.4)	63 (14.1)	79 (19.8)	36 (13.7)
Doctor's Note	518 (38.5)	152 (34.1)	161 (40.4)	128 (48.9)
Operation/Pathology Report	567 (42.2)	227 (50.9)	155 (38.9)	93 (35.5)
Other/Unknown	12 (0.89)	4 (0.90)	4 (1.0)	5 (1.9)

SD = standard deviation; BMI=body mass index; CVD=cardiovascular disease; MI=myocardial infarction; VT=venous thrombosis; BSO=Bilateral salpingo-oophorectomy

Table 2.2. Risk of VT associated with combined hysterectomy with and without BSO and current HT use status.

	No HT Use*					HT Use*				
	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value
Venous Thrombosis										
No Hysterectomy	2,332	752	No HT	1.0 (ref)		435	160	E+P	1.5 (1.2-1.9)	<0.001
Hysterectomy-Alone	362	118	No HT	1.0 (0.80-1.3)	0.96	186	38	E-alone	0.76 (0.53-1.1)	0.15
Hysterectomy with BSO	481	203	No HT	1.3 (1.0-1.5)	0.02	316	87	E-alone	1.0 (0.78-1.3)	0.93

BSO=Bilateral Salpingo-Oophorectomy; E=Estrogen; P=Progestogen; HT=Hormone Therapy; VT=Venous Thrombosis; OR=Odds Ratio; CI=Confidence Interval.

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, more than high school education, current smoking status.

*P-value from likelihood ratio test of interaction between hysterectomy/oophorectomy and HT status = 0.0002.

Table 2.3. Risk of VT associated with hysterectomy with BSO compared with hysterectomy-alone, on average and stratified by menopausal status and age at hysterectomy, and by current HT use at the index date.

	Hysterectomy-Alone (reference)		Hysterectomy with BSO		Hysterectomy-Alone	Hysterectomy with BSO		
	Control n	Case n	Control n	Case n		Adjusted OR (95% CI)[a]	p-value	Likelihood Ratio Test p-value
Venous Thrombosis								
Unstratified	548	156	797	290	1.0	1.3 (0.99-1.6)	0.057	-
Menopausal Status at Hysterectomy								
Pre/Perimenopause	412	108	641	213	1.0	1.3 (0.99-1.7)	0.06	
Postmenopause	90	35	147	70	1.0	1.2 (0.69-2.0)	0.57	0.46
Age at Hysterectomy								
<45 Years	360	90	366	116	1.0	1.3 (0.93-1.8)	0.12	
45-54 Years	131	38	310	113	1.0	1.2 (0.74-1.8)	0.53	
>=55 Years	57	28	121	61	1.0	0.95 (0.52-1.7)	0.87	0.62
Current Hormone Therapy Use at the Index Date								
Non-Use of HT	362	118	481	203	1.0	1.3 (0.95-1.6)	0.11	
Use of HT	186	38	316	87	1.0	1.2 (0.79-1.9)	0.36	0.97

BSO=Bilateral Salpingo-Oophorectomy; HT=Hormone Therapy; VT=Venous Thrombosis; OR=Odds Ratio; CI=Confidence Interval.

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, education (indicator variable), smoking status (dummy variable), and current hormone therapy use at index assuming 80% compliance.

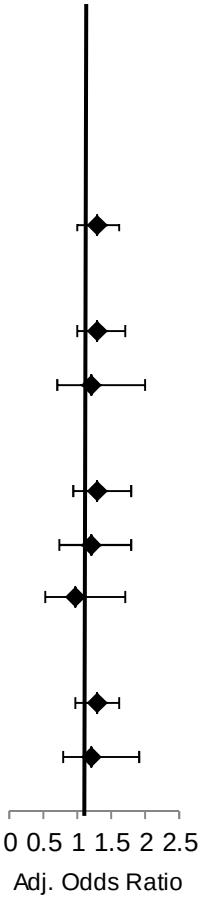

Table 2.A1. *International Classification of Diseases (ICD)*, *Ninth Revision* hospital discharge codes and *ICD-10* death record codes used to identify possible VT, MI, and stroke events.

Used to Identify:	*ICD-9* Codes	*ICD-10* Codes
VT (DVT and PE)	415.1	I26.x
	451.1-.2	
	451.81-.84	
	451.89-.9	
	453.0-.2	
	453.8-.9	
	671.3-.4	
	673.2	
	453.40-.42	
	38.7*	
MI	410	I21.x
	427.4-.5	I46.x
	427.5	I49.0
		I49.8-.9
		147.0-.9
		149.1-.4
Stroke	430	I60.x
	431	I61.x
	432.9	I62.9
	434	I63.x
	435	I64.x
	436	I69.x
		G45.x

DVT=Deep Vein Thrombosis;
PE=Pulmonary Embolism;
MI=Myocardial Infarction.

*Procedure Code.

Table 2.A2. Characteristics of controls by hysterectomy and oophorectomy status and current HT use at index assuming 80% compliance (n=4112).

	No Current HT Use			Current HT Use		
	n=3175			n=937		
	No Hysterectomy or Oophorectomy	Hysterectomy-Alone	Hysterectomy with BSO	No Hysterectomy or Oophorectomy	Hysterectomy-Alone	Hysterectomy with BSO
	n=2,332	n=362	n=481	n=435	n=186	n=316
Age, mean (SD), years	69.8 (9.6)	71.3 (9.0)	71.1 (10.0)	64.0 (8.7)	69.4 (8.7)	68.0 (10.8)
White race/ethnicity, No.(%)	2041 (87.6)	317 (87.6)	425 (88.4)	397 (91.3)	171 (91.9)	294 (93.0)
Greater than High School Education, No (%)	1153 (65.4)	174 (60.8)	222 (58.9)	276 (75.2)	99 (66.3)	153 (60.0)
BMI, mean (SD), (kg/m2)	28.0 (6.50)	28.8 (6.3)	28.7 (6.8)	27.3 (6.3)	28.5 (6.2)	28.5 (5.5)
Prevalent CVD, No. (%)	205 (8.8)	34 (9.4)	45 (9.4)	16 (3.7)	14 (7.5)	27 (8.5)
Diabetes Mellitus, No. (%)	219 (9.4)	41 (11.3)	50 (10.4)	18 (4.1)	10 (5.4)	24 (7.6)
Treated Hypertension, No. (%)	1157 (49.6)	194 (53.4)	282 (58.6)	174 (40.0)	96 (51.6)	197 (62.3)
Birth Decade						
<1910	9 (0.39)	4 (1.1)	1 (0.21)	0 (0.0)	1 (0.54)	1 (0.32)
1910-1919	219 (9.4)	37 (10.2)	56 (11.6)	20 (4.6)	14 (7.5)	33 (10.4)
1920-1929	815 (35.0)	131 (36.2)	167 (34.7)	118 (27.1)	75 (40.3)	102 (32.3)
1930-1939	616 (26.4)	109 (30.1)	152 (31.6)	126 (29.0)	64 (34.4)	96 (30.4)
1940-1949	481 (20.6)	62 (17.1)	62 (12.9)	146 (33.6)	25 (13.4)	50 (15.8)
1950-1959	187 (8.00	19 (5.3)	37 (7.8)	24 (5.5)	7 (3.8)	33 (10.4)
1960-1969	5 (0.21)	0 (0.0)	5 (1.0)	1 (0.23)	0 (0.0)	1 (0.32)
1970-1979	0 (0.0)	0 (0.0)	1 (0.21)	0 (0.0)	0 (0.0)	0 (0.0)
Characteristics among Women with Hysterectomy						
Age at Hysterectomy, No. (%)						
<=45 Years	-	84 (23.2)	178 (37.0)	-	47 (25.3)	132 (41.80
46-54 Years	-	234 (64.6)	217 (45.1)	-	126 (67.7)	149 (47.2)
>=55 Years	-	44 (12.2)	86 (17.9)	-	13 (7.0)	35 (11.1)
Years since Hysterectomy, mean (SD)	-	27.3 (10.8)	24.3 (12.7)	-	27.3 (11.1)	22.8 (11.9)
Medical Record Source of Hysterectomy Information, No. (%)						
Patient	-	52 (14.4)	52 (14.4)	-	100 (20.8)	64 (20.3)
Doctor's Note	-	161 (44.5)	161 (44.5)	-	164 (34.1)	98 (31.0)
Operation/Pathology Report	-	144 (39.8)	144 (39.8)	-	212 (44.1)	153 (48.4)
Other/Unknown	-	5 (1.4)	5 (1.4)	-	5 (1.0)	1 (0.32)

HT = hormone therapy; SD = standard deviation; BMI=body mass index; CVD=cardiovascular disease; MI=myocardial infarction; VT=venous thrombosis; BSO=Bilateral salpingo-oophorectomy.

Table 2.A3. Risk of MI and ischemic stroke associated with combined hysterectomy with and without BSO and current HT use status.

	No HT Use*					HT Use*				
	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value
Myocardial Infarction										
No Hysterectomy	2,332	644	No HT	1.0 (ref)		435	141	E+P	1.1 (0.88-1.4)	0.41
Hysterectomy-Alone	362	114	No HT	1.1 (0.85-1.4)	0.54	186	56	E-alone	0.94 (0.68-1.3)	0.71
Hysterectomy with BSO	481	137	No HT	0.97 (0.78-1.2)	0.80	316	92	E-alone	0.93 (0.72-1.2)	0.57
Ischemic Stroke										
No Hysterectomy	2,332	367	No HT	1.0 (ref)		435	75	E+P	1.2 (0.87-1.5)	0.33
Hysterectomy-Alone	362	74	No HT	1.2 (0.92-1.6)	0.17	186	37	E-alone	1.1 (0.78-1.7)	0.49
Hysterectomy with BSO	481	96	No HT	1.2 (0.88-1.5)	0.32	316	55	E-alone	0.97 (0.71-1.3)	0.84

BSO=Bilateral Salpingo-Oophorectomy; E=Estrogen; P=Progestogen; HT=Hormone Therapy; OR=Odds Ratio; CI=Confidence Interval; MI=Myocardial Infarction

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, more than high school education, current smoking status.

*P-value from likelihood ratio test of interaction between hysterectomy/oophorectomy and HT status = 0.51 for MI analyses; 0.38 for ischemic stroke analyses.

Table 2.A4. Risk of MI and ischemic stroke associated with hysterectomy with BSO compared with hysterectomy-alone, on average and stratified by menopausal status and age at hysterectomy, and by current HT use at the index date.

	Hysterectomy-Alone		Hysterectomy with BSO		Hysterectomy-Alone	Hysterectomy with BSO		
	Control n	Case n	Control n	Case n		Adjusted OR (95% CI)[a]	p-value	Likelihood Ratio Test p-value
Myocardial Infarction								
Unstratified	548	170	797	229	1.0	0.93 (0.74-1.2)	0.55	-
Pre/Perimenopause	412	122	641	182	1.0	0.97 (0.74-1.3)	0.81	
Postmenopause	90	33	147	40	1.0	0.77 (0.44-1.3)	0.35	0.14
<45 Years	360	109	366	111	1.0	0.98 (0.72-1.3)	0.91	
45-54 Years	131	38	310	93	1.0	1.2 (0.76-1.9)	0.44	
>=55 Years	57	23	121	25	1.0	0.43 (0.21-0.89)	0.02	0.42
Current Hormone Therapy Use at the Index Date								
Non-Use of HT	362	114	481	137	1.0	0.90 (0.67-1.2)	0.47	
Use of HT	186	56	316	92	1.0	1.1 (0.72-1.6)	0.69	0.79
Ischemic Stroke								
Unstratified	548	111	797	151	1.0	0.91 (0.69-1.2)	0.50	-
Pre/Perimenopause	412	84	641	129	1.0	0.95 (0.70-1.3)	0.74	
Postmenopause	90	19	147	20	1.0	0.69 (0.33-1.4)	0.33	0.99
<45 Years	360	73	366	74	1.0	0.96 (0.67-1.4)	0.84	
45-54 Years	131	27	310	55	1.0	0.99 (0.58-1.7)	0.96	
>=55 Years	57	11	121	22	1.0	0.93 (0.38-2.3)	0.88	0.29
Current Hormone Therapy Use at the Index Date								
Non-Use of HT	362	74	481	96	1.0	0.95 (0.68-1.3)	0.78	
Use of HT	186	37	316	55	1.0	0.83 (0.52-1.4)	0.45	0.65

BSO=Bilateral Salpingo-Oophorectomy; HT=Hormone Therapy; VT=Venous Thrombosis; OR=Odds Ratio; CI=Confidence Interval; MI=myocardial infarction

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, education (indicator variable), smoking status (dummy variable), and current hormone therapy use at index assuming 80% compliance.

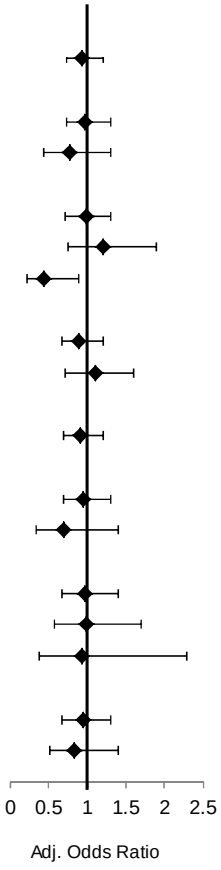

Table 2.A5. Risk of VT, MI, and ischemic stroke associated with combined hysterectomy with and without BSO and current HT use status, excluding women diagnosed with any type of cancer prior to the index date.

	No HT Use					HT Use				
	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value
Venous Thrombosis										
No Hysterectomy	1,995	484	No HT	1.0 (ref)		428	138	E+P	1.9 (1.5-2.4)	<0.001
Hysterectomy-Alone	291	69	No HT	0.96 (0.72-1.3)	0.77	182	30	E-alone	0.84 (0.56-1.3)	0.41
Hysterectomy with BSO	379	116	No HT	1.2 (0.95-1.5)	0.13	290	71	E-alone	1.2 (0.92-1.6)	0.16
Myocardial Infarction										
No Hysterectomy	1,995	564	No HT	1.0 (ref)		428	134	E+P	1.1 (0.84-1.3)	0.61
Hysterectomy-Alone	291	97	No HT	1.1 (0.85-1.4)	0.49	182	52	E-alone	0.87 (0.62-1.2)	0.43
Hysterectomy with BSO	379	112	No HT	0.99 (0.78-1.3)	0.97	290	84	E-alone	0.92 (0.70-1.2)	0.52
Ischemic Stroke										
No Hysterectomy	1,995	315	No HT	1.0 (ref)		428	70	E+P	1.1 (0.81-1.5)	0.54
Hysterectomy-Alone	291	65	No HT	1.31 (0.97-1.8)	0.08	182	32	E-alone	1.0 (0.67-1.5)	0.98
Hysterectomy with BSO	379	75	No HT	1.1 (0.85-1.5)	0.39	290	51	E-alone	0.97 (0.70-1.4)	0.88

BSO=Bilateral Salpingo-Oophorectomy; E=Estrogen; P=Progestogen; HT=Hormone Therapy; VT=Venous Thrombosis; OR=Odds Ratio; CI=Confidence Interval; MI=Myocardial Infarction

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, more than high school education, current smoking status

Table 2.A6. Risk of VT, MI, and ischemic stroke associated with combined hysterectomy with and without BSO and current HT use status, restricted to index years >=1999.

	No HT Use					HT Use				
	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value	Control n	Case n	HT Type	Adjusted OR (95% CI)[a]	p-value
Venous Thrombosis										
No Hysterectomy	1,794	624	No HT	1.0 (ref)		256	117	E+P	1.7 (1.3-2.1)	<0.001
Hysterectomy-Alone	272	102	No HT	1.1 (0.84-1.4)	0.52	118	30	E-alone	0.87 (0.57-1.3)	0.51
Hysterectomy with BSO	359	179	No HT	1.4 (1.1-1.7)	0.001	199	70	E-alone	1.2 (0.88-1.6)	0.26
Myocardial Infarction										
No Hysterectomy	1,794	443	No HT	1.0 (ref)		256	90	E+P	1.3 (1.0-1.8)	0.05
Hysterectomy-Alone	272	74	No HT	1.0 (0.79-1.4)	0.74	118	32	E-alone	1.0 (0.67-1.6)	0.93
Hysterectomy with BSO	359	103	No HT	1.1 (0.87-1.5)	0.37	199	62	E-alone	1.1 (0.81-1.5)	0.54
Ischemic Stroke										
No Hysterectomy	1,794	263	No HT	1.0 (ref)		256	43	E+P	1.2 (0.82-1.7)	0.35
Hysterectomy-Alone	272	46	No HT	1.1 (0.77-1.5)	0.63	118	25	E-alone	1.4 (0.86-2.2)	0.19
Hysterectomy with BSO	359	66	No HT	1.2 (0.87-1.6)	0.31	199	39	E-alone	1.2 (0.80-1.7)	0.40

BSO=Bilateral Salpingo-Oophorectomy; E=Estrogen; P=Progestogen; HT=Hormone Therapy; VT=Venous Thrombosis; OR=Odds Ratio; CI=Confidence Interval; MI=Myocardial Infarction

[a]Adjusted for continuous age, hypertension, index year (indicator variable), white race, continuous BMI, more than high school education, current smoking status.

CHAPTER 3: The association between vasomotor symptoms and hemostatic factor levels in postmenopausal women

Abstract

Background: Vasomotor symptoms (VMS) may be a marker of increased cardiovascular risk. VMS frequency has been associated with hemostatic factor levels among midlife women, but the association of VMS with hemostatic factor levels among postmenopausal women has not been evaluated. We evaluated the cross-sectional association of VMS presence and severity with hemostatic factor levels measured at baseline among Women's Health Initiative (WHI) Hormone Therapy trial participants.

Methods: This cross-sectional analysis included 2,149 postmenopausal women with measures of VMS severity in the 4 weeks prior reported at WHI baseline, who were not using warfarin or hormone therapy and for whom the following baseline hemostatic factors were measured within the WHI Cardiovascular Disease (CVD) Biomarker Case-Control Study: antithrombin, plasminogen activator inhibitor-1, protein C antigen, total and free protein S antigen, total and free tissue factor pathway inhibitor, D-dimer and normalized activated protein C sensitivity ratio (nAPCsr). Using multiple linear regression, we estimated the adjusted average difference in each hemostatic factor associated with VMS presence and severity. A multiple comparisons corrected p-value was computed using the P-min procedure and compared with a level of 0.05 to determine statistical significance of our smallest observed p-value.

Results: Women were 67 years of age on average and 34% of women reported VMS present at baseline. There was some suggestion that VMS presence may be associated with a -0.34 adjusted difference in nAPCsr compared with no VMS (SE=0.13; p=0.009), but this association was not significant after correction for multiple comparisons (p=0.064). VMS presence or severity was not significantly associated with the other hemostatic factors.

Conclusions: We found no evidence that VMS presence or severity was associated with levels of hemostatic factors among postmenopausal women.

Introduction

Vasomotor symptoms (VMS), defined as hot flashes and night sweats, are experienced by most women at some point during the menopausal transition, with estimates of VMS prevalence in postmenopause ranging widely from 30-80%.[25] Evidence suggests that in addition to impacting quality of life, VMS may be a marker for vascular change, potentially leading to greater cardiovascular event risk[28]. VMS have previously been associated with biomarkers associated with cardiovascular disease[26,27,82-87]. Given the high prevalence of VMS, it has also been suggested that VMS characteristics with greater inter-individual variability, such as VMS severity, timing, and duration, may provide information helpful in cardiovascular risk stratification as women transition through menopause.[28]

The etiology of VMS is incompletely understood, but a combination of hormonal and thermoregulatory factors is hypothesized to contribute to their genesis.[81-83] Low endogenous estradiol (E2) and high follicle stimulating hormone (FSH) levels are associated with VMS presence and greater VMS frequency and severity,[84,85] hormonal patterns which are also associated with mostly prothrombotic changes in hemostatic factors among midlife women.[14,15] This suggests that VMS may plausibly be a marker of clinically unmeasured endogenous hormonal changes associated with thrombotic risk. Frequent hot flashes have been associated with some hemostatic factor levels among pre- and perimenopausal women, including higher factor VIIc (FVIIc) and tissue plasminogen activator antigen (tPA-ag) levels,[86] but the association of VMS with most hemostatic factors among postmenopausal women has not been evaluated.

In a cross-sectional analysis among postmenopausal women not currently using hormone therapy (HT), we tested the hypothesis that VMS presence and severity at baseline in the Women's Health Initiative Hormone Therapy trials (WHI-HT) would be associated with higher levels of hemostatic activation markers and pro-coagulant and anti-fibrinolytic factors, and lower levels of anticoagulant hemostatic factors. In secondary analyses, we hypothesized

that longer VMS duration and postmenopausal status at VMS onset, after the hormonal fluctuations that occur during the menopausal transition, would be positively associated with higher levels of hemostatic activation markers and pro-coagulant and anti-fibrinolytic factors, and lower levels of anticoagulant factors.

Methods

Setting and Design

The data for this cross-sectional study are from a nested, case-control study of cardiovascular biomarkers in relation to cardiovascular disease (CVD), set within the WHI-HT trials. The WHI-HT trials enrolled 27,347 postmenopausal women ages 50 to 79 years of age from areas surrounding 40 clinical centers from 1993 to 1998, as previously described.[10,11,87,88] The WHI-HT trials were approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

Nested Case-Control Study Participants

Women eligible for this study were participants in the CVD Biomarker Case-Control Studies, which included centrally-adjudicated cases of coronary heart disease (CHD), defined as myocardial infarction (MI) or coronary death, (n=401) and venous thrombosis (VT) (n=221) occurring between randomization and February 28, 2001, and all centrally-adjudicated cases of stroke occurring between randomization and September 12, 2005 (n=572). Some participants experienced multiple case event types. One control with no MI, VT, or stroke was selected for each case event, with controls selected at the time of the case's event and matched on age, randomization date, hysterectomy status, and prevalent CVD at baseline (n=1,169).

Excluded were women for whom hemostatic factor assays of interest were not completed (n=14), with unknown VMS presence and severity at baseline (n=20), and who had not completed a full 3-month HT washout prior to the date of blood sample collection or the date

of VMS report (n=139) (eligible n=2,149). No eligible participants used warfarin at baseline. Data analyzed in this cross-sectional study were collected at WHI baseline, which collectively encompassed an initial telephone screening eligibility interview and 3 in-person study screening visits; this baseline screening period was prior to the WHI-HT trial randomization of women to treatment or placebo.

Vasomotor Symptoms

At the second baseline screening visit, using standardized study forms, women were asked whether hot flashes and night sweats had occurred in the past 4 weeks and whether they were mild, moderate, or severe. Eligible women who were using HT before this screening visit had completed a 3-month period off HT by the time of reporting on current symptoms. Mild symptoms were those that did not interfere with usual activities, moderate symptoms interfered somewhat with usual activities, and severe symptoms were so bothersome that usual activities could not be performed. Women with any mild, moderate, or severe hot flashes were considered to have VMS present at baseline.

Secondary exploratory analyses relied on historical VMS and menopause data reported by participants at the second baseline screening visit. Separately from the report of VMS presence and severity, women were asked whether they had ever had menopausal symptoms such as hot flashes and night sweats. If they had, they reported their age when they first started having symptoms as well as their age at last symptom, or current age if still having symptoms. Age at menopause was determined using methods previously published[89], using the age at which a women last had any menstrual bleeding, had a bilateral oophorectomy, or began using HT. For women with a prior hysterectomy without bilateral oophorectomy, age at menopause was defined as the age at HT initiation or the onset of menopausal symptoms; therefore, women with a prior hysterectomy without bilateral oophorectomy were excluded from analyses in which menopausal status at VMS onset was the exposure of interest (n=502).

In secondary analyses, menopausal status at VMS onset was determined by comparing age at menopause with age at VMS onset and women were thus classified 3 ways: as having experienced VMS onset (1) prior to age at menopause (in pre/perimenopause), (2) within the same year as the age at menopause (year of menopause), or (3) after the age at menopause (postmenopause). VMS duration at the time of WHI baseline was determined by subtracting age at first VMS from age at last VMS, or, age at WHI baseline if VMS were present at baseline or if no VMS end date was reported. Of note, the full VMS duration for women still having VMS at WHI baseline is truncated.

Hemostatic Factor Measures

Blood specimens were collected from all participants at baseline, prior to the collection of baseline VMS information for 1609 women (mean=38.5 days prior; range=1-238 days), after the collection of VMS information for 62 women (mean=45.1 days after; range=1-256 days), and on the same day as the collection of VMS information for 478 women. Eligible women who had used HT previously had not used HT for at least 3 months prior to the date of blood specimen collection. Blood samples were collected into tubes containing 1.8% sodium citrate, centrifuged at 1300 g for 10 minutes at 4°C within 2 hours, and stored at -70°C.[90] Batches used for hemostatic factor assays included both case and control subjects.[90]

Available hemostatic factor measures were divided into two groups *a priori* (Table 3.1), a group of primary interest (group 1) and a group of secondary interest (group 2), based on the strength of the published evidence for the association of exogenous HT with factor levels.[34-36,91-99] The hemostatic factors of primary interest were antithrombin (AT), fibrin D-dimer (D-dimer), normalized activated protein C sensitivity ratio (nAPCsr), plasminogen activator inhibitor-1 antigen (PAI-1), protein C antigen, protein S antigen (free and total), and tissue factor protein inhibitor (TFPI) (free and total). The remaining 7 hemostatic factors (factor FVIII activity [FVIIIc], fibrinogen, plasmin-alpha 2-antiplasmin complex [PAP], prothrombin antigen, prothrombin

fragment F 1.2 [F1.2], thrombin activatable fibrinolysis inhibitor [TAFI], and von Willebrand factor [vWF]) were of secondary interest based on weaker evidence of an association between exogenous HT and these factors.

The hemostatic factor assays have been described in prior WHI publications and will be summarized in brief.[90,100] The measurement of the endogenous thrombin potential-based nAPCsr was completed at the Department of Biochemistry at the University of Maastricht, the Netherlands, and expressed the ratio of thrombin generation without and with added APC, normalized against normal plasma.[90,101] Total and free TFPI antigen were assayed using the Asserochrom enzyme-linked immunosorbent assay (Diagnostica Stago, www.stago-us.com) at the Department of Hematology, Oslo University Hospital, Oslo. Fibrinogen, FVIIIc, vWF, ATIII, D-dimer, PAI-1, PAP, prothrombin, F1.2, Protein C, free and total S, and TAFI were measured by the Laboratory for Clinical Biochemistry Research, University of Vermont. Fibrinogen was measured via a clot-rate assay using a STA-R instrument (Diagnostica Stago), FVIIIc by clotting time on mixing with FVIII deficient plasma using STA-Deficient VIII (Diagnostica Stago), vWF, ATIII, and D-dimer by immunoturbidometric or colorimetric assays using a STA-R instrument (Liatest von Willebrand factor, Liatest D-di, Stachrom ATIII, Diagnostica Stago), PAI-1, PAP, and prothrombin by in-house immunoassay[102], prothrombin fragment 1.2 by ELISA (Dade-Behring), protein C, and free and total protein S by Asserachrom ELISA (Diagnostica Stago), and TAFI by immunoassay with antibodies from Affinity Biologicals.

Covariates

Baseline demographic, medical history, and lifestyle covariates were collected at baseline screening visits.[87] Self-reported current age, race/ethnicity, age at last menstrual bleeding, prior hysterectomy, education level, smoking status and physical activity were collected using standardized questionnaires. Participants also self-reported medical history characteristics including prevalent MI, prevalent stroke, prevalent VT, history of DVT or PE among family

members, history of treated diabetes, high cholesterol requiring treatment, and physician-diagnosed hypertension. Weight and height were measured at the initial screening visit and were used to calculate body mass index (BMI) in kg/m^2. At the initial telephone eligibility screening interview at baseline, participants reported current use of estrogen and progesterone in the form of pills, skin patches, implants, creams, suppositories, shots, and birth control pills (excluding birth control pills used prior to age 50). Current use of opposed and unopposed estrogen in the form of pills and patches was also reported at the first in-person screening visit at baseline. We considered women to be current users of HT at baseline if any HT use was reported on either of these 2 baseline questionnaires. Since we excluded any women without 3 months of HT washout prior to blood draw or VMS report, any HT use that eligible women reported referred to use prior to the 3-month HT washout period.

Statistical Analysis

We tabulated demographic and medical history characteristics by VMS presence and severity at baseline. We also reported hemostatic factor level medians and interquartile ranges (IQRs) by VMS presence and severity.

Using multiple linear regression, we estimated the association of VMS presence with each hemostatic factor separately, adjusting for matching variables and potential confounders: age (linear), hysterectomy (yes/no), prevalent myocardial infarction (MI), stroke, and VT at baseline, nested case-control study group (control, MI case, stroke case, VTE case, multiple case types), race/ethnicity (categorical), BMI (linear, kg/m^2), smoking status (categorical), and current HT use at baseline, which was at least 3 months prior to blood specimen collection and VMS report (yes/no). In multiple linear regression analyses, missing covariate data were imputed separately for each hemostatic factor of interest using chained equations, which allows the specification of different analytic models for each variable requiring imputation[77,103]; linear regression analytic models were specified to impute missing BMI values (0.3% missing) and

multinomial logistic regression models were specified to impute race/ethnicity (0.2% missing), and smoking status (1.6% missing) using Stata version 13.1 statistical software.[46] Predictors included in the chained equations were vasomotor symptom presence, hysterectomy, prevalent MI, stroke, and VT, nested case-control study group, current HT use at baseline, and the hemostatic factor of interest; ten datasets were imputed.

Linear regression models estimated the adjusted average difference in hemostatic factors associated with VMS presence (any) compared with absence. We also reported the percent difference in standard deviation (SD) units for each hemostatic factor level associated with VMS presence compared with absence, calculated by dividing the estimated beta by the hemostatic factor's SD and multiplying by 100. Similar analyses evaluated the association between VMS severity (mild, moderate, or severe) and hemostatic factor levels among women with baseline VMS, estimating differences in hemostatic factor levels associated with moderate VMS and severe VMS compared with mild VMS. We tested for a linear trend between VMS severity and hemostatic factor levels using multiple linear regression models with VMS severity included as a grouped-linear term.

Among women who reported any history of VMS prior to or at baseline, secondary analyses evaluated the relation between VMS timing (VMS onset in premenopause, in the same year as menopause, or in postmenopause) and VMS duration (<5 years, 5-9 years, 10-14 years, ≥15 years) and each hemostatic factor, separately. Note that some women reported a history of VMS prior to WHI baseline but did not report VMS presence in the 4-weeks prior to the second baseline screening visit; therefore, a larger number of women are included in the VMS timing and duration analyses than in the VMS severity analyses which are only among women with VMS present at WHI baseline. We tested for a linear trend in the menopausal status at VMS onset and hemostatic factor relation, and separately, in the VMS duration and hemostatic factor relation by modeling these exposures using grouped-linear terms.

Although multiple linear regression analyses adjusted for nested case-control study group, we conducted sensitivity analyses restricted to controls. Since HT use at baseline, but prior to blood specimen collection and VMS report, may have impacted VMS presence and severity at WHI baseline due to fluctuations in endogenous hormones caused by recent cessation of HT, and because past HT use may have impacted menopausal stage at VMS onset or total VMS duration, we conducted 2 sensitivity analyses related to HT use: (1) we excluded women who currently used HT at baseline, prior to the report of VMS and blood specimen collection; (2) we excluded women who reported current or any past use of HT at baseline.

Since we were evaluating 9 hemostatic factors within group 1 in relation to each VMS exposure of interest, and because some hemostatic factor levels were correlated, we used a P-min procedure to account for multiple comparisons.[44] VMS presence was permuted in 10,000 replications across all group 1 hemostatic factors in each replication, resulting in a p-value that indicated the number of times out of 10,000 replications that the test statistic was by chance lower than our observed smallest p-value. This p-value was compared to an alpha level of 0.05 to determine statistical significance of our smallest observed p-value.

Results

Table 3.2 presents demographic and health characteristics of eligible WHI-HT CVD Biomarkers Study participants, by VMS presence and severity. At baseline, 66% of eligible participants reported no VMS in the prior 4 weeks (n=1,429), 22% reported mild (n=467), 9.2% reported moderate (n=199) and 2.5% reported severe VMS (n=54). At baseline, women were 67.2 years of age on average and 43% of women had experienced menopause ≥20 years earlier. Women with severe VMS were more likely to have experienced VMS <10 years earlier (24.1%), to be of Black race (38.9%) and to be current smokers (22.2%) than women with no VMS or less severe VMS. On average, women with severe VMS also had a higher BMI (31.7 kg/m^2) than women

with no VMS or less severe VMS. Table 3.3 presents unadjusted medians and interquartile ranges of hemostatic factor levels by VMS presence and severity.

In adjusted analyses evaluating the association between VMS presence and severity and group 1 hemostatic factors (Table 3.4), there was some suggestion that the presence of any VMS at baseline was associated with lower nAPCsr values than VMS absence (β=-0.34; SE=0.13; p=0.009); however this did not survive correction for multiple comparisons (p=0.064). There was no evidence of an association between VMS presence or severity and any other group 1 hemostatic factor levels. In sensitivity analyses (1) restricted to controls, (2) restricted to women who did not currently use HT at baseline, prior to VMS report and blood draw, and (3) restricted to women who never used HT, results were similar. We found no evidence of an association of VMS presence or severity (Table 3.5) with any of the group 2 hemostatic factor levels.

In secondary analyses among women who ever had VMS, there were no associations of any hemostatic factor with menopausal status at VMS onset (Appendix Table 3.A1). There was also no evidence of an association between linear VMS duration and any hemostatic factor, other than some suggestion of a linear trend between longer VMS duration and higher PAI-1 antigen levels p=0.026), an association that was non-significant when using a multiple comparisons corrected p-value estimated using permutation (p=0.18) (Appendix Table 3.A2).

Discussion

In this cross-sectional analysis among postmenopausal women not currently using HT, there was no significant evidence of an association between VMS presence, severity, duration, or timing and measures of hemostasis. These data are not supportive of the hypothesis that VMS is a marker of a more thrombotic profile among postmenopausal women.

VMS and Hemostasis

The lack of an association between VMS presence and hemostatic factor levels observed here contrasts with results of a longitudinal study of pre- and perimenopausal women. In Study of Women's Health Across the Nation (SWAN), participants aged 42 to 52 years at baseline (n=3,199), more frequent hot flashes were associated with higher FVIIc and tPA-ag levels, which is suggestive of an association between greater VMS frequency and a more thrombotic hemostatic profile.[86] In the SWAN study, PAI-1 and fibrinogen were also positively associated with more frequent VMS, but not after adjustment for confounders. Measures of tPA-ag and FVIIc were unavailable in the WHI-HT CVD Biomarkers Study for evaluation in relation to VMS.

Several differences between the WHI-HT and SWAN study populations may contribute to the presence of a VMS-hemostatic factor association in the SWAN study but not in this WHI-HT analysis. The SWAN analysis evaluated associations between VMS and hemostatic factors longitudinally across 8 study visits over 8 years, among women with an average age of 46 years at cohort entry.[86] At each study visit, women reported VMS frequency in the 2 weeks prior. The average age of SWAN participants at cohort entry is over 20 years younger than that of women included in this WHI-HT analysis (mean=67 years). In SWAN, 54% of participants were premenopausal and 46% were early perimenopausal, in contrast to the predominantly late postmenopausal population included in this WHI-HT analysis. Therefore, VMS reported in SWAN were occurring in the context of the menopausal transition but in WHI-HT were occurring in postmenopause, among women for whom the transition into postmenopause occurred ≥20 years prior in 43% of participants. It has been previously proposed that VMS occurring during the menopausal transition and in postmenopause may be symptoms of different underlying physiologic processes[28], with this hypothesis partially stemming from differential responses of VMS to HT treatment dependent on menopausal status.[104] However, differences in the etiology of VMS by menopausal status are poorly understood.

Biologic Plausibility

In addition to their relation to low E2 and high FSH levels,[84,85] VMS are thought to be thermoregulatory events that follow an increase in core body temperature.[81] VMS have been acutely related to peripheral vasodilation, as measured by higher skin temperature and increased blood flow[81], and thus, have been proposed as potential markers of underlying vascular change.[105] Whether VMS are associated with cardiovascular biomarkers and events is controversial,[105] with some studies suggesting positive associations between VMS and biomarkers associated with greater cardiovascular risk[83,106-108], and others suggesting an association between VMS and markers associated with less cardiovascular risk.[109] In a WHI Observational Study analysis of the relation between VMS and cardiovascular events, the timing of VMS onset seemed to be important, with perimenopausal VMS associated with a lower risk of stroke, total CVD events, and all-cause mortality but postmenopausal VMS associated with a greater risk of coronary heart disease and all-cause mortality.[28]

In this study, we found no evidence of an association between VMS and hemostatic factors among postmenopausal women, but VMS have been associated with hemostatic factors in the SWAN study of premenopausal and early perimenopausal women.[86] One proposed biologic pathway potentially linking VMS to hemostatic factors is via endothelial dysfunction, as measured by flow-mediated dilation (FMD).[83,110] VMS severity among women aged 45 to 58 years[83] and among early postmenopausal women aged 42-55 years[110] has been associated with impaired FMD.[83,110] Endothelial dysfunction has the potential to impact blood coagulation, given that activated endothelial cells may express tissue factor and that the extrinsic pathway of the coagulation cascade is activated by the exposure of tissue factor, and by the interaction of tissue factor with activated factor VII.[56,111] However, the results of our analysis were not supportive of an association of VMS presence or severity with a hemostatic profile in postmenopausal women.

Strengths and Limitations

A limitation of this cross-sectional study is that the temporality and directionality of the association between VMS and hemostatic factor levels cannot be determined. Furthermore, although we adjusted regression analyses for known potential confounders, the possibility of residual confounding by unidentified confounders remains.

Our study evaluated a large number of hemostatic factors in relation to VMS, which increases our possibility of Type I error. However, to increase the threshold for statistical significance due to multiple comparisons, we used a multiple comparisons-corrected p-value estimated using a P-min procedure and divided hemostatic factors into 2 groups *a priori*. We found no statistically significant evidence of an association between VMS presence or severity and any group 1 hemostatic factor levels.

nAPCsr values in this study were higher than those typically reported for normal individuals (values typically <2).[101] The high nAPCsr values in this population have been discussed in a prior publication[90] and may be due to differences in sample storage and handling between WHI plasma and the normal pooled plasma used to normalize the ETP-based APC resistance test.

This secondary analysis was conducted using a subset of WHI-HT participants for whom hemostatic factor levels were already measured within the scope of a nested case-control study of CHD, stroke, and VT. We also conducted sensitivity analyses restricted to controls from the WHI-HT CVD Biomarkers study and results were similar to those conducted among the population including both cases and controls from the nested case-control study. The distribution of VMS severity in our study population is unlikely representative of the general United States population of postmenopausal women, however, since the WHI-HT trials excluded women unwilling to discontinue HT use and who reported severe VMS that would make placebo treatment intolerable.[88]

VMS is a self-reported measure, which can be seen as both a weakness and strength. While self-reported VMS are minimally invasive to collect and can be reported in-clinic, self-

reported VMS are dependent on an individual's perception of discomfort. There will likely be some misclassification of "true" VMS. However, such misclassification is likely to be nondifferential, which typically biases effect estimates towards the null. VMS presence and severity in the 4-weeks prior to the second baseline screening visit is unlikely to be impacted by substantial recall bias, due to the recency of the symptoms reported. In contrast, exposures of interest in secondary analyses, VMS duration and menopausal stage at onset, were created using a retrospectively reported measure of age at VMS onset, which is susceptible to recall bias if women do not remember the age at which VMS began. Due to this potential for recall bias, these are secondary analyses with tables reported as supplementary digital content and results should be interpreted cautiously.

HT use at baseline, but at least 3 months prior to the reporting of VMS and to the collection of blood specimens, should not have impacted reports of VMS presence and severity. These VMS measures were reported following a 3-month HT washout period and reflected VMS presence and severity only during the past 4-weeks, which was during a period of HT non-use for all eligible participants. To address the possibility that the cessation of HT that occurred as part of this washout may have impacted reports of VMS presence and severity as well as hemostatic factor levels, due to an instability in hormone levels, we conducted a sensitivity analysis restricted to women who reported no current HT use at WHI baseline and results were similar.

Strengths of this study include the inclusion of more than 2,000 women with measured hemostatic factor levels and the measurement and adjustment for important confounders related to both VMS and hemostatic factor levels. In addition, although VMS data in our study was cross-sectional, primary analyses used VMS presence and severity information from the 4 weeks prior, which is unlikely to be strongly impacted by recall bias. Furthermore, because all women eligible for this study underwent a 3-month HT washout period prior to their report of

VMS if HT had been previously used, women were not using HT at the time of VMS report, which provides VMS information unsuppressed by HT use.

Conclusions

In this cross-sectional analysis, there was no evidence of an association between VMS presence, severity, timing or duration with hemostatic factors among postmenopausal women. Given that VMS have been previously associated with hemostatic factors among pre- and perimenopausal women, future studies that attempt to characterize potential differences and similarities in the etiology of VMS by menopausal stage and age would improve our understanding of VMS across the menopausal transition and into postmenopause.

Table 3.1. Hemostatic factors for evaluation in relation to vasomotor symptoms, by group.

Group 1 (Primary Hypotheses)

Name	Abbreviation	Units	Measurement of:	Hypothesized Direction of Association*
antithrombin	ATIII	%	anticoagulation	-
fibrin D-dimer	D-dimer	ug/mL	global	+
normalized activated protein C sensitivity ratio	nAPCsr	ratio	APC resistance	+
plasminogen activator inhibitor-1 antigen	PAI-1	ng/mL	fibrinolysis	+
protein C	-	%	anticoagulation	-
protein S (free and total)	-	%	anticoagulation	-
tissue factor protein inhibitor (free and total)	TFPI	ng/mL	anticoagulation	-

Group 2 (Secondary Hypotheses)

Name	Abbreviation	Units	Measurement of:	Hypothesized Direction of Association*
factor VIII activity	FVIIIc	%	procoagulation	+
fibrinogen	-	mg/dL	procoagulation	+
plasmin-alpha 2-antiplamin complex	PAP	nmol/L	fibrinolysis	+
prothrombin antigen	prothrombin	ug/mL	procoagulation	+
prothrombin fragment F 1.2	F1.2	nmol/L	procoagulation	+
thrombin activatable fibrinolysis inhibitor	TAFI	ug/mL	fibrinolysis	+
von Willebrand factor	vWF	%	procoagulation	+

*Hypothesized association between VMS presence, stronger VMS severity, longer VMS duration, and later menopausal stage at VMS onset and hemostatic factor levels. This is also the hypothesized direction of the association between the hemostatic factor and VT risk.

Table 3.2. Participant characteristics at WHI baseline, by baseline VMS presence and severity.

	All Participants (n=2,149)	No VMS (n=1,429)	Any VMS (n=720)		
			Mild (n=467)	Moderate (n=199)	Severe (n=54)
Age at Enrollment, mean (SD), y	67.2 (6.6)	68.3 (6.2)	65.4 (6.7)	63.8 (7.1)	63.1 (6.7)
CVD Nested Case/Control Event Status, n (%)					
CHD Case	380 (17.7)	241 (16.9)	84 (18.0)	40 (20.1)	15 (27.8)
Stroke Case	525 (24.4)	341 (23.9)	107 (22.9)	61 (30.7)	16 (29.6)
VT Case	212 (9.9)	145 (10.2)	50 (10.7)	13 (6.5)	4 (7.4)
No Event (Control)	1021 (49.8)	727 (50.9)	236 (50.5)	88 (44.2)	20 (37.0)
HT Use at Baseline, Pre- 3-month Washout, n (%)					
Never	1148 (53.4)	809 (56.6)	226 (48.4)	85 (42.7)	28 (51.9)
Past	951 (44.3)	596 (41.7)	221 (47.3)	109 (54.8)	25 (46.3)
Current	50 (2.3)	24 (1.7)	20 (4.3)	5 (2.5)	1 (1.9)
Years since menopause, no. (%)					
<10 years	246 (11.5)	120 (8.4)	76 (16.3)	37 (18.6)	13 (24.1)
10-19 years	664 (30.9)	434 (30.4)	152 (32.6)	64 (32.2)	14 (25.9)
>=20 years	926 (43.1)	647 (45.3)	176 (37.7)	78 (39.2)	25 (46.3)
Unknown	313 (14.6)	228 (16.0)	63 (13.5)	20 (10.1)	2 (3.7)
Ethnicity, no. (%)					
American Indian/Alaskan Native	13 (0.60)	6 (0.42)	6 (1.3)	0 (0.0)	1 (1.9)
Asian/Pacific Islander	24 (1.1)	21 (1.5)	3 (0.64)	0 (0.0)	0 (0.0)
Black	244 (11.4)	98 (6.9)	78 (16.7)	47 (23.6)	21 (38.9)
Hispanic	64 (3.0)	40 (2.8)	11 (2.4)	11 (5.5)	2 (3.7)
White not of Hispanic Origin	1778 (82.7)	1246 (87.2)	363 (77.7)	139 (69.9)	30 (55.6)
Unknown	26 (1.2)	18 (1.3)	6 (1.3)	2 (1.0)	0 (0.0)
Education, no. (%)					
High school diploma or less	678 (31.6)	421 (29.5)	154 (33.0)	74 (37.2)	29 (53.7)
School after high school	847 (39.4)	556 (38.9)	189 (40.5)	83 (41.7)	19 (35.2)
College degree or higher	608 (28.3)	445 (31.1)	120 (25.7)	38 (19.1)	5 (9.3)
Unknown	16 (0.74)	7 (0.49)	4 (0.86)	4 (2.0)	1 (1.9)
Family history of VT, no. (%)	193 (9.0)	136 (9.5)	32 (6.9)	15 (7.5)	10 (18.5)
Ever Hypertension, no. (%)	966 (45.0)	605 (42.3)	222 (47.5)	103 (51.8)	36 (66.7)
High Cholesterol, no. (%)	365 (17.0)	247 (17.3)	75 (16.1)	29 (14.6)	14 (25.9)
Ever Diabetes, no. (%)	267 (12.4)	157 (11.0)	69 (14.8)	28 (14.1)	13 (24.1)
BMI, mean (SD), kg/m2	29.2 (5.8)	28.8 (5.7)	29.6 (5.6)	30.5 (6.5)	31.7 (4.6)
Smoking, no. (%)					
Never	1073 (49.9)	734 (51.4)	229 (49.0)	91 (45.7)	19 (35.2)
Past	808 (37.6)	538 (37.7)	173 (37.0)	76 (38.2)	21 (38.9)
Current	234 (10.9)	135 (9.5)	58 (12.4)	29 (14.6)	12 (22.2)
Unknown	34 (1.6)	22 (1.5)	7 (1.5)	3 (1.5)	2 (3.7)
Physical activity, no. (%)					
<5 MET h/wk	876 (40.8)	566 (39.6)	181 (38.8)	97 (48.7)	32 (59.3)
5-<12 MET h/wk	427 (19.9)	284 (19.9)	103 (22.1)	33 (16.6)	7 (13.0)
>=12 MET h/wk	599 (27.9)	414 (28.9)	122 (26.1)	54 (27.1)	9 (16.7)
Unknown	247 (11.5)	165 (11.6)	61 (13.1)	15 (7.5)	6 (11.1)

WHI=Women's Health Initiative; VMS=vasomotor symptoms; SD=standard deviation; y=years; CVD=cardiovascular disease; CHD=coronary heart disease; VT=venous thrombosis; HT=hormone therapy; BMI=body mass index; MET=metabolic equivalent of task; h=hours; wk=week.

Table 3.3. Unadjusted median measures and interquartile ranges of hemostatic factor levels, by VMS presence and severity.

	All Participants		No VMS		Any VMS					
					Mild VMS		Moderate VMS		Severe VMS	
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)
Group 1 Hemostatic Factors										
ATIII (%)	1002	91.0 (20.0)	674	91.0 (19.0)	228	89.0 (20.0)	79	92.0 (22.0)	21	91.0 (16.0)
D-Dimer (ug/mL)	1609	0.37 (0.41)	1060	0.39 (0.41)	357	0.32 (0.36)	149	0.32 (0.39)	43	0.32 (0.35)
nAPCsr (ratio)	1601	3.4 (3.3)	1073	3.4 (3.3)	349	3.1 (3.0)	141	3.6 (3.6)	38	3.3 (3.2)
PAI-1 (ng/mL)	1501	41.5 (50.7)	989	39.6 (48.5)	332	45.4 (52.8)	140	49.5 (59.1)	40	51.3 (64.3)
Protein C (%)	732	109.1 (26.7)	487	107.5 (26.0)	171	113.6 (31.9)	57	109.1 (21.8)	17	104.2 (22.3)
Protein S, Total (%)	730	107.7 (22.1)	487	107.3 (23.1)	170	107.9 (20.3)	56	110.6 (18.8)	17	115.3 (25.1)
Protein S, Free (%)	725	100.6 (25.5)	483	99.9 (25.5)	171	103.7 (26.4)	55	102.7 (31.5)	16	98.8 (32.5)
TFPI, Total (ng/mL)	1811	86.5 (25.5)	1221	86.5 (26.3)	390	85.7 (24.5)	159	87.8 (27.3)	41	81.9 (26.0)
TFPI, Free (ng/mL)	1827	15.5 (8.7)	1231	15.6 (8.7)	393	15.4 (8.3)	162	15.8 (11.0)	41	14.9 (9.5)
Group 2 Hemostatic Factors										
FVIIIc (%)	1613	110.0 (71.0)	1061	110.0 (64.0)	358	106.5 (75.0)	151	110.0 (84.0)	43	115.0 (72.0)
Fibrinogen (mg/dL)	1613	317.0 (118.0)	1061	315.0 (113.0)	358	307.0 (123.0)	151	327.0 (127.0)	43	345.0 (160.0)
PAP (nmol/L)	1500	4.4 (2.3)	988	4.5 (2.4)	332	4.3 (2.2)	140	4.1 (2.5)	40	3.7 (2.0)
Prothrombin (ug/mL)	999	108.0 (26.0)	671	107.7 (25.5)	227	108.9 (26.5)	80	112.2 (27.1)	21	107.6 (29.3)
F1.2 (nmol/L)	1490	1.3 (0.47)	984	1.3 (0.46)	329	1.3 (0.49)	139	1.3 (0.50)	38	1.3 (0.42)
TAFI (ug/mL)	1572	5.0 (2.4)	1032	5.1 (2.4)	350	5.0 (2.4)	147	5.4 (2.6)	43	4.5 (1.7)
vWF (%)	1606	95.0 (58.0)	1059	97.0 (59.0)	355	95.0 (62.0)	149	93.0 (51.0)	43	89.0 (59.0)

VMS = vasomotor symptoms; IQR = interquartile range; ATIII = Antithrombin; nAPCsr = normalized activated protein C sensitivity ratio; PAI-1 =Plasminogen Activator Inhibitor-1 antigen; TFPI = Tissue factor pathway inhibitor; PAP = plasmin-alpha 2-antiplasmin complex; tPA = tissue plasminogen activator; SE = standard error; SD = standard deviation.

Table 3.4. Adjusted associations between VMS presence, severity and group 1 hemostatic factor levels.

			VMS Presence (No VMS as Reference)				VMS Severity (Mild VMS as Reference)						
							Moderate			Severe			
	Mean (SD)	n (no VMS/ VMS)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD	n (mild/ moderate/ severe)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD	p-trend
Group 1 Hemostatic Factors													
ATIII (%)	91.2 (16.3)	674 / 328	0.30 (1.1)	0.79	1.8%	228 / 79 / 21	0.50 (2.1)	0.81	3.1%	-5.7 (3.7)	0.12	-35.0%	0.36
D-Dimer (ug/mL)	0.52 (0.57)	1060 / 549	-0.040 (0.031)	0.20	-7.0%	357 / 149 / 43	0.030 (0.049)	0.54	5.3%	-0.086 (0.082)	0.29	-15.1%	0.77
nAPCsr (ratio)	3.9 (2.3)	1073 / 528	-0.34 (0.13)	0.009	-14.8%	349 / 141 / 38	0.21 (0.22)	0.34	9.1%	0.28 (0.39)	0.48	12.2%	0.29
PAI-1 (ng/mL)	59.8 (56.2)	989 / 512	-1.1 (3.2)	0.73	-2.0%	332 / 140 / 40	0.46 (5.5)	0.93	0.82%	1.9 (9.2)	0.84	3.4%	0.85
Protein C (%)	110.9 (20.5)	487 / 245	1.3 (1.7)	0.46	6.3%	171 / 57 / 17	0.75 (3.3)	0.82	3.7%	-2.5 (5.7)	0.66	-12.2%	0.85
Protein S, Total (%)	108.4 (17.3)	487 / 243	0.062 (1.5)	0.97	0.36%	170 / 56 / 17	1.9 (2.7)	0.49	11.0%	2.8 (4.6)	0.54	16.2%	0.41
Protein S, Free (%)	102.2 (19.6)	483 / 242	1.6 (1.6)	0.35	8.2%	171 / 55 / 16	1.4 (3.1)	0.66	7.1%	-3.7 (5.4)	0.50	-18.9%	0.82
TFPI, Total (ng/mL)	88.6 (20.7)	1221 / 590	0.54 (1.1)	0.62	2.6%	390 / 159 / 41	1.2 (2.0)	0.56	5.8%	-1.8 (3.5)	0.60	-8.7%	0.98
TFPI, Free (ng/mL)	18.9 (11.4)	1231 / 596	0.96 (0.59)	0.10	8.4%	393 / 162 / 41	0.11 (1.2)	0.93	0.96%	-1.0 (2.1)	0.62	-8.8%	0.77

[a]Adjusted for matching variables (age in years (continuous linear), hysterectomy status (dichotomous yes/no), prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent VTE at WHI baseline, and case-control status (control, MI case, stroke case, VTE case)) and potential confounders including any current HT at WHI baseline, prior to VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), BMI (continuous linear in kg/m2), smoking status

VMS = vasomotor symptoms; ATIII = Antithrombin; nAPCsr = normalized activated protein C sensitivity ratio; PAI-1 =Plasminogen Activator Inhibitor-1 antigen; TFPI = Tissue factor pathway inhibitor; PAP = plasmin-alpha 2-antiplasmin complex; tPA = tissue plasminogen activator; SE = standard error; SD = standard deviation

Table 3.5. Adjusted associations between VMS presence, severity and group 2 hemostatic factor levels.

			VMS Presence			VMS Severity (Mild VMS as Reference)						
							Moderate			Severe			
	Mean (SD)	n (no VMS/ VMS)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	n (mild/ moderate/ severe)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	p-trend
Group 2 Hemostatic Factors													
FVIIIc (%)	115.0 (54.5)	1061 / 552	2.2 (3.0)	0.47	4.0%	358 / 151 / 43	3.3 (5.8)	0.57	6.1%	0.39 (9.8)	0.97	0.72%	0.73
Fibrinogen (mg/dL)	318.3 (87.6)	1061 / 552	-4.2 (4.7)	0.37	-4.8%	358 / 151 / 43	16.1 (8.5)	0.058	18.3%	17.9 (14.3)	0.21	16.3%	0.050
PAP (nmol/L)	4.9 (2.4)	988 / 512	-0.074 (0.13)	0.58	-3.1%	332 / 140 / 40	-0.078 (0.22)	0.72	-3.3%	-0.49 (0.36)	0.18	-20.4%	0.24
Prothrombin (ug/mL)	109.8 (20.6)	671 / 328	1.5 (1.5)	0.30	7.3%	227 / 80 / 21	0.64 (2.8)	0.82	3.1%	-4.0 (5.0)	0.43	-19.4%	0.68
F1.2 (nmol/L)	1.8 (1.8)	984 / 506	0.098 (0.11)	0.36	5.4%	329 / 139 / 38	-0.034 (0.21)	0.87	-1.9%	-0.15 (0.36)	0.68	-8.3%	0.70
TAFI (ug/mL)	5.1 (1.8)	1032 / 540	-0.061 (0.10)	0.55	-3.4%	350 / 147 / 43	0.25 (0.17)	0.14	13.9%	-0.40 (0.28)	0.15	-22.2%	0.80
vWF (%)	106.4 (52.7)	1059 / 547	0.32 (2.9)	0.91	0.61%	355 / 149 / 43	-1.5 (5.0)	0.77	-2.8%	-9.2 (8.4)	0.28	-17.5%	0.34

[a]Adjusted for matching variables (age in years (continuous linear), hysterectomy status (dichotomous yes/no), prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent VTE at WHI baseline, and case-control status (control, MI case, stroke case, VTE case)) and potential confounders including any current HT at WHI baseline, prior to VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), BMI (continuous

VMS = vasomotor symptoms; SE = standard error; SD = standard deviation; FVIIIc = Factor VIII activity; PAP = plasmin-alpha 2-antiplasmin complex; F1.2 = prothrombin fragment F1.2; TAFI = thrombin activatable fibrinolysis inhibitor; vWF = von Willebrand factor.

Table 3.A1. Adjusted associations between menopausal status at VMS onset and group 1 hemostatic factor levels.

			Menopausal Status at VMS Onset (Perimenopause as Reference)						
			Year of Menopause			Postmenopause			
	Mean (SD)	n (pre or perimenopause/ year of menoause/ postmenopause)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	p-trend
Group 1 Hemostatic Factors									
ATIII (%)	91.2 (16.3)	191 / 210 / 139	-2.3 (1.9)	0.23	14.1%	-0.023 (1.9)	0.99	-0.14%	0.92
D-Dimer (ug/mL)	0.52 (0.57)	307 / 320 / 239	0.018 (0.042)	0.67	3.2%	0.046 (0.041)	0.26	8.1%	0.27
nAPCsr (ratio)	3.9 (2.3)	340 / 302 / 240	-0.15 (0.22)	0.51	-6.5%	0.31 (0.21)	0.14	13.5%	0.18
PAI-1 (ng/mL)	59.8 (56.2)	286 / 301 / 232	-5.6 (5.6)	0.32	-10.0%	-0.11 (5.4)	0.98	-0.20%	0.94
Protein C (%)	110.9 (20.5)	150 / 157 / 104	0.30 (3.1)	0.92	1.5%	-0.36 (3.0)	0.91	-1.8%	0.91
Protein S, Total (%)	108.4 (17.3)	150 / 156 / 103	-2.1 (2.5)	0.40	-12.1%	-0.95 (2.5)	0.71	-5.5%	0.67
Protein S, Free (%)	102.2 (19.6)	149 / 154 / 103	-2.1 (2.5)	0.40	-10.7%	-0.94 (2.5)	0.71	-4.8%	0.67
TFPI, Total (ng/mL)	88.6 (20.7)	373 / 351 / 277	0.30 (1.9)	0.87	1.4%	-0.38 (1.9)	0.84	-1.8%	0.85
TFPI, Free (ng/mL)	18.9 (11.4)	379 / 352 / 279	0.65 (1.1)	0.54	5.7%	1.1 (1.0)	0.30	9.6%	0.77

[a]Adjusted for matching variables (age in years (continuous linear), hysterectomy status (dichotomous yes/no), prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent VTE at WHI baseline, and case-control status (control, MI case, stroke case, VTE case)) and potential confounders including any current HT at WHI baseline, prior to VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), BMI (continuous linear in kg/m2), smoking status (categorical).

VMS = vasomotor symptoms; ATIII = Antithrombin; nAPCsr = normalized activated protein C sensitivity ratio; PAI-1 =Plasminogen Activator Inhibitor-1 antigen; TFPI = Tissue factor pathway inhibitor; PAP = plasmin-alpha 2-antiplasmin complex; tPA = tissue plasminogen activator; SE = standard error; SD = standard deviation

Table 3.A2. Adjusted associations between VMS duration at WHI baseline and group 1 hemostatic factor levels.

		VMS Duration as of WHI Enrollment (<5 Years as Reference)										
			5-10 Years			10-15 Years			>=15 Years			
	Mean (SD)	n (<5 / 5-10 / 10-15 / >=15)	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	Adjusted Average Difference (SE)[a]	p-value	Adjusted Average Difference in %SD Units[a]	p-trend
Group 1 Hemostatic Factors												
ATIII (%)	91.2 (16.3)	185 / 122 / 92 / 148	-0.61 (1.8)	0.74	-3.7%	1.2 (2.0)	0.54	7.4%	2.7 (1.8)	0.13	16.6%	0.45
D-Dimer (ug/mL)	0.52 (0.57)	290 / 194 / 140 / 250	0.00094 (0.040)	0.98	0.16%	-0.010 (0.045)	0.026	-1.8%	-0.041 (0.038)	0.29	-7.2%	0.25
nAPCsr (ratio)	3.9 (2.3)	298 / 199 / 140 / 252	-0.046 (0.21)	0.83	-2.0%	-0.14 (0.23)	0.54	-6.1%	0.023 (0.20)	0.91	1.0%	0.88
PAI-1 (ng/mL)	59.8 (56.2)	273 / 183 / 136 / 235	-3.0 (5.3)	0.58	-5.3%	-7.2 (5.9)	0.22	-12.8%	8.0 (5.1)	0.11	14.2%	0.026
Protein C (%)	110.9 (20.5)	138 / 90 / 76 / 113	-1.7 (2.8)	0.55	-8.3%	2.3 (3.0)	0.44	11.2%	5.5 (2.7)	0.045	26.8%	0.22
Protein S, Total (%)	108.4 (17.3)	138 / 88 / 76 / 113	-3.2 (2.3)	0.17	-18.5%	-2.7 (2.5)	0.27	15.6%	0.27 (2.2)	0.90	1.6%	0.64
Protein S, Free (%)	102.2 (19.6)	137 / 88 / 75 / 112	-6.2 (2.6)	0.018	-31.6%	-7.2 (2.8)	0.011	-36.7%	-3.7 (2.5)	0.15	-18.9%	0.26
TFPI, Total (ng/mL)	88.6 (20.7)	338 / 227 / 166 / 276	-1.8 (1.8)	0.31	-8.7%	0.86 (1.9)	0.66	4.2%	2.5 (1.7)	0.14	12.1%	0.071
TFPI, Free (ng/mL)	18.9 (11.4)	342 / 229 / 167 / 279	-1.2 (1.0)	0.23	-10.5%	0.027 (1.1)	0.98	0.24%	0.92 (0.97)	0.35	8.1%	0.32

[a]Adjusted for matching variables (age in years (continuous linear), hysterectomy status (dichotomous yes/no), prevalent myocardial infarction at WHI baseline, prevalent stroke at WHI baseline, prevalent VTE at WHI baseline, and case-control status (control, MI case, stroke case, VTE case)) and potential confounders including any current HT at WHI baseline, prior to VMS report and blood specimen collection (dichotomous yes/no), race/ethnicity (categorical), BMI (continuous linear in kg/m2), smoking status (categorical).

ATIII = Antithrombin; nAPCsr = normalized activated protein C sensitivity ratio; PAI-1 =Plasminogen Activator Inhibitor-1 antigen; TFPI = Tissue factor pathway inhibitor; PAP = plasmin-alpha 2-antiplasmin complex; tPA = tissue plasminogen activator.

CHAPTER 4: Vasomotor symptom presence, severity, timing and duration and the risk of incident venous thrombosis among postmenopausal women

Abstract

Background: Vasomotor symptoms (VMS) have been proposed as markers of vascular change but it is unknown whether VMS are associated with the risk of venous thrombosis (VT). We evaluated the association between VMS presence, VMS severity, menopausal status at VMS onset, and VMS duration and the risk of VT among postmenopausal women.

Methods: This cohort study included participants of the Women's Health Initiative (WHI) Hormone Therapy (WHI-HT) trials (n=24,508) and the WHI Observational Study (WHI-OS) (n=87,783), analyzed separately. At WHI baseline, women reported whether hot flashes or night sweats were present in the 4 weeks prior and if so, their severity. Women also reported their age at first and last hot flash or night sweat, and from this we calculated the menopausal status at VMS onset (VMS timing) and VMS duration. Using Cox proportional hazards models, we estimated the risk of incident VT associated with VMS presence, severity, timing, and duration, stratified by time-varying current hormone therapy use and adjusted for age, BMI, smoking status, and race/ethnicity. Statistical significance was determined using a Bonferroni-adjusted alpha of 0.0125.

Results: At WHI baseline, WHI-HT participants were 64 years of age on average and WHI-OS participants were 63 years of age on average. In the WHI-HT study, 522 women experienced a VT event during a median of 8.2 years of follow-up and in the WHI-OS, 1,103 women experienced a VT event during a median of 7.9 years of follow-up. In adjusted analyses, we found no evidence of an association between VMS presence (WHI-HT HR_{adj}=0.91 [95% CI: 0.75, 1.1]; WHI-OS HR_{adj}=1.1 [95% CI: 0.99, 1.3]), severity (WHI-HT severe VMS HR_{adj}=0.99 [95% CI: 0.53, 1.9], p-trend: 0.62; WHI-OS severe VMS HR_{adj}=1.3 [95% CI: 0.89, 2.0], p-trend: 0.55), duration (WHI-HT >15 years HR_{adj}=1.1 [95% CI: 0.81, 1.5], p-trend: 0.42; WHI-OS >15 years HR_{adj}=1.1 [95% CI: 0.92, 1.3], p-trend: 0.33), or menopausal status at VMS onset and the

risk of incident VT (WHI-HT postmenopausal onset HR_{adj}=1.2 [95% CI: 0.88, 1.6], p-trend: 0.23; WHI-OS postmenopausal onset HR_{adj}=1.2 [95% CI: 0.96, 1.4], p-trend: 0.13).

Conclusions: We found no evidence that VMS presence, severity, timing, or duration were associated with VT risk. Our findings do not suggest that VMS are a marker of VT risk.

Introduction

Vasomotor symptoms (VMS), which include hot flashes and night sweats, are a common feature of the menopausal transition, with VMS prevalence estimates reported by a National Institutes of Health State-of-the Science Panel ranging widely from 35 to 50% among perimenopausal women, and 30 to 80% among postmenopausal women.[25] It is hypothesized that a combination of hormonal and thermoregulatory factors contribute to the etiology of VMS[82], but their physiology is incompletely understood. Recent research posits that vascular biology may play a role in the etiology of VMS, suggesting that VMS are possible markers of underlying vascular change. Several biomarkers associated with the risk of arterial thrombotic events have been associated with VMS presence or severity, including reduced flow-mediated dilation (FMD)[83,110], greater aortic calcification[83], higher cholesterol levels[106], higher body mass index (BMI)[106], higher blood pressure levels[106,107], and greater carotid intima media thickness[108]. VMS beginning in postmenopause have been associated with a greater risk of coronary heart disease (CHD) and all-cause mortality than never VMS.[28] Whether VMS presence, severity, timing, and duration are associated with the risk of venous thrombosis (VT) events, including deep vein thrombosis (DVT) and pulmonary embolism (PE), is unclear.

Given that endogenous hormone levels are thought to be associated with VMS[84,112,113], and the strong association between the use of exogenous hormones and the risk of VT[8-11,31,32], it is plausible that VMS may be a marker of hormonally-related vascular changes that may ultimately impact VT risk. VMS have not consistently been associated with markers of anticoagulation and fibrinolysis[86] (Harrington; unpublished), but differences in findings may be due to differences in study populations, such as age and menopausal status of participants. In one study of pre- and early perimenopausal women, VMS presence was associated with elevated levels of Factor VII activity and tissue plasminogen activator antigen (tPA-ag).[86] In contrast, a recent analysis within a subset of Women's Health Initiative Hormone Therapy (WHI-HT) trial postmenopausal participants reported no evidence of an association between VMS

presence, severity, timing or duration with levels of hemostatic measures that included antithrombin, plasminogen activator inhibitor-1, protein C antigen, total and free protein S antigen, total and free tissue factor pathway inhibitor, D-dimer, and normalized activated protein C sensitivity ratio (nAPCsr) (Harrington; unpublished).

The objective of this study was to further characterize the risk of incident VT associated with VMS presence, severity, timing and duration in a cohort of postmenopausal women. We hypothesized that VMS presence and greater VMS severity would be associated with a greater risk of incident VT and that later menopausal status at VMS onset and longer VMS duration would be positively associated with VT risk. Given the relation between late-onset VMS and other cardiovascular event outcomes[28], we also hypothesized that the VMS presence-VT risk association may differ by years since menopause and age at WHI baseline, which was the time of VMS report.

Methods

Study and Design

The data for this cohort study are from 2 studies within the Women's Health Initiative (WHI): the WHI-HT trial and the WHI Observational Study (WHI-OS), with analyses conducted separately in these two study settings. The WHI-HT enrolled 27,347 postmenopausal women ages 50 to 79 years of age from areas surrounding 40 clinical centers from 1993 to 1998[87,88] and randomized women to oral hormone therapy (HT) or placebo. Women who had a prior hysterectomy were randomized to estrogen-alone (E-alone) or placebo, and women without a prior hysterectomy were randomized to estrogen plus progesterone or placebo (E+P). WHI-OS enrolled 93,676 postmenopausal women ages 50 to 79 years of age between 1994 and 1998 and followed them for health outcomes including cardiovascular events; HT use was not randomized in the WHI-OS. WHI baseline occurred prior to the WHI-HT trial's randomization of HT and collectively refers to 4 contacts with study participants including an initial telephone screening interview and

3 baseline screening visits. Participants of the WHI-HT trials who were using HT at baseline underwent a 3-month period of HT non-use prior to the study's randomization to HT or placebo. Additional study details of the WHI-HT[10,11,87,88] and WHI-OS[114] have been published previously. *A priori*, we determined that we would conduct analyses separately among WHI-HT and WHI-OS participants due to differences in HT use at baseline between the two populations and differences in VT-report, which are explained in more detail below in this methods section. The WHI studies were approved by the human subjects review committees at each participating institution and all participants provided written informed consent.

Eligible Participants

From all WHI-HT (n=27,347) and all WHI-OS (n=93,676) participants, we excluded women missing data regarding the presence and severity of hot flashes or night sweats during the 4 weeks prior to second WHI baseline screening visit (WHI-HT n=163; WHI-OS n=516), with a history of VT prior to WHI baseline (WHI-HT n=312; WHI-OS n=4,135), missing data regarding the number of days between enrollment and end of follow-up (WHI-HT n=92; WHI-OS n=474), women currently using coumarin anticoagulants at WHI baseline (WHI-HT n=1; WHI-OS n=1,115), and WHI-HT participants who had not completed a full 3-month period of HT washout by the time of their VMS report (WHI-HT n=2,273).

Vasomotor Symptoms

At the second baseline screening visit, as part of a 34-symptom questionnaire, women reported whether they had experienced hot flashes or night sweats in the 4 weeks prior and if so, whether they were mild, moderate or severe. The questionnaire defined mild symptoms as those that did not interfere with usual activities, moderate symptoms as those that interfered somewhat with usual activities, and severe symptoms as those so bothersome that usual

activities could not be performed. Women with any mild, moderate, or severe hot flashes or night sweats were considered to have VMS present at baseline.

Analyses using menopausal status at VMS onset and VMS duration relied on historical VMS information and age at which the participant transitioned into postmenopause, among women ever reporting hot flashes or night sweats. As part of a reproductive history questionnaire, separate from the collection of VMS presence and severity data, women reported whether they had ever had menopausal symptoms such as hot flashes and night sweats and if they had, they reported their age at the first and last of these symptoms. Women currently experiencing VMS entered their current age. Age at menopause was determined using previously published methods[89] and was defined as the first of the age at which the woman last had any menstrual bleeding, had a bilateral oophorectomy, or began using HT. For women with a prior hysterectomy without bilateral oophorectomy, age at menopause was determined using the age at HT initiation or the onset of menopausal symptoms including hot flashes and night sweats. Since VMS were used to determine age at menopause for these women, women with a prior hysterectomy without bilateral oophorectomy were excluded from analyses with menopausal status at VMS onset as the exposure of interest.

Using the reported age at first VMS and the calculated age at menopause, we determined menopausal status at VMS onset by comparing age at menopause with age at VMS onset. Women were classified as having VMS onset (1) prior to completion of the menopausal transition (pre/perimenopause), (2) in the same year as completion of the menopausal transition (year of menopause), or (3) in postmenopause. VMS duration by the time of WHI baseline was determined by subtracting age at first VMS from age at last VMS, or age at WHI baseline if VMS were present at baseline. Of note, some women reported VMS at some point prior to WHI baseline, but did not report VMS presence at WHI baseline; these women were categorized as having absent VMS at WHI baseline and were not included in severity analyses using VMS

severity reported at WHI baseline, but were eligible for inclusion in menopausal status at VMS onset and in VMS duration analyses.

Venous Thrombosis Events

In the WHI-HT study, participants reported inpatient and outpatient (since 1999) venous thrombosis (VT) events, including both deep vein thrombosis (DVT) and pulmonary embolism (PE) during contact every 6 months and at annual in-clinic visits. Possible VT diagnoses from these reports were reviewed at clinical centers using standardized criteria by trained local adjudicators.[115] Central adjudication followed this local adjudication process. Additional details regarding event adjudication have been previously published.[31] VT events included in this WHI-HT analysis were centrally adjudicated diagnoses identified prior to the end of the WHI core study on April 8, 2005.

In the WHI-OS, inpatient and outpatient VT events were self-reported on follow-up forms mailed annually in years 1 and 3 through 8, structured the same as those used for the initial participant-report of VT events within the WHI-HT study. There was no local or central adjudication of self-reported VT events in the WHI-OS study. This WHI-OS analysis includes events reported by the end of the last follow-up form mailed as part of the core WHI study, which includes data collected through September 12, 2005.

Medical History and Demographic Characteristics

At baseline, all WHI-HT and WHI-OS participants completed questionnaires that collected demographic, lifestyle, and medical history information[87] including self-reported current age, race/ethnicity, age at last menstrual bleeding, history of and age at hysterectomy or bilateral oophorectomy, smoking status, education level, and physical activity. Participants reported whether they had treated diabetes (excluding gestational diabetes), high cholesterol requiring pills, had ever been told by a physician that they had hypertension or high blood pressure, or

whether they had a family history of DVT or PE among their parents, full-blooded siblings, or children. Weight and height were measured at the initial screening visit and this information was used to calculate body mass index (BMI) in kg/m^2.

During the telephone screening interview at WHI baseline, which was prior to the start of any 3-month HT washout required for WHI-HT trial participants and prior to the WHI-HT trial's randomization of HT, both WHI-OS and WHI-HT participants reported whether they were currently using or had ever previously used any estrogen or progesterone HT including pills, patches, implants, creams, suppositories, shots, or birth control pills (included only if used after age 50). During follow-up, HT use was reported differently by the trial and observational study participants. For WHI-HT study participants, information regarding adherence to the study pills (HT or placebo) was collected. This information included whether the participant was not taking study pills for all or part of the most recent 1-year study period and whether study pill use was resumed if previously stopped. We used this information to determine time-varying current HT use, with cessation of HT study pills during any 1-year period categorized as non-use of HT for that complete time period and thereafter, unless HT use was later resumed. Women randomized to placebo pills were always categorized as current non-users of HT. Participants of the WHI-OS reported the use of any estrogen or progesterone in the forms of pills and patches on annual mailed follow-up forms completed in years 1 and 3 through 8 and this information was used to determine time-varying current HT use.

Statistical Analyses

For both WHI-HT and WHI-OS participants, we described the baseline demographic characteristics and selected medical history characteristics by VMS presence and severity at WHI baseline. Separate Cox proportional hazards models stratified by time-varying current HT use (any vs. none) evaluated the association of baseline VMS presence, baseline VMS severity among those with VMS, menopausal status at first VMS (pre/perimenopause, year of

menopause, postmenopause) among those with VMS, and VMS duration (<5, 5-10, 10-15, >15 years) among those with VMS and time-to-VT event. Time-to-event was defined as the number of days from WHI randomization to first VT diagnosis in the WHI-HT and as the number of days from WHI enrollment to the first VT diagnosis in the WHI-OS, with removal from observation at the earliest date of either death, loss to follow-up or end of follow-up. Results are presented as hazard ratios (HRs), 95% confidence intervals (CIs), number of VT cases, and VT event rate per 1,000 person-years. Analysis of the relationship between VMS severity, menopausal status at VMS onset, and VMS duration and VT risk included a test for linear trend by modeling these exposures using grouped linear forms of these variables.

In VMS presence analyses, VMS absence was referent. VMS severity analyses were conducted among women with any VMS present at WHI baseline and mild VMS was referent. Menopausal status at VMS onset analyses were conducted among women who ever reported hot flashes or night sweats and the pre/perimenopausal status at VMS onset category was referent. In VMS duration analyses, <5 years VMS duration was referent. Statistical significance was determined using a Bonferroni-adjusted 2-sided alpha level of 0.0125 (0.05/4 VMS exposures).

For all VMS exposures, age-adjusted models were adjusted for linear age and multiple-adjusted models for likely confounders identified *a priori*: linear age, linear BMI, indicator variables for smoking status (never, former, current), and categorized race/ethnicity (White not of Hispanic origin, Black or African-American, other). Covariates with missing data (BMI, 0.1% missing; smoking status, 1.2% missing; race/ethnicity, 0.27% missing; HT use in the past year in the WHI-OS study, missing in 1.1 to 2.3% across years 1-8,) were multiply imputed using 5 imputations using chained equations[77,103]; linear regression models were specified to impute missing BMI values and multinomial logistic regression was used to impute missing smoking status, race/ethnicity, and time-varying current HT use in the WHI-OS study. Predictors in the imputation models were days from WHI enrollment to event or censoring, VMS presence, and

linear age in addition to the other variables being imputed. The assumption of proportional hazards was evaluated by testing Schoenfeld residuals.

A priori, we hypothesized that the association between VMS presence and time-to-VT event may differ by age group at WHI baseline (50-59 years, 60-69 years, 70-79 years) and years since menopause at WHI baseline (<10 years, 10-19 years, ≥20 years), with the hypothesis that VMS presence at WHI baseline may be associated with a greater VT risk among older women and women with more years since menopause at WHI baseline than among younger women and women with fewer years since menopause at WHI baseline. We evaluated interaction separately between VMS presence and both of these factors (as indicator variables) using likelihood ratio tests. Estimates among subgroups of women by years since menopause and age at WHI baseline were reported only if there was statistically significant evidence of interaction at a Bonferroni-adjusted p-value of 0.0125.

Sensitivity analyses for the exposures of VMS presence and severity in the WHI-HT and WHI-OS analyses excluded all women with any current HT use at WHI baseline (WHI-HT n excluded=695; WHI-OS n excluded=42,347), since current HT use may alter VMS presence or severity reported at WHI baseline. Sensitivity analyses for the exposures of menopausal status at VMS onset and VMS duration in both cohorts excluded all women who reported any HT use prior to or at WHI baseline (WHI-HT n excluded=10,260; WHI-OS n excluded=60,171), since this prior HT usage may have altered the timing of VMS onset or the duration of symptoms. Analyses were performed using Stata software, version 13.1.[46]

Results

The eligible study population was comprised of 24,508 participants of the WHI-HT study and 87,783 participants of the WHI-OS. At baseline, WHI-HT study participants were 64 years of age on average and menopause had occurred an average of 16 years prior; WHI-OS study participants were 63 years of age on average and menopause had occurred an average of 15

years prior. At the initial telephone screening interview during WHI baseline (before the HT washout period for WHI-HT participants), 4% of WHI-HT participants and 49% of WHI-OS participants were currently using HT. In the WHI-HT, after the 3-month washout period for women using HT, 61% of eligible participants reported no VMS in the 4 weeks prior to baseline (n=15,061), 26% reported mild (n=6,285), 10% reported moderate (n=2,504), and 3% reported severe VMS (n=658) (Table 4.1). In the WHI-OS, 69% of women reported no VMS in the 4 weeks prior to baseline and only 2% reported severe VMS (n=1,607). In the WHI-HT study, women with severe VMS after the washout period were slightly more likely to have used HT before the washout (5%) than women with no VMS (2%). In contrast, in the WHI-OS study, women with severe VMS were less likely to currently use HT at baseline (35%) than women with mild (45%) or no VMS (52%).

In both the WHI-HT and WHI-OS, women with severe hot flashes were more likely to have had a prior bilateral oophorectomy, ever have been hypertensive, have treated diabetes, to be current smokers, and were less likely to have a college degree or higher than were women with no VMS or less severe VMS. A greater proportion of women with severe hot flashes were of Black race than were women with no or less severe VMS. Average follow-up was 8.1 years among WHI-HT participants and 7.5 years among WHI-OS participants and ranged from 1 days to 11.4 years.

VMS Presence, Severity, Timing, and Duration

In multiple-adjusted analyses, we found little evidence of an association between VMS presence and time-to-incident VT event in the WHI-HT study (HR_{adj}=0.91; 95% CI: 0.75, 1.1) (Table 4.2). Although VMS presence at baseline in the WHI-OS was associated with a 20% greater risk of incident VT in age-adjusted analyses (HR_{ageadj}=1.2; 95% CI: 1.0, 1.3), this estimated greater risk shifted toward the null after adjusting for additional covariates (HR_{adj}=1.1; 95% CI: 0.99, 1.3). Among women with any VMS at WHI baseline, we found no evidence that VMS severity was

associated with VT risk in the WHI-HT study (p-trend=0.62) or in the WHI-OS study (p-trend=0.55).

In the WHI-HT and WHI-OS study settings, among women who reported ever having VMS, there was no significant evidence of an association of VT with menopausal status at VMS onset (WHI-HT p-trend=0.23; WHI-OS p-trend=0.13) or with duration of VMS (WHI-HT p-trend=0.42; WHI-OS p-value for linear trend=0.33; Table 4.3). We found no statistically significant evidence of non-proportional hazards in the WHI-HT analysis or the WHI-OS analysis at a Bonferroni corrected alpha of 0.0125.

Tests for Interaction

In the WHI-HT and WHI-OS cohorts, we found no evidence of interaction between VMS presence and, separately, age group at baseline (WHI-HT likelihood ratio test p=0.16; WHI-OS p=0.45) or years since menopause at baseline (WHI-HT p=0.50; WHI-OS p=0.12), on the risk of VT. Subgroup-specific risk estimates are not presented since we found no statistically significant evidence of interaction.

Sensitivity Analyses

In sensitivity analyses that excluded women using HT at WHI baseline, there remained no evidence of an association between VMS presence and VT risk among WHI-HT participants (HR_{adj}=0.94; 95% CI: 0.78, 1.1) or among WHI-OS participants (HR_{adj}=1.2; 95% CI: 0.97, 1.4). There was also no evidence of an association between VMS severity and VT risk in either cohort and risk estimates were similar to those without this current HT use exclusion. In sensitivity analyses for the exposure of menopausal status at VMS onset that excluded women who reported any current or past use of HT, estimates of VT risk associated with postmenopausal status at VMS onset shifted closer toward the null among WHI-HT participants (HR_{adj} in year of menopause=1.4; 95% CI: 0.94, 2.0; HR_{adj} in postmenopause=0.94; 95% CI:

0.60, 1.5) and among WHI-OS participants (HR_{adj} in year of menopause=1.0; 95% CI: 0.74, 1.4; HR_{adj} in postmenopause=1.0; 95% CI: 0.68, 1.4). Sensitivity analyses using the VMS duration exposure with current and past HT users excluded provided relative risk estimates similar to those in the full eligible cohorts.

Discussion

In this study of postmenopausal participants of the WHI-HT trials and WHI-OS, we found no evidence of an association of VMS presence, severity, timing, or duration with VT risk. This lack of evidence for an association between VMS and VT risk aligns with findings from a study conducted previously in the setting of the WHI-HT that suggested no evidence of an association between VMS and hemostatic factor levels, which are markers of thrombotic risk (Harrington; unpublished).

VMS and Thrombotic Event Risk

No studies have evaluated the association between VMS and the risk of incident VT and few have investigated the association of VMS with other CVD clinical endpoints or mortality.[28,116,117] The presence of night sweats, but not hot flashes, was associated with a modestly greater risk of CHD than VMS absence in a population-based study of Dutch and Swedish women 46 to 64 years of age (HR_{adj}=1.3; 95% CI: 1.1-1.7).[116] Timing of VMS in relation to menopause was important in relation to cardiovascular event risk in another WHI-OS analysis of major CHD, stroke, total CVD, and all-cause mortality (n=60,027).[28] Women with VMS present at menopausal onset that did not persist into postmenopause (at WHI-OS enrollment) had a lower risk of stroke (HR_{adj}=0.83; 95% CI: 0.72, 0.96), total CVD events (major CHD and stroke) (HR_{adj}=0.89; 95% CI: 0.81, 0.97), and all-cause mortality (HR_{adj}=0.92; 95% CI: 0.85, 0.99) than women who never reported VMS.[28] Women who did not experience VMS at menopausal onset but who did experience VMS in postmenopause, at WHI-OS enrollment which was on average

13-18 years after menopausal onset, had a higher risk of major CHD (HR_{adj}=1.3; 95% CI: 1.01, 1.7) and all-cause mortality (HR_{adj}=1.3; 95% CI: 1.1, 1.5) than women never reporting VMS[28].

In the present analysis, however, we found no evidence that VMS presence or that menopausal status at VMS onset was associated with VT risk. Although our VT relative risk estimates associated with VMS onset in postmenopause among participants of the WHI-OS (HR_{adj}=1.2; 95% CI: 0.96, 1.4) were similar to those published for the risk of major CHD and all-cause mortality[28], we found no statistically significant evidence of association between VMS timing and VT risk. Several differences between these 2 studies should be noted. Our study evaluated VT rather than arterial thrombotic event risk and it is plausible that differences in the etiologies of arterial and venous thrombosis may lead to differences in the association between VMS and cardiovascular event risk, by event type. Our analyses of VMS timing were restricted to women who had ever reported VMS and used pre/perimenopausal onset of VMS as referent, in contrast to the published study of CHD and all-cause mortality, which compared risk to a reference group of women without VMS. In addition, our models were stratified by time-varying current HT use, which may provide for more complete adjustment than baseline HT use.

Biologic Plausibility

In this study, we found no evidence of an association of VMS presence, severity, duration, or timing with VT risk, aligning with results from our WHI-HT cross-sectional analysis of VMS and hemostatic factors that also suggested no evidence of an association. The association between VMS and hemostatic factors, which are biomarkers associated with VT risk, has been inconsistent. In a previous study conducted within a subset of WHI-HT postmenopausal participants, there was no strong evidence that VMS presence or severity were associated with levels of antithrombin, plasminogen activator inhibitor-1, protein C antigen, total and free protein S antigen, total and free tissue factor pathway inhibitor, D-dimer, and nAPCsr (Harrington; unpublished). In contrast, among Study of Women's Health Across the Nation (SWAN)

perimenopausal participants (n=3,199), more frequent hot flashes were associated with higher factor VII activity and tissue plasminogen activator antigen, suggesting a more thrombotic profile.[86] Differences in results from these 2 studies may stem from differences in participant characteristics between studies. Participants of the WHI-HT study were 67 years of age on average and were all postmenopausal, while SWAN participants were perimenopausal women with an average age of 46 years.

Limitations and Strengths

VMS data used in this study was self-reported. Although self-reported VMS data are easy to collect in a clinic setting, inexpensive, and reflect a woman's perception of her VMS, self-reported VMS are also subjective measures. The objective measurement of VMS, for example by sternal skin conductance, is not part of routine clinical care. There is likely some misclassification of self-reported VMS in relation to objectively-measured VMS[118]. Given that VT event status was unknown at the time of VMS report at WHI baseline, the misclassification of VMS is likely to be nondifferential by VT event, which should lead to bias toward the null.

Other limitations of this study include the retrospective reporting of VMS presence and severity in the 4-weeks prior to WHI baseline as well as the use of participant recall for age at first and last VMS. In particular, women may inaccurately recall their age at first VMS, given that most participants were in the late postmenopausal stage at WHI baseline (WHI-HT average age=64; WHI-OS average age=63) and that VMS are most common in late perimenopause and in early postmenopause.[26] Ideally, VMS information would be collected prospectively, with women reporting VMS events as they occur or via the recording of VMS information in daily diaries.[118] Given that women were postmenopausal at baseline in WHI, however, such data collection that prospectively captured the age at first VMS onset was not possible. We have no reason to believe that misclassification of age at first and last VMS would be differential by VT risk, however, and this misclassification should lead to estimates biased toward the null.

A complicating factor in this evaluation of VT risk associated with VMS is the use of HT, which is known to be associated with an increased risk of VT[31], and which also effectively reduces VMS in most women. In this study, we stratified analyses by time-varying current HT use because of the association of HT use with both VMS and with greater VT risk. The potential for residual confounding remains, however, if current HT use is misclassified or if recent HT use is associated with VMS or VT risk. In addition, given that some participants were using HT at the time of VMS self-report at WHI baseline in the WHI-OS setting, it is unknown whether these women using HT would have had a different VMS experience had they not been using HT. Similarly, past use of HT prior to WHI baseline may potentially alter the age at first and last VMS. In VMS presence and severity sensitivity analyses that excluded current HT users at WHI baseline, and in VMS timing and duration sensitivity analyses that excluded current and past HT users, most relative risk estimates remained similar to estimates from the full eligible cohort, but the VT relative risk associated with VMS that began in postmenopause shifted closer to the null. Women eligible for inclusion in our sensitivity analyses are not representative of the more general population of women, however, since women with VMS who choose not to use HT might have different cardiovascular risk or other health factors than women with VMS who do choose to use HT.

Due to the unique setting of WHI, which included both an observational component and randomized HT trial component, we were able to conduct our analyses in two populations from whom the same data were collected. Analyses were conducted separately for WHI-HT and WHI-OS participants due to differences in use of HT at WHI baseline by VMS presence and severity between the two studies and because VT events were adjudicated in the WHI-HT study but were self-reported in the WHI-OS study. Among women without VMS present at baseline after the washout period in the WHI-HT, only 2% of WHI-HT participants used HT at baseline prior to washout, in contrast to the 52% of WHI-OS participants without VMS present who used HT at baseline. Among women with severe VMS at baseline, 5% of women in the WHI-HT and

35% of women in the WHI-OS used HT at WHI baseline. The inclusion of both the WHI-HT and WHI-OS populations in this study allows for replication of analyses in two large populations with different VMS qualities and differing HT use patterns; however, replication efforts in a non-WHI population would further improve our understanding of VMS in relation to VT risk.

Other strengths of our study include a large number of participants, our ability to evaluate VMS characteristics (severity, duration, and timing) in addition to VMS presence, and the adjudication of VT events in the WHI-HT setting. In the WHI-OS, VT events were self-reported; however, in the WHI-HT, the confirmation rate of self-reported VT when compared with central adjudication was 80.4%[119], suggesting that the WHI-OS self-reported VT events may include only moderate levels of misclassification.

Conclusions

In conclusion, we found no evidence that VMS presence, severity, timing, or duration were associated with VT incidence. Given that VMS have been previously associated with an increased risk of CHD[28,116] and all-cause mortality[28], future studies of VMS as a possible marker for cardiovascular event risk are warranted.

Table 4.1. Selected baseline characteristics of participants, by baseline vasomotor symptom presence and severity.

	WHI-HT (n=24,508)				WHI-OS (n=87,783)			
		Any VMS (n=9,447)				Any VMS (n=27,067)		
	No VMS (n=15,061)	Mild (n=6,285)	Moderate (n=2,504)	Severe (n=658)	No VMS (n=60,716)	Mild (n=19,469)	Moderate (n=5,991)	Severe (n=1,607)
HT Use at Enrollment, no. (%)								
Never Use	8646 (59.7)	3230 (53.4)	1204 (49.6)	276 (43.1)	17,783 (29.8)	5,921 (30.9)	1,864 (31.7)	501 (31.6)
Past Use	5556 (38.3)	2576 (42.6)	1099 (45.3)	334 (52.1)	10,823 (18.2)	4,709 (24.6)	1,762 (29.9)	530 (33.4)
Current Use	293 (2.0)	246 (4.1)	125 (5.2)	31 (4.8)	31,022 (52.0)	8,506 (44.5)	2,263 (38.4)	556 (35.0)
Randomized HT arm, no. (%)								
E-alone treatment	2694 (17.9)	1145 (18.2)	551 (22.0)	157 (23.9)	-	-	-	-
E-alone placebo	2731 (18.1)	1192 (19.0)	536 (21.4)	174 (26.4)	-	-	-	-
E+P treatment	4914 (32.6)	2018 (32.1)	731 (29.2)	173 (26.3)	-	-	-	-
E+P placebo	4722 (31.4)	1930 (30.7)	686 (27.4)	154 (23.4)	-	-	-	-
Years since menopause, mean (SD)	17.4 (8.9)	13.6 (9.4)	13.0 (9.6)	12.9 (10.3)	15.9 (9.2)	13.6 (9.3)	13.5 (9.8)	13.8 (10.2)
Age, years, mean (SD)	65.2 (6.9)	61.4 (6.9)	59.8 (6.7)	58.9 (6.6)	64.4 (7.2)	61.7 (7.2)	60.9 (7.2)	59.9 (7.2)
Prior bilateral oophorectomy, no. (%)	2124 (14.1)	822 (13.1)	378 (15.1)	107 (16.3)	12328 (20.3)	3685 (18.9)	1152 (19.2)	355 (22.1)
Family history of VT, no. (%)	1368 (9.1)	636 (10.1)	246 (9.8)	64 (9.7)	4574 (7.5)	1562 (8.0)	543 (9.1)	158 (9.8)
Hypertension Ever, no. (%)	4991 (33.1)	2087 (33.2)	919 (36.7)	268 (40.7)	19284 (31.8)	6415 (33.0)	2243 (37.4)	666 (41.4)
High cholesterol, no. (%)	1940 (12.3)	742 (11.8)	313 (12.5)	98 (14.9)	8709 (14.3)	2803 (14.4)	925 (15.4)	271 (16.9)
Treated diabetes, no. (%)	1063 (7.1)	479 (7.6)	212 (8.5)	69 (10.5)	2835 (4.7)	1195 (6.1)	521 (8.7)	175 (10.9)
BMI, mean (SD)	28.9 (6.0)	29.3 (6.0)	30.1 (6.2)	30.9 (6.3)	28.8 (5.6)	27.6 (5.8)	28.8 (6.3)	30.0 (6.8)
Smoking, no. (%)								
Never	7715 (51.2)	2982 (47.5)	1156 (46.2)	287 (43.6)	31,066 (51.2)	9,605 (49.3)	2,794 (46.6)	718 (44.7)
Past	5745 (38.1)	2538 (40.4)	973 (38.9)	244 (37.1)	25,592 (42.2)	8,292 (42.6)	2,565 (42.8)	646 (40.2)
Current	1437 (9.5)	701 (11.2)	346 (13.8)	118 (17.9)	3,298 (5.4)	1,331 (6.8)	551 (9.2)	218 (13.6)
Unknown	164 (1.1)	64 (1.0)	29 (1.2)	9 (1.4)	760 (1.3)	241 (1.2)	81 (1.4)	25 (1.6)
Ethnicity, no. (%)								
American Indian/Alaskan Native	68 (0.45)	33 (0.53)	13 (0.52)	5 (0.76)	225 (0.37)	92 (0.47)	46 (0.77)	16 (1.0)
Asian/Pacific Islander	326 (2.2)	100 (1.6)	36 (1.4)	4 (0.61)	2,057 (3.4)	421 (2.2)	112 (1.9)	15 (0.93)
Black	969 (6.4)	815 (13.0)	492 (19.7)	211 (32.1)	3,288 (5.4)	2,272 (11.7)	1,085 (18.1)	443 (27.6)
Hispanic	677 (4.5)	309 (4.9)	244 (9.7)	83 (12.6)	1,913 (3.2)	822 (4.2)	423 (7.1)	144 (9.0)
White not of Hispanic origin	12830 (85.2)	4925 (78.4)	1679 (67.1)	345 (52.4)	52,426 (86.4)	15,585 (80.1)	4,226 (70.5)	954 (59.4)
Other	165 (1.1)	79 (1.3)	37 (1.5)	9 (1.4)	637 (1.1)	227 (1.2)	80 (1.3)	29 (1.8)
Unknown	26 (0.17)	24 (0.38)	3 (0.12)	1 (0.15)	170 (0.28)	50 (0.26)	19 (0.32)	6 (0.37)
Education. no. (%)								
High school diploma or less	4029 (26.8)	1857 (29.6)	940 (37.5)	277 (42.1)	11,880 (19.6)	4,263 (21.9)	1,693 (28.3)	557 (34.7)
School after high school	5937 (39.4)	2580 (41.1)	995 (39.7)	265 (40.3)	21,423 (35.3)	7,243 (37.2)	2,344 (39.1)	649 (40.4)
College degree or higher	4999 (33.2)	1800 (28.6)	550 (22.0)	111 (16.7)	26,943 (44.4)	7,803 (40.1)	1,891 (31.6)	386 (24.0)
Unknown	96 (0.64)	48 (0.76)	19 (0.76)	5 (0.76)	470 (0.77)	160 (0.82)	63 (1.1)	15 (0.93)
Physical activity, MET h/wk, mean (SD)	11.2 (13.5)	10.1 (12.4)	9.6 (12.7)	9.1 (12.3)	13.9 (14.6)	13.1 (14.7)	11.7 (14.1)	10.2 (13.4)

*WHI-HT=Women's Health Initiative Hormone Therapy trials; WHI-OS=Women's Health Initiative Observational Study; VMS=vasomotor symptoms; HT=hormone therapy; E=estrogen; P=progestogen; no.=number; SD=standard deviation; VT=venous thrombosis; BMI=body mass index; MET=metabolic equivalent of task.

Table 4.2. Venous thrombosis events by baseline vasomotor symptom presence and severity.								
				Age-Adjusted		Multiple-Adjusted*		p-trend
	Total n	Cases	Rate/1000 Person Years (95% CI)	HR (95% CI)	p-value	HR (95% CI)	p-value	
WHI-HT								
VMS Presence at Baseline								
No VMS	15,061	354	2.9 (2.6, 3.3)	1.0	-	1.0	-	-
VMS	9,447	168	2.2 (1.9, 2.5)	0.93 (0.77, 1.1)	0.47	0.91 (0.75, 1.1)	0.34	
VMS Severity at Baseline among Women with VMS								
Mild VMS	6,285	119	2.3 (1.9, 2.8)	1.0	-	1.0	-	
Moderate VMS	2,504	38	1.9 (1.4, 2.6)	0.89 (0.62, 1.3)	0.52	0.86 (0.60, 1.2)	0.43	
Severe VMS	658	11	2.1 (1.2, 3.8)	1.1 (0.59, 2.0)	0.78	0.99 (0.53, 1.9)	0.98	0.62
WHI-OS								
VMS Presence at Baseline								
No VMS	60,716	761	1.7 (1.5-1.8)	1.0	-	1.0	-	-
VMS	27,067	342	1.7 (1.5-1.9)	1.2 (1.0, 1.3)	0.011	1.1 (0.99, 1.3)	0.079	
VMS Severity at Baseline among Women with VMS								
Mild VMS	19,469	246	1.7 (1.5, 1.9)	1.0	-	1.0	-	
Moderate	5,991	69	1.5 (1.2, 2.0)	0.97 (0.74, 1.3)	0.80	0.91 (0.70, 1.2)	0.50	
Severe VMS	1,607	27	2.3 (1.6, 3.3)	1.5 (1.0, 2.3)	0.039	1.3 (0.89, 2.0)	0.16	0.55

*Analysis stratified by time-varying current HT use (any vs. none) using true stratification. Multiple-adjusted model adjusted for age in years, BMI (kg/m^2), current/former/never smoking status and categorized race/ethnicity.

Table 4.3 Venous thrombosis events by menopausal status at VMS onset and VMS duration.

				Age-Adjusted		Multiple-Adjusted*		
	Total n	Cases	Rate/1000 Person-Years	HR (95% CI)	p-value	HR (95% CI)	p-value	p-trend
WHI-HT								
Menopausal Status at VMS Onset								
Pre/Perimenopause	5,463	98	2.3 (1.9, 2.8)	1.0	-	1.0	-	
Year of Menopause	5,444	109	2.5 (2.1, 3.1)	1.2 (0.89, 1.6)	0.24	1.2 (0.93, 1.6)	0.15	
Postmenopause	4,024	83	2.6 (2.1, 3.3)	1.2 (0.88, 1.6)	0.25	1.2 (0.88, 1.6)	0.25	0.23
VMS Duration among women with VMS								
<5 Years	5,933	90	1.9 (1.5, 2.3)	1.0	-	1.0	-	
5-10 Years	3,458	72	2.6 (2.1, 3.3)	1.4 (1.0, 1.9)	0.027	1.4 (1.0, 1.9)	0.046	
10-15 Years	2,344	56	3.0 (2.3, 3.9)	1.5 (1.1, 2.1)	0.017	1.4 (1.0, 2.0)	0.046	
>15 Years	3,327	75	2.9 (2.3, 3.7)	1.2 (0.88, 1.6)	0.25	1.1 (0.81, 1.5)	0.53	0.42
WHI-OS								
Menopausal Status at VMS Onset								
Pre/Perimenopause	22,321	258	1.5 (1.3, 1.7)	1.0	-	1.0	-	
Year of Menopause	22,085	276	1.6 (1.5, 1.9)	1.1 (0.91, 1.3)	0.36	1.1 (0.90, 1.3)	0.46	
Postmenopause	15,914	225	1.9 (1.7, 2.2)	1.2 (0.99, 1.4)	0.070	1.2 (0.96, 1.4)	0.130	0.13
VMS Duration								
<5 Years	27,576	323	1.5 (1.4, 1.7)	1.0	-	1.0	-	
5-10 Years	13,221	136	1.3 (1.1, 1.6)	0.89 (0.72, 1.1)	0.22	0.86 (0.71, 1.1)	0.16	
10-15 Years	8,589	108	1.7 (1.4, 2.0)	1.0 (0.81, 1.3)	0.91	0.97 (0.78, 1.2)	0.77	
>15 Years	11,361	195	2.3 (2.0, 2.7)	1.2 (0.99, 1.4)	0.06	1.1 (0.92, 1.3)	0.27	0.33

*Analysis stratified by time-varying current HT use (any vs. none) using true stratification. Multiple-adjusted model adjusted for age in years, BMI (kg/m^2), current/former/never smoking status, and categorized race/ethnicity.

CONCLUSION

In this dissertation, we attempted to further characterize the endogenous hormonal milieu associated with the risk of venous thrombosis (VT) in postmenopausal women. To this end, we conducted 4 studies within the settings of the Seattle Heart and Vascular Health (HVH) Study and the Women's Health Initiative (WHI). One of these studies directly evaluated the relation between endogenous sex hormone levels and hemostatic factor levels, and the other 3 studies evaluated VT risk or hemostatic factor levels in relation to events associated with changes in these endogenous hormones (vasomotor symptoms (VMS) and hysterectomy and oophorectomy).

In a cross-sectional evaluation of the association between endogenous sex hormone, sex hormone binding globulin (SHBG), and prohormone levels among postmenopausal women, we did not find statistical evidence that levels of endogenous sex hormones were associated with hemostatic factor levels. Without correcting for multiple comparisons, there was some suggestion that higher levels of estradiol (E2), estrone (E1), sex hormone binding globulin (SHBG), total testosterone (T), and dehydroepiandrosterone (DHEA) may be associated with lower levels of an anticoagulant measure, total protein S, and that higher E1 levels may be associated with lower levels of another anticoagulant measure, antithrombin (ATc); lower levels of these anticoagulant measures are associated with greater thrombotic propensity. There was also some suggestion that higher levels of the adrenal proandrogens, dehydroepiandrosterone-sulfate (DHEAS) and DHEA, may be associated with lower levels of the global coagulation measures, thrombin generation (TG) peak and TG endogenous thrombin potential (ETP), and with lower levels of a marker of APC resistance, normalized activated protein C sensitivity ratio (nAPCsr); the directions of these differences are all associated with less thrombotic propensity. Higher DHEA levels may also be associated with lower levels of the procoagulant, Factor VII activity (FVIIc). Due to the large number of comparisons made in this study, however, we could not rule out that these associations were chance findings. Because our analysis was based on a

limited sample, additional studies of a larger sample size would contribute to our understanding of the relation between endogenous hormone and hemostatic factor levels.

Hysterectomy, with and without oophorectomy, is associated with decreases in ovarian androgen and estrogen levels.[16,17,65] In a population-based, case-control study of postmenopausal women without a history of reproductive cancer, we found that women who had undergone a prior hysterectomy with bilateral salpingo-oophorectomy (BSO) and who were not currently using HT had a greater risk of incident VT than women with no prior hysterectomy who were not currently using HT; the risk of VT was attenuated for women with a prior hysterectomy with BSO with current HT use. In addition, we found some evidence that among women with prior hysterectomy, hysterectomy with BSO may be associated with a greater VT risk than hysterectomy-alone. Among women in the United States who undergo a hysterectomy, approximately 54% receive a BSO concurrently.[30] BSOs performed for benign indications at the time of hysterectomy are typically performed for ovarian cancer prevention.[120] Possible differences in the risk of a cardiovascular event, including VT, associated with hysterectomy with BSO versus hysterectomy with ovarian conservation may be important considerations when weighing the risks and benefits of elective BSO at the time of hysterectomy. However, given that other studies have reported no statistically significant evidence of a greater VT risk associated with hysterectomy with BSO[20,23,24], our findings require replication for clinical significance.

In 2 studies of vasomotor symptoms (VMS) in relation to thrombotic risk in postmenopausal women an average of 15 years since menopause, one of which evaluated VMS in relation to hemostatic factor levels and the other which evaluated the association between VMS and incident VT risk, we did not find evidence that VMS presence was associated with greater thrombotic risk. In a cross-sectional study of postmenopausal women, we found no evidence that VMS presence or severity was associated with levels of hemostatic factors among postmenopausal women. VMS have previously been associated with some hemostatic factors

among pre- and perimenopausal women.[86] It is plausible that self-reported VMS occurring during the menopausal transition are symptoms of a different underlying physiologic process than VMS occurring in postmenopause, however, and that the underlying processes causing VMS at these different stages of reproductive aging may be differentially associated with hemostatic factor levels.

We also found no evidence of an association between VMS presence, severity, timing, or duration and the risk of incident VT in a cohort study of postmenopausal women. VMS onset in postmenopause has previously been associated with the risk of coronary heart disease and all-cause mortality[28], suggesting that the timing of VMS onset in relation to menopause may be associated with the risk of cardiovascular disease. Such an association with VMS beginning in postmenopause was not evident in our study of VT risk, however.

This dissertation project aimed to contribute an improved understanding of thrombotic risk associated with the endogenous hormonal milieu in postmenopause. Results from this dissertation work reinforce the hypothesis that endogenous and exogenous hormones differently impact thrombotic risk. Although the use of exogenous HT is positively associated with the risk of VT, we found little evidence that endogenous hormone levels were associated with hemostatic factors, we found evidence that hysterectomy with BSO and no current HT use (both associated with lower E2 levels) were associated with a greater risk of incident VT than no hysterectomy and no current HT use, and although VMS are hypothesized to be hormonally-related, we found no evidence that VMS are associated with hemostatic factor levels or with the risk of incident VT.

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