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Charles P. Ko Date

Hot Flashes, Hormone Therapy and Breast Cancer Risk in the Women's Health Initiative Clinical Trials

By

Charles Perry Ko
Master of Public Health
Hubert Department of Global Health

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Ghada N. Farhat, PhD, MPH
Committee Chair

Hot Flashes, Hormone Therapy and Breast Cancer Risk in the Women's Health Initiative Clinical Trials

By

Charles Perry Ko

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Abstract

Hot Flashes, Hormone Therapy and Breast Cancer Risk in the
Women's Health Initiative Clinical Trials

By Charles Perry Ko

Background: The association of hot flashes with breast cancer risk is not clearly elucidated, although evidence suggests that their new onset with anti-estrogen therapy predicts breast cancer prognosis and that they modify the effects of dietary interventions on breast cancer risk and prognosis.

Objective: We investigated the association of baseline hot flash status with the subsequent development of invasive breast cancer (Aim 1) and assessed whether baseline hot flash status modifies the effect of estrogen plus progestin (E+P) and estrogen alone (E-alone) therapy on invasive breast cancer risk (Aim 2).

Methods: We performed a prospective analysis within the Women's Health Initiative Hormone Therapy Clinical Trials. The E+P trial included 16,608 women aged 50-79 years with an intact uterus and no history of breast cancer, and the E-alone trial 10,739 women aged 50-79 years with prior hysterectomy and no history of breast. During an average follow-up of 14.4 years extending post intervention, 1,460 cases of breast cancer were diagnosed (960 in E+P and 500 in E-alone).

Results: For Aim 1, combining the trials' placebo groups, hot flash status was not associated with breast cancer risk. In adjusted models, the hazard ratio (HR) was 1.05 (95% CI 0.85-1.29) for women with mild hot flashes and 1.16 (95% CI 0.89-1.53) for women with moderate/severe symptoms, compared to women without symptoms. For Aim 2, breast cancer risk associated with E+P treatment tended to increase with higher severity of hot flashes (none: HR= 1.16, 95% CI 0.98-1.37; mild: HR= 1.19, 95% CI 0.88-1.63; moderate/severe: HR= 1.46, 95% CI 0.96-2.24); however, interactions of E+P with hot flash status categories were not statistically significant. Breast cancer risk associated with E-alone treatment did not vary significantly by hot flash status (none: HR=0.89, 95% CI 0.70-1.12; mild: HR=0.59, 95% CI 0.38-0.92; moderate/severe: HR=0.72, 95% CI 0.43-1.23; p-values for interaction terms >0.05).

Conclusions: In postmenopausal women, hot flashes did not predict breast cancer risk, and they did not modify the effect of E+P or E-alone therapy on breast cancer risk. Further studies are needed to evaluate the possibility that women with moderate/severe symptoms have a higher breast cancer risk with E+P treatment.

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This study utilized data from the Women's Health Initiative (WHI) study[1]. We gratefully acknowledge the dedication of WHI investigators, staff and participants. Opinions, ideas and interpretations included in the thesis are mine alone and not those of the WHI investigators.

1. https://www.whi.org/SitePages/WHI%20Home.aspx

Table of Contents

Chapter 1: Introduction

Vasomotor symptoms, which include hot flashes and night sweats are the most common symptoms accompanying menopause, with an estimated 65-76% of women experiencing hot flashes during this transition [1]. The pathophysiology of vasomotor symptoms remains not fully understood. Hot flashes were found to be associated with fluctuating levels of estradiol (E2), decreased levels of inhibin B and increased levels of follicle stimulating hormone (FSH) [2]; however, these endogenous hormone levels are not enough to explain vasomotor symptoms since hormone fluctuation occurs in all women transitioning through menopause and not only in symptomatic women [3]. The surgical induction of menopause through hysterectomy for example, also causes levels of estrogen to decrease and results in hot flashes in some women. The administration of estrogen in the form of hormone therapy has long been used and found to be effective at reducing hot flashes [4], further demonstrating the role of hormones in the causation of vasomotor symptoms.

A narrowed thermal neutral zone, defined by a lowered threshold for sweating and an increased threshold for shivering, has been recently demonstrated among symptomatic women [3]. In addition, core body temperature was shown to slightly increase before hot flashes [5]. The combination of these two effects cause symptomatic women to reach the upper sweating threshold with only small increases in their core body temperature. Estrogen administration in symptomatic women was found to increase the upper threshold for sweating, significantly reducing the number of hot flash episodes [6]. While the exact mechanism is not fully understood, estrogen remains a critical factor in the occurrence of hot flashes. Other factors

associated with hot flashes include increased abdominal adiposity, increased body mass index (BMI), age, race, education status and smoking status [7].

Although hot flashes are most commonly thought to affect the quality of life, emerging research has also linked hot flash symptoms to coronary heart disease (CHD) [8, 9] and more recently, hot flashes were found to play a role in breast cancer incidence and prognosis [10, 11].

The Study of Women's Health Across the Nation Heart Study examined the associations between hot flashes and various cardiovascular diseases, reasoning that risk factors for cardiovascular diseases and hot flashes coincide, such as obesity, age and smoking. The study found hot flashes to be significantly associated with flow-mediated dilation (FMD) and also with aortic calcification [8]. The Women's Health Initiative clinical trials found that women who experienced vasomotor symptoms at baseline had an increased risk of hormone therapy associated CHD with older age ($p=0.04$) and longer time since menopause ($p=0.06$). These trends were not significant in women who did not experience vasomotor symptoms at baseline [12]. Another study, the Heart Estrogen/Progestin Replacement Study (HERS) found that among women who experienced hot flashes at baseline, those assigned to the estrogen plus progestin group were 9-times more likely to experience CHD than those assigned to the placebo group (Hazard Ratio [HR]=9.01, 95% CI 1.15-70.35). Among women who did not experience hot flashes at baseline, there was no significant association between hormone therapy and CHD (HR=1.32, 95% CI 0.86-2.03) [9].

For many perimenopausal and postmenopausal breast cancer patients, chemotherapy as well as adjuvant endocrine therapy such as tamoxifen, result in severe side effects, one of the most common being hot flashes. Hot flashes are therefore more common among women with breast cancer than among healthy women [10]. Tamoxifen acts as an antiestrogen, preventing the normal role of estrogen in the body by blocking estrogen receptors [10]. Cytochrome P450 2D6 (CYP2D6) metabolizes tamoxifen into its two more active metabolites, endoxifen and 4-hydroxytamoxifen [13]. Polymorphisms exist for the CYP2D6 enzyme with some variations having a poorer metabolizing rate of tamoxifen. Women with these genetic variations to their CYP2D6 and also women who take CYP2D6 inhibitors have been shown to have lower levels of endoxifen [14]. The North Central Center Treatment Group (NCCTG) found the most common CYP2D6 polymorphism to be associated with low incidence of hot flashes after the initiation of tamoxifen therapy ($p=0.064$), and also demonstrated worse relapse-free time ($p=0.023$) and worse disease-free survival ($p=0.012$) when compared to the normal functioning CYP2D6 [15]. This suggests a role for hot flashes in predicting CYP2D6 activity and drug efficacy, with the presence of hot flashes indicating normal CYP2D6 activity and less chance of breast cancer recurrence.

The Women's Healthy Eating and Living (WHEL) study further investigated hot flashes as a predictive marker for the efficacy of tamoxifen. Among women being treated with tamoxifen, those who experienced hot flashes at baseline were less likely to have a recurrence of breast cancer (HR=0.50, 95% CI 0.36-0.69) [16]. The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial further supported these findings and not only looked at tamoxifen as an adjuvant therapy, but also arimidex, and their effect on hormone receptor-positive breast cancer either

alone or in combination. Analysis of the trial concluded that women with new onset of vasomotor symptoms within three months of adjuvant therapy initiation had lower recurrence of breast cancer compared to those women who did not experience vasomotor symptoms (HR=0.84, 95% CI 0.71-1.00) [11].

Hot flashes were also observed to modify the effects that dietary interventions had on the risk of invasive breast cancer. In the Women's Health Initiative (WHI) Dietary Modification Trial, the low-fat dietary intervention resulted in a greater decrease in the risk of breast cancer among women who experienced hot flashes (HR= 0.65, 95% CI 0.42-1.01) than among women who did not experience hot flashes (HR= 0.93, 95% CI 0.84-1.03) [17].

To our knowledge, only three studies have assessed the association of vasomotor symptoms with breast cancer risk. Data from a case-control study evaluating the relationship between menopausal hormone therapy and risk of varying types of invasive breast cancer was used to assess the relationship between vasomotor symptoms and the risk of breast cancer. Results from the analysis showed a decreasing risk of breast cancer with increasing intensity of hot flashes for all three types of invasive breast cancer, invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and invasive ductal-lobular carcinoma (IDLC). Women who ever experienced a hot flash had decreased risks of IDC (OR=0.5, 95% CI 0.3-0.7), ILC (OR=0.5, 95% CI 0.3-0.7), and IDLC (OR=0.7, 95% CI 0.4-1.2) [18]. The Two Sister Study looked at women diagnosed with breast cancer before the age of 50 and their sisters who had not been diagnosed with breast cancer. Results showed that women who ever experienced hot flashes had a decreased odds of young-onset breast cancer compared to women who never experienced hot

flashes (OR=0.47, 95% CI 0.37-0.60). When the analysis was done separately comparing ER-positive and ER-negative breast cancer, odds were lower in the ER-positive cases (OR=0.40, 95% CI 0.30-0.54), but the p-value for heterogeneity between ER-positive and ER-negative was not statistically significant [19]. On the other hand, data from the Australian Longitudinal Study of Women's Health (ALSWH) which prospectively evaluated the association of hot flashes with breast cancer incidence, observed no association (HR=1.09, 95% CI 0.84-1.33). Analysis could not be done separately for the different subtypes due to insufficient information, nor was data collected for family history of breast cancer and endogenous hormone levels [20].

Further research is needed to investigate the role of hot flashes in breast cancer incidence, and to our knowledge, no study has investigated the effect of hot flashes on the association of hormone therapy with breast cancer risk. Given the association of hot flashes with lower levels of estradiol, we expect that women who experience hot flashes will have a decreased risk for developing breast cancer compared to women who do not report this symptom. In the WHI clinical trials, the effect of hormone therapy on breast cancer risk differed in the estrogen plus progestin (E+P) and estrogen alone (E-alone) trials [21, 22]. Additional results from the WHI clinical trials indicated an interaction between baseline estradiol level and E+P therapy on breast cancer risk. Women with low levels of estradiol were found to have the highest increase in breast cancer risk during E+P treatment [23]. Therefore, it is reasonable to hypothesize that the effect of hormone therapy on breast cancer risk will vary by baseline hot flash status and will differ in the E-alone and E+P trials. Using data from the WHI Hormone Therapy Clinical Trials, we propose to investigate the association of baseline hot flash status with the subsequent development of

invasive breast cancer (Aim 1) and to assess whether baseline hot flash status modifies the effect of E+P and E-alone therapy on invasive breast cancer risk (Aim 2).

Chapter 2: Review of the Literature

I. Breast Cancer

Epidemiology

Breast cancer is the most common type of cancer among women, with 1 in 9 women expected to be diagnosed in their lifetime [10]. While breast cancer occurs mostly in women, men are also susceptible, albeit at a low risk. Between the period of 2009 to 2013, the average annual incidence rate for female breast cancer was estimated to be 125 per 100,000 women in the United States, and the average mortality rate was 21.5 per 100,000 women [24]. Beginning in the early 1980's, breast cancer incidence rates saw a dramatic increase largely due to a rapid increase in early detection due to mammography screening. Other factors such as postmenopausal hormone therapy (PHT) use and increased obesity rates also added to the rapid increase in breast cancer incidence. The years 2002-2003 witnessed a reduction in breast cancer rates, with one explanation being the abrupt succession of PHT following the 2002 release of findings from the WHI Hormone Therapy Clinical Trial indicating an increased risk for heart disease and breast cancer with E+P therapy. Between 2003 and 2013, incidence rates for breast cancer have been rather stable [24]. Although overall incidence rates stabilized, rates among black women during this period continued to increase while rates among white women did not [25]. As with other types of cancer, the risk of breast cancer increases with age, with the median age at diagnosis estimated at 61 years between 2008 and 2012 in the United States [24].

Subtypes

In the early 1900's, breast cancer incidence by age demonstrated two distinct peaks with one peak among women aged 50 years and the other around 60 years. This led researchers to believe

that breast cancer could in fact be divided into different subgroups [25]. More recently, invasive breast cancers have been divided into several subgroups based on different gene expressions of the breast cancer cells. Identification of these subgroups have become increasingly helpful in determining prognosis and treatment, for example the likelihood of relapse and whether the use of hormone therapy is beneficial to the patient or not [26]. Hormone receptor status as well as the human epidermal growth factor receptor 2 status (HER2) are two important factors in determining subtypes. Breast cancer can be classified as either being hormone receptor-positive or hormone receptor-negative, with emerging evidence that risk factors are associated differently with each. Hormone receptor-positive cancers require the presence of hormones in order for the cancer to proliferate. More specifically, the estrogen-receptors and progesterone-receptors play a critical role in defining the type of breast cancer. Breast cancers can be estrogen receptor-positive (ER-positive) or estrogen receptor-negative (ER-negative) depending on the number of receptors present in the breast tumor tissue. Likewise, breast cancer can be progesterone receptor-positive or progesterone receptor-negative [27]. HER2 proteins are also found on the surface of some breast cancer cells and are involved in cell growth. Similar to hormone receptors, breast cancer cells with little to no HER2 proteins are classified as HER2-negative, while cells containing many HER2 proteins are classified as HER2-positive [28].

Based on tumor expression of hormone receptors and HER2 proteins, four major molecular subtypes of breast cancer have emerged. Luminal subtypes include those breast cancers that are hormone receptor-positive and are further divided into luminal A and luminal B. The luminal A breast cancers are classified as being ER-positive and/or PR-positive and HER2-negative, while the luminal B group breast cancers are ER-positive and/or PR-positive and HER2-positive. A

third subtype, called HER2-enriched, includes breast cancers that are both ER-negative and PR-negative, but HER2-positive. The last subtype, known as basal-like, consist of breast cancers that are ER-negative, PR-negative and HER2-negative. HER2-enriched and basal-like breast cancers are less common and also associated with lower overall survival rates [29].

Luminal subtypes A and B are the most common types of breast cancer [26] and as it might be inferred, breast cancers that are hormone receptor-positive will respond better to hormone therapy implemented after surgery or chemotherapy. With hormone therapy, hormone receptors are either blocked or hormone production is halted. One type of therapy using tamoxifen, a selective estrogen receptor modulator (SERM) blocks estrogen receptors prohibiting estrogen to bind to the receptors and therefore halting the growth of the tumor. Another type of hormone therapy using aromatase inhibitors stops the productions of estrogen in a woman's body. This is done by stopping the body from converting androgens into estrogen. Aromatase inhibitors are only used in postmenopausal women [10].

Etiology

Family history and reproductive factors

One of the most recognized risk factors for breast cancer is family history of breast cancer, specifically in a first-degree relative. Disease risk increases with having two first degree relatives with breast cancer, and even more so, if incidence occurred before menopause. Another set of risk factors included reproductive history. Early menarche, late menopause, nulliparity, and first full term pregnancy after the age of 30 all increase the risk of breast cancer. On the contrary, artificial induction of menopause, through hysterectomy for example, and first full term

pregnancy before the age of 20 have a protective effect against breast cancer. Parity also has a protective effect, with each successive birth having incremental protection, no matter at what age [30].

Several studies have looked into the etiology of breast cancer and created models linking reproductive risk factors and breast cancer incidence. One of the first models proposed by Pike et al., took into account breast cancer tissue aging with respect to reproductive risk factors and breast cancer incidence. Pike et al. explained that breast tissue does not age at a consistent rate with calendar time, but instead ages constantly from menarche to age of first full term birth, and then decreases from there until menopause. Therefore, in order to calculate breast tissue age and use Pike's model, one must know age at menarche, age at first full term birth and age at menopause. The model found that early first full term pregnancy, defined as pregnant before the age of 19, had a protective effect against breast cancer. While women with late first full term pregnancy, defined as over 35 years, had an increased risk of breast cancer when compared to nulliparous women. This model was the first to take into account breast tissue age and also explored how endogenous hormone levels affect the breast tissue age [31].

A later model, modified the Pike model to further account for other reproductive risk factors that were not accounted for. This model, by Rosner and Colditz, made it possible to consider factors such as age at first term birth greater than 40 years, premenopause, and also birth spacing. Compared to nulliparous women, the model demonstrated that the risk of breast cancer for parous women was dependent upon age at first birth and age at subsequent births. The general trend showed that compared to nulliparous women, younger parous women had a slightly higher

risk of breast cancer. The risk of breast cancer among older parous women, on the other hand, was dependent upon their age at first birth. Those with an early age at first birth were at a decreased risk of breast cancer, while those with an older age at first birth were at an increased risk of breast cancer compared to nulliparous women [32].

Hormonal factors

Endogenous sex hormones and breast cancer

Several studies have associated higher levels of endogenous sex hormones with increased breast cancer risk [33-36]. The WHI[37] observational study data was used to examine the relationship between endogenous hormone levels and both ER-positive and ER-negative breast cancers. Endogenous levels of testosterone and estradiol were measured at baseline and breast cancer type was measured through pathology reports and medical records. It was found that among postmenopausal women, higher levels of endogenous testosterone were associated with a lower risk of ER-negative breast cancer. Using women in the lowest quartile for testosterone level as a reference group, there was a 56% lower risk (HR=0.44, 95% CI: 0.23-0.85), 45% lower risk (HR=0.55, 95% CI: 0.30-1.01), and 49% lower risk (HR=0.51, 95% CI: 0.28-0.94) of ER-negative breast cancer for women in the second, third and fourth quartiles respectively. Endogenous levels of estradiol were not significantly associated with ER-negative breast cancer. For ER-positive breast cancer, women with higher levels of endogenous estradiol levels were at higher risk for cancer. Using women in the lowest quartile for estradiol level as a reference group, there was a 2.14-fold increased risk (HR=2.14, 95% CI: 1.11-3.71), 1.90-fold increased risk (HR=1.90, 95% CI: 1.08-3.36), and 1.86-fold increased risk (HR=1.86, 95% CI: 1.00-3.45)

of ER-positive breast cancer in the second, third and fourth quartiles respectively. This trend was not seen for higher levels of testosterone after adjusting for estradiol [33].

Postmenopausal hormone therapy and breast cancer risk

Evidence from a few clinical trials and observational studies suggest increased breast cancer with hormone therapy, particularly the E+P formulation. The WHI Hormone Therapy Clinical Trials, looked at the effects of E+P therapy on major health outcomes in healthy postmenopausal women. This trial was terminated early when it was found that E+P increased the risk of breast cancer for healthy postmenopausal women. After a mean of 5.2 follow-up years, a 26% increase in invasive breast cancer cases was seen in the E+P group (HR=1.26, 95% CI: 1.00-1.59). Almost reaching nominal statistical significance, this was enough to terminate the E+P portion of the WHI clinical trials [21].

Another component of the WHI Hormone Therapy Clinical Trials assessed the effects of conjugated equine estrogen therapy on postmenopausal women with prior hysterectomy. After a mean of 6.8 follow-up years, the E-alone trial was terminated early due to a lack of perceived benefit from hormone use, with cardiovascular disease being the main outcome of interest. Invasive breast cancer was used as the main safety outcome for this trial, and a 23% decrease in invasive breast cancer cases was seen among the E-alone group (HR=0.77, 95% CI: 0.59-1.01). Unlike the E+P trial, results from the E-alone trial suggested no elevated breast cancer risk for this type of hormone therapy among postmenopausal women with prior hysterectomy [22].

The Million Women Study, an observational study, recruited 1,084,110 women aged 50-64 years between 1996 and 2001 to examine the association between hormone therapy and incidence of breast cancer. With an average follow-up of 2.6 years, there was a 66% increased risk of breast cancer among ever users compared to never users of hormone therapy (RR=1.66, 95% CI: 1.58-1.75) with 22% increased risk in death due to breast cancer among ever users (RR=1.22, 95% CI: 1.00-1.48). It was also seen that for current users, E-alone therapy and E+P therapy both had an increased risk for developing breast cancer (RR=1.3, 95% CI: 1.22-1.38 and RR=2.00, 95% CI: 1.91-2.09, respectively). Among the current users, the relative risk of breast cancer also increased as the duration of use increased for both E-alone and E+P therapies [38].

The Heart and Estrogen/progestin Replacement Study (HERS) was a randomized, double-blinded, placebo-controlled trial that looked at the effects of E+P therapy on the risk of nonfatal myocardial infarction (MI) and CHD death among postmenopausal women with preexisting coronary disease. One of the secondary noncardiovascular outcomes measured at each follow-up visit was breast cancer. With an average of 4.1 follow-up years, there was a 30% increase in breast cancer among women taking E+P therapy compared to women in the placebo group (HR=1.30, 95% CI: 0.77-2.19), but was not statistically significant. Unlike other observational studies that have looked at the association between PHT and breast cancer for an extended period of time, the duration of hormone therapy in the HERS trial was less than five years [39].

Beginning in 1976, the Nurse's Health Study followed 121,700 registered nurses with the intent to look at the association of oral contraceptives with different types of cancers. Baseline characteristics were gathered using a mailed questionnaire and included oral contraception use,

breast cancer risk factors, and PHT use. Follow-up questionnaires were mailed out every two years that included detailed information on breast cancer risk factors, duration of hormone use and type of hormones used. The National Death Index was also used to obtain the status of those women who did not respond. Data provided from 1978 to 1992 demonstrated an increased risk for invasive cancer in both E-alone therapy (RR=1.32, 95% CI: 1.14-1.54) and E+P therapy (RR=1.40, 95% CI: 1.15-1.74). The relative risks between the two hormone therapy groups did not significantly differ, concluding that the addition of progestin did not reduce the risk of invasive breast cancer. An important point to consider is that the addition of progestin was not significant until 1986 when 18% of the women taking PHT used progestin and rose to 30% in 1990 [40]. Further analysis done on an eligible cohort of postmenopausal women within the Nurse's Health Study between 1998 to 2000, examined the association of PHT to estrogen receptor (ER) and progesterone receptor (PR) status in invasive breast cancer. Among women who took PHT for 10 or more years, hormone therapy users were more likely to develop ER-positive/PR-positive breast cancer (RR=1.80, 95% CI: 1.52-2.12) but not more likely to develop ER-negative/PR-negative breast cancer (RR=1.00, 95% CI: 0.72-1.39) [41].

Endogenous sex hormone, postmenopausal hormone therapy and breast cancer risk

Ancillary analysis from the WHI E+P trial looked into endogenous hormone levels to determine if pretreatment levels of endogenous sex hormones modified the effects of the E+P therapy on breast cancer incidence. It was found that there was an increased risk of breast cancer with E+P therapy for women in the lowest quartiles of endogenous estrogen, more specifically total estradiol, bioavailable estradiol, estrone and estrone sulfate [23]. The observed effect modification between endogenous hormone and PHT on breast cancer risk could be of use in

determining which women are most suitable for E+P therapy whose risks would not outweigh the benefits from therapy.

Other risk factors

Other risk factors for breast cancer include age, race, breast density, bone mineral density, inherited mutations in BRCA1 and BRCA2, obesity, tobacco use, alcohol consumption, physical activity and diet [42].

Differences in etiology by breast cancer subtype

Beginning in 2010, population based cancer registries in the U.S. were required to document both hormone receptor status and HER2 status for all breast cancer cases [25]. Although this information was being recorded prior to 2010 in some registries, the implementation of this policy nationally allowed for even further research of risk factors pertaining to different breast cancer subtypes. Data from the Multiethnic Cohort Study, a prospective study examining incidence of cancer among five ethnic groups, was used to further examine differences in risk factors pertaining to hormone receptor status. Risk factors were assessed for the following combinations of hormone receptor status: ER-positive/PR-positive, ER-positive/PR-negative, and ER-negative/PR-negative. In regards to race/ethnicity, ER-negative/PR-negative cancers were most common among African American and Latina women. And while two or more alcoholic beverages per day increased risk for all ER/PR combinations of breast cancer, ER-negative/PR-negative cancers had the highest association when compared to women who did not drink (HR=1.71, 95% CI: 1.19-2.46). ER-positive/PR-positive breast cancers were strongly associated with many of the reproductive risk factors previously mentioned including late

menopause having an increased risk as well as late age at first full term birth also having an increased risk among parous women. Among postmenopausal women, the use of PHT had a significant increased risk of breast cancer with E+P therapy having the highest association (HR=2.28, 95% CI: 1.97-2.64), while the use of E-alone therapy had a lower, yet significant association as well (HR=1.63, 95% CI: 1.15-2.33). Factors such as late menarche and parity were shown to have a protective effect against ER-positive/PR-positive breast cancers. Body mass index was also found to be significantly associated with ER-positive/PR-positive breast cancers, with risk increasing with increasing BMI when compared to a normal BMI measurement. BMI was not found to be significantly associated with the other ER/PR combinations. [27].

With the availability of HER2 status for national cancer registries, risk factors and demographic information more specific to breast cancer subtypes could be calculated. According to the Annual Report to the Nation on the Status of Cancer, 1975-2011, Luminal A subtype breast cancers were the most common subtype with about 76% of all breast cancer cases being Luminal A, at a rate of 86.5 per 100,000 women. Among all race/ethnic groups, Luminal A breast cancers also demonstrated the highest rates within each race/ethnic group. Basal-like breast cancers represented 13% of all breast cancer cases, at a rate of 15.5 per 100,000 women. Among all race/ethnic groups, the highest rates of Basal-like breast cancers were seen among Non-Hispanic black women [25].

II. Menopause transition and vasomotor symptoms

Women begin their lives with a full set of follicles containing oocytes that are slowly depleted throughout their lifetime through ovulation. As a woman reaches menopause, the lower number

of follicles in her body results in lower amounts of inhibin b being produced by the follicles. A negative feedback system between inhibin b and follicle stimulating hormone (FSH), consequently results in increased levels of FSH [43]. Increased levels of FSH result in further loss of follicles and abnormal reproductive cycles. These non-regular cycles result in a fluctuation of estrogen levels and estrogen levels decrease with the depletion of all follicles. High levels of FSH and low levels of estradiol mark a woman's transition into the postmenopausal period [44].

Menopause is marked by the discontinued regular cycle of menstrual bleeding often accompanied by a variety of symptoms. These symptoms, along with menstrual regularity and a woman's last menstrual period can help define the stages of menopause defined as: premenopausal, early perimenopausal, late perimenopausal and postmenopausal. Premenopause can be defined as bleeding in the past three months with normal predictability of menses in the past year. Women enter early perimenopause when predictability in the past year decreases and late perimenopause when no menses have occurred in the past 3-11 months. Postmenopause is defined as no menses in the past 12 or more months [45].

Symptoms that have been documented as accompanying menopause include vasomotor symptoms, joint aches, depression, decreased libido and poor sleep. One of the more common symptoms are vasomotor symptoms such as night sweats and hot flashes, with an estimated 65-76% of women experiencing hot flashes during their menopausal transition [1]. The Massachusetts Women's Health Study followed a cohort of originally premenopausal women to look at factors that determine the length of perimenopause and the frequency of vasomotor

symptoms among other things. The study found that for those women who experience hot flashes and night sweats, the majority experience these symptoms between the perimenopausal and postmenopausal stage for an average of about 4 years [46]. The Penn Ovarian Aging Study further explored the relationship between common menopausal symptoms and the stages of menopause while also looking at the levels of various reproductive hormones. Results found menopausal stage to be associated with hot flashes, demonstrating an increased reporting of hot flashes through the menopausal stages, peaking at postmenopause. Hot flashes were also found to be associated with fluctuating levels of estradiol (E2), decreased levels of inhibin b and increased levels of FSH [2].

Biologically, these changing levels of reproductive hormones mark the stages of menopause and as estrogen levels decrease, some women begin to experience hot flashes. The surgical induction of menopause through hysterectomy for example, also cause levels of estrogen to decrease and for some women to experience hot flashes. Administration of estrogen in the form of hormone therapy has been effective at reducing hot flashes [4] further demonstrating the role of hormones in the causation of vasomotor symptoms. Estrogen levels are not enough to explain these symptoms though because decreased levels of estrogen occur in all women going through menopause and not only in those women experiencing hot flashes [3].

One major difference between symptomatic and asymptomatic women is found when comparing their thermal neutral zone, defined by the upper threshold of sweating and the lower threshold for shivering [3]. In symptomatic women, this thermal neutral zone is narrowed causing the upper threshold of sweating to be lowered and the lower threshold of shivering to be raised. In addition,

core body temperature was shown to slightly increase before hot flashes [5]. The combination of these two effects cause symptomatic women to reach the upper sweating threshold with only small increases in their core body temperature. Once a woman's core body temperature reaches this sweating threshold, the body sweats in attempt to lower the core body temperature back within the thermal neutral zone. Estrogen administration to symptomatic women found that the upper threshold for sweating actually increased, significantly reducing the number of hot flash episodes [6]. While the exact mechanism is not fully understood, estrogen remains a critical factor to hot flash occurrence.

Aside from reproductive hormone levels, abdominal adiposity has also been shown to be significantly associated with hot flash symptoms [7]. More specifically, with every one standard deviation (SD) increase in subcutaneous adiposity, which provides insulation to the body, there was a 30% increased odds in hot flashes (OR=1.30, 95% CI: 1.07-1.58). This coincides with the thermoregulatory theory described above. As a woman's body reaches the sweating threshold and attempts to dissipate heat, factors such as subcutaneous abdominal adiposity will further cause a woman to sweat. Other factors that have been found to be associated with hot flashes are age, race, education status and smoking status [7].

PHT was the most common treatment for hot flashes among menopausal women. When placed on estrogen therapy, women reported a decrease in hot flashes. However, after results from the WHI clinical trials suggested an increased risk for breast cancer, CHD, stroke and thromboembolism for women using E+P therapy [21] and an increased risk for CHD for women using E-alone therapy [22], hormone therapy to treat hot flashes is now being given at lower

doses [3]. Results from the WHI clinical trials prompted the use of non-hormonal treatment for women who suffer from hot flashes as well. One such treatment, clonidine, has been shown to lower norepinephrine release and widen the thermal neutral zone [47]. Behavioral treatment has also been explored as an alternative to hormone therapy. These treatments have included progressive muscle relaxation and paced respiration exercises [48]. Other suggestions to treat hot flashes without the use of hormone therapy are drinking cold beverages, dressing in layers, using a fan or air conditioner, smoking cessation and weight loss.

III. Vasomotor symptoms and breast cancer

Vasomotor symptoms and women's health outcomes

Although hot flashes are most commonly thought to affect the quality of life, emerging research has also linked hot flash symptoms to coronary heart disease (CHD) and most recently, breast cancer.

The Study of Women's Health Across the Nation Heart Study examined the associations between hot flashes and various cardiovascular diseases, reasoning that risk factors for cardiovascular diseases and hot flashes coincide, such as obesity, age and smoking. The study found hot flashes to be significantly associated with flow-mediated dilation (FMD) and also with aortic calcification [8]. A secondary data analysis of the WHI clinical trials found that women who experienced vasomotor symptoms at baseline had an increased risk of HT-associated CHD, with older age ($p=0.04$) and longer time since menopause ($p=0.06$) [12]. These trends were not significant in women who did not experience vasomotor symptoms at baseline. The Heart Estrogen/Progestin Replacement Study (HERS) was another randomized, placebo-controlled

trial that looked at the effects of E+P therapy and found a significant association of E+P with an increased risk in CHD. Among women who experienced hot flashes at baseline, those assigned to the E+P group were 9-times more likely to experience CHD than those assigned to the placebo group (HR=9.01, 95% CI: 1.15-70.35) [9]. Among women who did not experience hot flashes at baseline, there was no significant association between hormone therapy and CHD (HR=1.32, 95% CI: 0.86-2.03).

Vasomotor symptoms and breast cancer prognosis

For many perimenopausal and postmenopausal breast cancer patients, chemotherapy results in severe side effects, one of the most common being hot flashes. Hot flashes are therefore more common among women with breast cancer, with the symptoms being more severe than those experienced by healthy women [10]. With results from the WHI clinical trials suggesting E+P therapy to increase the risk for breast cancer [21], it is crucial that alternative forms of therapy to relieve hot flash symptoms among breast cancer patients be explored.

In addition to chemotherapy, adjuvant endocrine therapy such as tamoxifen is used to treat and prevent breast cancer, more specifically hormone receptor positive breast cancer [13]. Tamoxifen acts as an antiestrogen, preventing the normal role of estrogen in the body by blocking estrogen receptors [10]. One of the most common side effects from tamoxifen use are hot flashes. Tamoxifen is metabolized into its two more active metabolites, endoxifen and 4-hydroxytamoxifen, both more potent antiestrogens than tamoxifen. This is done by the cytochrome P450 2D6 (CYP2D6) enzyme [13]. Polymorphisms exist for the CYP2D6 enzyme with some variations having a poorer metabolizing rate of tamoxifen. Women with these genetic

variations to their CYP2D6 and also women who take CYP2D6 inhibitors have been shown to have lower levels of endoxifen [14]. In a study done by the North Central Center Treatment Group (NCCTG), the most common CYP2D6 polymorphism was found to be associated with low incidence of hot flashes after the initiation of tamoxifen therapy ($p=0.064$), and also demonstrated worse relapse-free time ($p=0.023$) and worse disease-free survival ($p=0.012$) when compared to women with normal functioning CYP2D6 [15]. This suggests that for tamoxifen treated women, hot flashes could have the potential to predict CYP2D6 activity and drug efficacy, with the presence of hot flashes indicating normal CYP2D6 activity and less chance of breast cancer recurrence.

The Women's Healthy Eating and Living (WHEL) study further investigated the a priori hypothesis of a hot flash's ability to predict the efficacy of tamoxifen in preventing the recurrence of breast cancer. For this hypothesis, 864 women with early stage breast cancer were randomized to the control arm with hot flash severity being reported at baseline as well as tamoxifen use. It was reported that among women being treated with tamoxifen, those who experienced hot flashes at baseline were less likely to have a recurrence of breast cancer (HR=0.50, 95% CI: 0.36-0.69) [16]. This finding was further supported in the analysis from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. This trial not only looked at tamoxifen as an adjuvant therapy, but also arimidex, and their effect on hormone receptor-positive breast cancer either alone or in combination. Analysis of the trial concluded that women with new onset of vasomotor symptoms within three months of adjuvant therapy initiation had lower recurrence of breast cancer compared to those women who did not experience vasomotor symptoms (HR=0.84, 95% CI: 0.71-1.00) [11].

Vasomotor symptoms and breast cancer incidence

The presence of hot flashes was observed to modify the effects that dietary interventions had on the risk of invasive breast cancer. In the WHI Dietary Modification trial, the effects of a low-fat diet among women who self-reported hot flashes at baseline were assessed. It was found that the low-fat dietary intervention had a greater decrease in risk among women who experienced hot flashes (HR= 0.65, 95% CI: 0.42-1.01) than among women who did not experience hot flashes (HR= 0.93, 95% CI: 0.84-1.03) [17].

To our knowledge, only three studies assessed the association of vasomotor symptoms with breast cancer risk. Data from a case-control study evaluating the relationship between menopausal hormone therapy and risk of varying types of invasive breast cancer was used to assess the relationship between vasomotor symptoms and the risk of breast cancer. Vasomotor symptoms and severity were assessed using a questionnaire that asked participants to recall vasomotor episodes before a reference date. Reference dates for cases were their date of breast cancer diagnosis and reference dates for controls were given according to the distribution of reference dates of the cases. The three types of invasive breast cancer assessed were invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and invasive ductal-lobular carcinoma (IDLC). Vasomotor intensity was evaluated based on perspiration and awakening at night. Results from the analysis showed a decreasing risk of breast cancer with increasing intensity of hot flashes for all three types of invasive breast cancer. Women who ever experienced a hot flash had decreased risks of IDC (OR=0.5, 95% CI: 0.3-0.7), ILC (OR=0.5, 95% CI: 0.3-0.7), and IDLC (OR=0.7, 95% CI: 0.4-1.2) [18].

The Two Sister Study, a controlled case-control study, looked at women diagnosed with breast cancer before the age of 50 and their sisters who had not been diagnosed with breast cancer. The study's aim was to evaluate the association between menopausal symptoms and risk of early-onset breast cancer. Results showed that women who ever experienced hot flashes had a decreased odds of early-onset breast cancer compared to women who never experienced hot flashes (OR=0.47, 95% CI: 0.37-0.60). When the analysis was done separately comparing ER-positive and ER-negative breast cancer, odds were lower in the ER-positive cases (OR=0.40, 95% CI: 0.30-0.54), but the p-value for heterogeneity between ER-positive and ER-negative was not statistically significant [19].

Instead of a case-control study, data from the Australian Longitudinal Study of Women's Health (ALSWH) was used to prospectively look at the association of hot flashes and breast cancer incidence. The ALSWH followed a cohort of 11,297 women from 1998-2010 and collected data on these women every three years, with vasomotor symptoms being one of the variables reported at each survey. Breast cancer incidence was obtained from state cancer registries. Results from the analysis showed no association between hot flashes and incident breast cancer (HR=1.09, 95% CI: 0.84-1.33). Analysis could not be done separately for the different subtypes because data was not available, nor was data collected for family history of breast cancer and endogenous hormone levels [20].

IV. Limitations in the existing literature on vasomotor symptoms and breast cancer

Further research is needed to investigate the role of hot flashes in breast cancer incidence, and to our knowledge, no study has investigated the effect of hot flashes on the association of hormone therapy with breast cancer risk. Given the association of hot flashes with lower levels of estradiol, we expect that women who experience hot flashes will have a decreased risk for developing breast cancer compared to women who do not report this symptom. In the WHI clinical trials, the effect of hormone therapy on breast cancer risk differed in the E+P and E-alone trials [21, 22]. Additional results from the WHI clinical trials indicated an interaction between baseline estradiol level and E+P therapy on breast cancer risk. Women with low levels of estradiol were found to have the highest increase in breast cancer risk during E+P treatment [23]. Therefore, it is reasonable to hypothesize that the effect of hormone therapy on breast cancer risk will vary by baseline hot flash status and will differ in the E+P and E-alone trials.

V. Purpose statement and research questions

The purpose of the proposed analysis is to fill in the gaps of current literature regarding the role of hot flashes in the etiology of invasive breast cancer. Using data from the WHI Hormone Therapy Clinical Trials, we conducted analysis with the specific aims to:

1) Investigate the association of baseline hot flash status with the subsequent development of invasive breast cancer.
2) To assess whether baseline hot flash status modifies the effect of E+P and E-alone therapy on invasive breast cancer risk.

Regarding the first aim, the null hypothesis states that breast cancer incidence will not differ among women not taking hormone therapy with regards to hot flash status. The second aim has two null hypotheses, the first being that the risk of invasive breast cancer associated with E+P treatment will not differ by hot flash status. The second is that the risk of invasive breast cancer associated with E-alone treatment will not differ by hot flash status.

VI. Significance statement

Results of these analyses can have significant clinical importance. In looking at the association of baseline hot flash status with the subsequent development of invasive breast cancer, there is a potential to advise menopausal women about their risk of invasive breast cancer based on menopausal symptoms. More specifically, the severity of a woman's hot flashes has the possibility of advising on subsequent breast cancer risk.

Furthermore, results could help to advise whether or not a woman should be put on PHT. Knowing the severity of a woman's hot flashes can further help to determine if a woman is a good candidate for therapy or not based on her risk of invasive breast cancer.

Chapter 3: Manuscript

Hot Flashes, Hormone Therapy and Breast Cancer Risk in the Women's Health Initiative Clinical Trials

Abstract

Background: The association of hot flashes with breast cancer risk is not clearly elucidated, although evidence suggests that their new onset with anti-estrogen therapy predicts breast cancer prognosis and that they modify the effects of dietary interventions on breast cancer risk and prognosis.

Objective: We investigated the association of baseline hot flash status with the subsequent development of invasive breast cancer (Aim 1) and assessed whether baseline hot flash status modifies the effect of estrogen plus progestin (E+P) and estrogen alone (E-alone) therapy on invasive breast cancer risk (Aim 2).

Methods: We performed a prospective analysis within the Women's Health Initiative Hormone Therapy Clinical Trials. The E+P trial included 16,608 women aged 50-79 years with an intact uterus and no history of breast cancer, and the E-alone trial 10,739 women aged 50-79 years with prior hysterectomy and no history of breast. During an average follow-up of 14.4 years extending post intervention, 1,460 cases of breast cancer were diagnosed (960 in E+P and 500 in E-alone).

Results: For Aim 1, combining the trials' placebo groups, hot flash status was not associated with breast cancer risk. In adjusted models, the hazard ratio (HR) was 1.05 (95% CI 0.85-1.29) for women with mild hot flashes and 1.16 (95% CI 0.89-1.53) for women with moderate/severe

symptoms, compared to women without symptoms. For Aim 2, breast cancer risk associated with E+P treatment tended to increase with higher severity of hot flashes (none: HR= 1.16, 95% CI 0.98-1.37; mild: HR= 1.19, 95% CI 0.88-1.63; moderate/severe: HR= 1.46, 95% CI 0.96-2.24); however, interactions of E+P with hot flash status categories were not statistically significant. Breast cancer risk associated with E-alone treatment did not vary significantly by hot flash status (none: HR=0.89, 95% CI 0.70-1.12; mild: HR=0.59, 95% CI 0.38-0.92; moderate/severe: HR=0.72, 95% CI 0.43-1.23; p-values for interaction terms >0.05).

Conclusion: In postmenopausal women, hot flashes did not predict breast cancer risk, and they did not modify the effect of E+P or E-alone therapy on breast cancer risk. Further studies are needed to evaluate the possibility that women with moderate/severe symptoms have a higher breast cancer risk with E+P treatment.

Background

Vasomotor symptoms, which include hot flashes and night sweats are the most common symptoms accompanying menopause, with an estimated 65-76% of women experiencing hot flashes during this transition [1]. The pathophysiology of vasomotor symptoms remains not fully understood. Hot flashes were found to be associated with fluctuating levels of estradiol (E2), decreased levels of inhibin B and increased levels of follicle stimulating hormone (FSH) [2]; however, these endogenous hormone levels are not enough to explain vasomotor symptoms since hormone fluctuation occurs in all women transitioning through menopause and not only in symptomatic women [3]. The surgical induction of menopause through hysterectomy for example, also causes levels of estrogen to decrease and results in hot flashes in some women. The administration of estrogen in the form of hormone therapy has long been used and found to be effective at reducing hot flashes [4], further demonstrating the role of hormones in the causation of vasomotor symptoms.

A narrowed thermal neutral zone, defined by a lowered threshold for sweating and an increased threshold for shivering, has been recently demonstrated among symptomatic women [3]. In addition, core body temperature was shown to slightly increase before hot flashes [5]. The combination of these two effects cause symptomatic women to reach the upper sweating threshold with only small increases in their core body temperature. Estrogen administration in symptomatic women was found to increase the upper threshold for sweating, significantly reducing the number of hot flash episodes [6]. While the exact mechanism is not fully understood, estrogen remains a critical factor in the occurrence of hot flashes. Other factors

associated with hot flashes include increased abdominal adiposity, increased body mass index (BMI), age, race, education status and smoking status [7].

Although hot flashes are most commonly thought to affect the quality of life, emerging research has also linked hot flash symptoms to coronary heart disease (CHD) [8, 9] and more recently, hot flashes were found to play a role in breast cancer incidence and prognosis [10, 11].

The Study of Women's Health Across the Nation Heart Study examined the associations between hot flashes and various cardiovascular diseases, reasoning that risk factors for cardiovascular diseases and hot flashes coincide, such as obesity, age and smoking. The study found hot flashes to be significantly associated with flow-mediated dilation (FMD) and also with aortic calcification [8]. The Women's Health Initiative clinical trials found that women who experienced vasomotor symptoms at baseline had an increased risk of hormone therapy associated CHD with older age ($p=0.04$) and longer time since menopause ($p=0.06$). These trends were not significant in women who did not experience vasomotor symptoms at baseline [12]. Another study, the Heart Estrogen/Progestin Replacement Study (HERS) found that among women who experienced hot flashes at baseline, those assigned to the estrogen plus progestin (E+P) group were 9-times more likely to experience CHD than those assigned to the placebo group (Hazard Ratio [HR]=9.01, 95% CI 1.15-70.35). Among women who did not experience hot flashes at baseline, there was no significant association between hormone therapy and CHD (HR=1.32, 95% CI 0.86-2.03) [9].

For many perimenopausal and postmenopausal breast cancer patients, chemotherapy as well as adjuvant endocrine therapy such as tamoxifen, result in severe side effects, one of the most common being hot flashes. Hot flashes are therefore more common among women with breast cancer than among healthy women [10]. Tamoxifen acts as an antiestrogen, preventing the normal role of estrogen in the body by blocking estrogen receptors [10]. Cytochrome P450 2D6 (CYP2D6) metabolizes tamoxifen into its two more active metabolites, endoxifen and 4-hydroxytamoxifen [13]. Polymorphisms exist for the CYP2D6 enzyme with some variations having a poorer metabolizing rate of tamoxifen. Women with these genetic variations to their CYP2D6 and also women who take CYP2D6 inhibitors have been shown to have lower levels of endoxifen [14]. The North Central Center Treatment Group (NCCTG) found the most common CYP2D6 polymorphism to be associated with low incidence of hot flashes after the initiation of tamoxifen therapy ($p=0.064$), and also demonstrated worse relapse-free time ($p=0.023$) and worse disease-free survival ($p=0.012$) when compared to the normal functioning CYP2D6 [15]. This suggests a role for hot flashes in predicting CYP2D6 activity and drug efficacy, with the presence of hot flashes indicating normal CYP2D6 activity and less chance of breast cancer recurrence.

The Women's Healthy Eating and Living (WHEL) study further investigated hot flashes as a predictive marker for the efficacy of tamoxifen. Among women being treated with tamoxifen, those who experienced hot flashes at baseline were less likely to have a recurrence of breast cancer (HR=0.50, 95% CI 0.36-0.69) [16]. The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial further supported these findings and not only looked at tamoxifen as an adjuvant therapy, but also arimidex, and their effect on hormone receptor-positive breast cancer either

alone or in combination. Analysis of the trial concluded that women with new onset of vasomotor symptoms within three months of adjuvant therapy initiation had lower recurrence of breast cancer compared to those women who did not experience vasomotor symptoms (HR=0.84, 95% CI 0.71-1.00) [11].

Hot flashes were also observed to modify the effects that dietary interventions had on the risk of invasive breast cancer. In the WHI Dietary Modification Trial, the low-fat dietary intervention resulted in a greater decrease in the risk of breast cancer among women who experienced hot flashes (HR= 0.65, 95% CI 0.42-1.01) than among women who did not experience hot flashes (HR= 0.93, 95% CI 0.84-1.03) [17].

To our knowledge, only three studies have assessed the association of vasomotor symptoms with breast cancer risk. Data from a case-control study evaluating the relationship between menopausal hormone therapy and risk of varying types of invasive breast cancer was used to assess the relationship between vasomotor symptoms and the risk of breast cancer. Results from the analysis showed a decreasing risk of breast cancer with increasing intensity of hot flashes for all three types of invasive breast cancer, invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and invasive ductal-lobular carcinoma (IDLC). Women who ever experienced a hot flash had decreased risks of IDC (OR=0.5, 95% CI 0.3-0.7), ILC (OR=0.5, 95% CI 0.3-0.7), and IDLC (OR=0.7, 95% CI 0.4-1.2) [18]. The Two Sister Study looked at women diagnosed with breast cancer before the age of 50 and their sisters who had not been diagnosed with breast cancer. Results showed that women who ever experienced hot flashes had a decreased odds of young-onset breast cancer compared to women who never experienced hot

flashes (OR=0.47, 95% CI 0.37-0.60). When the analysis was done separately comparing ER-positive and ER-negative breast cancer, odds were lower in the ER-positive cases (OR=0.40, 95% CI 0.30-0.54), but the p-value for heterogeneity between ER-positive and ER-negative was not statistically significant [19]. On the other hand, data from the Australian Longitudinal Study of Women's Health (ALSWH) which prospectively evaluated the association of hot flashes with breast cancer incidence, observed no association (HR=1.09, 95% CI 0.84-1.33). Analysis could not be done separately for the different subtypes due to insufficient information, nor was data collected for family history of breast cancer and endogenous hormone levels [20].

Further research is needed to investigate the role of hot flashes in breast cancer incidence, and to our knowledge, no study has investigated the effect of hot flashes on the association of hormone therapy with breast cancer risk. Given the association of hot flashes with lower levels of estradiol, we expect that women who experience hot flashes will have a decreased risk for developing breast cancer compared to women who do not report this symptom. In the WHI clinical trials, the effect of hormone therapy on breast cancer risk differed in the estrogen plus progestin (E+P) and estrogen alone (E-alone) trials [21, 22]. Additional results from the WHI clinical trials indicated an interaction between baseline estradiol level and E+P therapy on breast cancer risk. Women with low levels of estradiol were found to have the highest increase in breast cancer risk during E+P treatment [23]. Therefore, it is reasonable to hypothesize that the effect of hormone therapy on breast cancer risk will vary by baseline hot flash status and will differ in the E+P and E-alone trials. Using data from the WHI Hormone Therapy Clinical Trials, we propose to investigate the association of baseline hot flash status with the subsequent development of

invasive breast cancer (Aim 1) and to assess whether baseline hot flash status modifies the effect of E+P and E-alone therapy on invasive breast cancer risk (Aim 2).

Methods

Study Design

The Women's Health Initiative, launched in 1993, is a long-term study investigating prevention strategies for heart disease, breast cancer, colorectal cancer and osteoporotic fractures in postmenopausal women. The study enrolled 161,808 women aged 50-79 years into two major components: the clinical trials and the observational study. The clinical trials consisted of the Dietary Modification Trial, the Calcium/Vitamin D trial, and the Hormone Therapy Trials [49], with the latter being the focus of this paper. The Hormone Therapy Trials included two parallel randomized, double-blind clinical trials investigating the effects of E+P and E-alone. At the end of the initial study period in 2005, the WHI Extension Studies (2005-2010 and 2010-2020) continued to follow up consenting study participants.

Estrogen Plus Progestin Trial

Two parallel randomized, double-blind clinical trials were performed for the postmenopausal therapy (PHT) study. The E+P trial focused on healthy postmenopausal women with an intact uterus to determine the type of impact hormone therapy had on CHD incidence among these women. Overall risks and benefits from E+P therapy were determined using a global index defined as the earliest onset of CHD, invasive breast cancer, colorectal cancer, endometrial cancer, hip fracture, pulmonary embolism (PE), stroke, or death due to another cause. Outcomes

were monitored on a semiannual basis, comparing the global index of the E+P group and the placebo group to an overall global index [21].

On May 31, 2002, the E+P portion of the study was halted [21]. A data safety and monitoring board (DSMB) independent of the WHI, performed interim analyses of the seven major outcomes on a semiannual basis starting in the fall of 1997. Predefined boundaries for the seven major outcomes were determined using the O'Brien-Fleming approach. Benefits were set at a 1-sided upper bound of 0.025 while risks were set at a 1-sided lower bound of 0.05. To further account for seven major outcomes, the risk boundaries were adjusted using a Bonferroni correction. Beginning in 1999 after five interim analyses, and through 2001, early adverse effects were seen for myocardial infarction (MI), stroke, and pulmonary embolisms (PE), although none crossed the boundaries previously set in place. After the 10^{th} interim analysis on May 31, 2002, the DSMB found that breast cancer had exceeded the previously set risk boundary while the cardiovascular diseases also continued although not exceeding their boundaries. With an average of 5.2 follow-up years, the E+P trial was halted seeing that the risk of breast cancer along with increased risk of CHD, stroke and PE outweighed any benefits of the hormone therapy.

Estrogen Alone Trial

Conjugated equine estrogen (CEE) therapy was used in women with prior hysterectomy to determine the type of impact hormone therapy had on CHD incidence among these women. The same global index used in the E+P study was used to help to determine risks and benefits from the use of E-alone.

Unlike the E+P, the E-alone trial continued through May 2002 because the health risks did not exceed the benefits at the time. Although the study continued, it was closely monitored by the same DSMB that monitored the E+P trial. The predefined boundaries for the seven major outcomes were calculated and set at the same levels as the E+P study. Participants in the E-alone trial were informed of the increased risk of breast cancer in the E+P study, and that no increased rates of breast cancer were seen in the E-alone trial. With the trial being scheduled to run until March 2005, the NIH found that after an average of 6.8 follow-up years, CEE had no effect on the risk of CHD. Although the boundary had not been crossed, the risk of stroke mirrored that of the E+P study. For these reasons, the NIH decided to stop the E-alone trial stating "the likelihood that neither cardio-protection nor breast cancer risk would be demonstrated in the remaining intervention period" [22]. Participants were informed to stop taking the study medication on March 1, 2004, one year prior to the original termination date.

Study Population

The majority of recruitment for all portions of the WHI were conducted via a mass mailing campaign disseminating the recruitment brochure. The brochure contained information about the WHI and also a prepaid return postcard for those interested in participating. Further eligibility screening was conducted by trained telephone interviewers for women who fit age requirements and either returned a postcard or called a clinical center. Enrollment into either portion of the PHT consisted of three visits to a clinic to determine eligibility, provide written informed consent and randomize into an intervention or placebo group [49].

Women were deemed eligible for the PHT portion of the WHI if they were age 50 to 79 at the time of initial screening, postmenopausal and were also likely to remain a resident of the area for 3 years. Postmenopausal was defined as no vaginal bleeding in the past 12 months for those age 50 to 54-years old, no vaginal bleeding in the past 6 months for those age 55 and older, having a hysterectomy or ever having used postmenopausal hormones. Women with an intact uterus were eligible for the E+P trial and women who had a hysterectomy were eligible for the E-alone trial. A total of 16608 women with an intact uterus were put into the E+P trial and randomized into either the combined hormone or placebo group [21]. A total of 10739 women with prior hysterectomy were recruited into the E-alone trial and randomized into either the hormone or placebo group [22].

Women were excluded from either trial if they had any competing risks with a survival time of less than three years, or if there were any concerns about safety and adherence. For those women already receiving postmenopausal hormones, a three-month washout period was required before beginning trial medication. All women in the study were required to provide written informed consent.

Treatment Assignment

Estrogen plus Progestin

One daily dose of the hormone therapy was administered and consisted of a single tablet containing 0.625 mg of conjugated equine estrogen and 2.5 mg of medroxyprogesterone acetate (MPA), also known as Prempro (Wyeth Ayerst, Philadelphia, PA). An identical placebo tablet was given to the control group. Randomization of women was conducted through the WHI

Clinical Coordinating Center and randomization was stratified by age range and clinic site. Double blinding was possible through unique bottle numbers and bar codes on all study medication. An unblinding officer was used in instances where safety or symptom management were of concern and trial medication was revealed to the clinic gynecologist.

Originally, the study design called for women with a uterus to be randomly assigned to an E+P, E-alone, or a placebo group. The protocol was altered after the results of the PEPI trial were published and the E-alone group was taken out. Women already assigned to the E-alone group were subsequently placed into the E+P group [50]. The resulting E+P group consisted of 8506 women while the placebo group consisted of 8102 women.

Estrogen Alone

5310 women were placed into the E-alone group while the placebo group consisted of 5429 women. One daily dose of the hormone therapy consisted of a single tablet containing 0.625 mg/d of CEE, also known as Permarin (Wyeth, St Davids, PA). An identical placebo tablet was given to the control group. Randomization of women was conducted in the same manner as the E+P trial, with randomization being stratified by age range and clinic site. Unique bottle numbers and bar codes also allowed for a double-blinded study.

Data Collection

To ensure adherence to medication and to assess any symptoms, participants in both trials were contacted six weeks after randomization. Thereafter, follow-up either by phone or clinic visits occurred every six months, with at least one clinic visit required annually. During each follow-up

phone call or clinic visit, the following were assessed: adherence to study medication, symptoms, participant concerns, and outcomes. At baseline as well as at year three, six, and nine, electrocardiograms were performed, while mammograms were required at each annual clinic visit. If these necessary precautions were not performed or if results could not be deduced, study medications were withheld. All participants were followed from date of entry until one of the following occurred: death, loss to follow-up, or request to leave the study.

A standardized form was provided by WHI to all clinics allowing a systematic data collection process. The WHI's Clinical Coordinating Center developed a database that was available to all study clinics for data to be entered. Local clinic databases allowed for data quality assurance through database checks, random audits and clinic visits.

Medication Adjustment/Discontinuation

If participants experienced symptoms hindering everyday comfort such as breast tenderness or vaginal bleeding, study medication dosage was altered. This was done by reducing the number of days the medication was taken depending on the severity of the symptoms. For women who began the E+P trial with an intact uterus and later had a hysterectomy, these women were then switched to either E-alone or replicate placebo pills. In these instances, where dosage was altered or study medication was switched, blinding remained.

Certain outcomes such as acute MI, stroke, and any major injury requiring hospitalization, recommend the termination of hormone therapy. When these incidences occurred in participants, the study medications were momentarily halted until patients were otherwise informed by the

study clinic. Other more major outcomes such as breast cancer, hyperplasia not responsive to treatment, DVT/PE, malignant melanoma, meningioma and prescription of estrogen or testosterone required the immediate termination of study medication as stated in the protocol [21].

Study Follow-up

Although both trials were terminated early, two WHI extension studies were initiated, the first beginning in 2005 for consenting participants and ending in 2010 (E+P N= 12,788; E-alone N= 7,645). At the conclusion of the first extension in 2010 participants were invited to continue in the second extension from 2010 through 2020 (E+P N= 9,891; E-alone N= 5,693). The purpose of these extension studies were to follow-up with participants in all components of the WHI to explain longer term outcomes, to describe any changes in hormone therapy for women in the WHI clinical trials, and to expand research questions that can be addressed by the unique qualities of a large longitudinal study that the WHI possesses. Data collection is primarily done by questionnaires sent to participants by mail, while external sources, such as the National Death Index (NDI), are used to collect any additional data. Data collected through and released in September 2015 were used in this study.

Ascertainment of Hot Flashes at Baseline

Hot flash status data was collected through a "thoughts and feelings questionnaire" from WHI administered at baseline. Participants were asked about the occurrence and severity of hot flashes during the previous four weeks. Hot flash was scored as: 0= none, 1= mild but not interfering

with usual activities, 2= moderate and interfering somewhat with usual activities, or 3= severe such that usual activities could not be performed.

Ascertainment of Breast Cancer

Pathology reports were used to confirm the diagnosis of all cancers in both trials, excluding melanoma skin cancers. 98.2% of all invasive breast cancers were reported using pathology reports.

Ascertainment of risk factors

Known risk factors for both breast cancer and hot flashes were determined prior to analysis. Baseline characteristics including demographic characteristics, reproductive and menstrual history, family history, and lifestyle habits, were collected through questionnaires at the beginning of the WHI clinical trials. Measurements such as height and weight were also collected at baseline visits.

Statistical analysis

For both the E+P trial and the E-alone trial, baseline characteristics between invasive breast cancer cases and non-cases were measured and compared using t-test or Wilcoxon rank sum test for continuous variables and Pearson's chi square test for categorical data. Hot flash status was re-categorized into three groups: 0= none, 1=mild, and 2= moderate/severe. Women with missing data for hot flash status (N= 234) were excluded from analysis.

For Aim 1, Cox proportional hazards regression was used to assess the effect of baseline hot flash status on the risk of invasive breast cancer. This analysis was restricted to the placebo arm of both the E+P and E-alone trials pooled together. Follow-up time was calculated from the date of trial randomization to the first diagnosis of invasive breast cancer, death, last contact with the participant, or the end of follow-up (September 30, 2015), whichever came first. Hazard ratios and 95% confidence intervals were measured for each hot flash category, with the referent being women who experienced no hot flashes. Both unadjusted and adjusted models were fitted, with potential confounders being those that were statistically significantly associated with breast cancer incidence at the 0.05 level of statistical significance. A p-value for the trend in hazard ratios was calculated by fitting hot flash status in the model as a continuous variable and obtaining the p-value for its coefficient. Models were adjusted for age, race, age at menarche, ever having needle aspiration and having a female relative with breast cancer.

For Aim 2, Cox proportional hazards regression was used to assess whether hot flash status modified the effect of hormone therapy on the risk of invasive breast cancer. Analysis were done separately for each hormone therapy trial. A product term for hot flash status and hormone therapy assignment was included in models. Follow-up time was calculated from the date of trial randomization to the first diagnosis of invasive breast cancer, death, last contact with the participant, or the end of follow-up (September 30, 2015), whichever came first. Both unadjusted and adjusted models were fitted to assess the risk of breast cancer associated with hormone therapy type for each hot flash category. Both models were adjusted for age, race, age at menarche, history of needle aspiration, family history of breast cancer and weight.

The level of statistical significance for all analyses was set at the 0.05 level. Analyses were done using SAS software version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Baseline Characteristics of Participants in Estrogen plus Progestin Trial

The mean age of participants with invasive breast cancer and non-cases was 63.2±6.8 years and 63.3±7.1 years, respectively, with no statistically significant difference. The mean age at menopause for women with invasive breast cancer was higher than for non-cases (50.3±4.6 years and 49.9±4.8 years respectively, p=0.02). Women with invasive breast cancer also had a higher percentage of first degree female relatives with breast cancer compared to non-cases (p<0.0001). Among invasive breast cancer cases, 68.8% did not experience hot flashes, 20.5% experienced mild hot flashes and 10.7% experienced moderate to severe symptoms. Among non-cases, 70.1% did not experience hot flashes, 20.6% experienced mild symptoms and 9.3% experienced moderate to severe symptoms (p=0.3) (Table 1).

Baseline Characteristics of Participants in Estrogen Alone Trial

The mean age of participants with invasive breast cancer and non-cases was 63.6±7.0 years and 63.6±7.3 years respectively, with no statistically significant difference. Compared to non-cases, women diagnosed with invasive breast cancer had a higher percentage of first-degree female relatives with breast cancer (p=0.0001), were more likely to have had a breast biopsy (p=0.06), were more likely to have had a needle aspiration of a breast lump (p=0.0004), and were less likely to have had bilateral oophorectomy (p=0.03). Among invasive breast cancer cases, 66.2% did not experience hot flashes, 21.4% experienced mild hot flashes and 12.4% experienced

moderate to severe symptoms. Among non-cases, 65.9% did not experience hot flashes, 21.5% experienced mild symptoms and 12.6% experienced moderate to severe symptoms (p=0.98) (Table1).

Baseline Hot Flash Status and Breast Cancer Risk

Among participants in the placebo arms of both trials, hot flash status was not associated with risk of breast cancer. Before adjusting for potential confounders, the hazard ratio for women experiencing mild hot flashes was 0.99 (95% CI 0.83-1.19) compared to women who experience no hot flashes. For women experiencing moderate to severe hot flashes, the hazard ratio was 1.00 (95% CI 0.78-1.28). Adjusting for confounders had a minimal effect on the associations. For women experiencing mild hot flashes, the hazard ratio was 1.05 (95% CI 0.85-1.29). The hazard ratio for women experiencing moderate to severe hot flashes was 1.17 (95% CI 0.89-1.53) (Table 2).

Effect of Estrogen plus Progestin on Breast Cancer Risk by Hot Flash Status

The interactions between mild hot flash status and E+P, and moderate/severe hot flash status and E+P were not statistically significant (p=0.87 and p=0.32, respectively). Compared to participants in the placebo arm, the hazard ratio associated with E+P treatment in women with no hot flash symptoms was 1.22 (95% CI 1.05-1.42), 1.20 (95% CI 0.91-1.59) for women experiencing mild symptoms, and 1.48 (95% CI 0.99-2.20) for women experiencing moderate to severe symptoms. After adjusting for possible confounders, hazard ratios remained not statistically significant when comparing treatment status at each hot flash symptom level (none

HR= 1.16, 95% CI 0.98-1.37; mild HR= 1.19, 95% CI 0.88-1.63; moderate/severe HR= 1.46, 95% CI 0.96-2.24) (Table 3).

Effect of Estrogen Only on Breast Cancer Risk by Hot Flash Status

The interactions between mild hot flash status and E-alone, and moderate/severe hot flash status and E-alone were not statistically significant (p=0.12 and p=0.49, respectively). The hazard ratio for E-alone compared to placebo was 0.88 (95% CI 0.71-1.09) in women with no hot flash symptoms, 0.68 (95% CI 0.46-1.00) for women experiencing mild hot flash symptoms and 0.81 (95% CI 0.49-1.34) for women experiencing moderate to severe hot flash symptoms. After adjusting for confounders, the hazard ratio for women experiencing no hot flashes and moderate/severe symptoms remained unchanged in magnitude and statistical significance (HR=0.89, 95% CI 0.70-1.12; and HR=0.72, 95% CI 0.43-1.23, respectively) (Table 3), while the hazard ratio for women experiencing mild symptoms became statistically significant (HR=0.59, 95% CI 0.38-0.92).

Discussion

In this prospective study, we observed that in postmenopausal women who were not on E+P or E-alone therapy, baseline hot flash status was not associated with risk of invasive breast cancer. In addition, hot flash status did not modify the effect of PHT, both the E+P and E-alone formulations, on breast cancer risk.

Our results regarding the association of hot flashes with breast cancer risk were consistent with the Australian Longitudinal Study of Women's Health (ALSWH) which found no association

between hot flashes and incident breast cancer (HR=1.09, 95% CI: 0.84-1.33) [20]. Two other studies found significant associations between hot flash symptoms, but also both differed in study design [18, 19]. The Two Sister Study, a controlled case-control study, focused solely on early-onset breast cancer [19]. The other population-based case control study, looked at all menopausal symptoms, not only hot flashes, and used a referent group of never experiencing menopausal symptoms during their analysis. Cases were also based on women diagnosed with invasive breast cancer, with the possibility of both cases and controls having current or past use of PHT [18].

To our knowledge, no other study has investigated the effect of hot flashes on the association of hormone therapy with invasive breast cancer risk. Ancillary analysis from the WHI E+P trial looked into endogenous hormone levels to determine if pretreatment levels of endogenous sex hormones modified the effects of the E+P on breast cancer incidence. It was found that there was an increased risk of breast cancer with E+P for women in the lowest quartiles of endogenous estrogen [23]. Furthermore, the Penn Ovarian Aging Study found hot flashes to be associated with fluctuating levels of estradiol (E2), decreased levels of inhibin b and increased levels of follicle stimulating hormone (FSH) [2]. Given the association of hot flashes with lower levels of estradiol [51] as well as an increased risk of breast cancer among women prescribed E+P with low levels of endogenous estrogen, we expected the risk of breast cancer to vary by hot flash status within each of the hormone therapy trials. While the overall interaction between hot flash status and E+P was not statistically significant, there was a hint of a trend where the effect of E+P on breast cancer risk was found to increase with higher severity of hot flashes. In women who experienced moderate/severe symptoms, E+P was associated with a 46% increased risk in

breast cancer compared to placebo; corresponding estimates in women who experienced mild hot flashes and in those with no symptoms were 19% and 16% respectively.

These findings are consistent with what we expected if hot flashes are associated with lower levels of estradiol, and the risk for breast cancer among women taking E+P increases with lower levels of endogenous estrogen. Further research is needed to confirm these results, but early findings have the potential for clinical importance. Hot flash symptoms could potentially help advise women whether or not to be put on E+P based on their risk for breast cancer.

Among women in the E-alone trial, the interaction between hot flash status and E-alone was not statistically significant. For women not experiencing hot flashes, E-alone had a 11% decreased risk in breast cancer compared to placebo. For women who experienced mild symptoms, E-alone had a 41% decreased risk compared to placebo and E-alone had a 28% decreased risk in breast cancer for women experiencing moderate/severe symptoms compared to the placebo. While the associations for no symptoms and moderate/severe symptoms were not statistically significant, the association for mild symptoms with invasive breast cancer was statistically significant (95% CI 0.38-0.92).

Strengths of this study include a large, ethnically diverse study population randomized by the study design of a double-blind, placebo-controlled trial. This prospective study also has the longest follow-up time and largest sample size of any study investigating hot flashes and breast cancer risk. Limitations of this study include not having baseline endogenous hormone measurements. Results from the study are also not generalizable due to the racial composition of

study participants, with the majority of women being white. It is also important to take into consideration that women in this study were well into menopause, compared to the other studies investigating this topic. The Two Sister Study investigated breast cancer incidence before the age of 50, while participants from the other population-based case control study ranged from 55-74 years old. Therefore, hot flash incidence in the WHI may be less common than in other studies to allow a detectable effect on risk or PHT effects.

Conclusion

Among participants in the placebo group of both trials, hot flashes did not predict invasive breast cancer risk in postmenopausal women. No statistically significant difference in breast cancer risk was seen between hot flash status. Hot flash status also did not modify the effect of E+P or E-alone therapy on breast cancer risk, although further studies are needed to evaluate the possibility that women with moderate/severe symptoms have a higher risk for breast cancer with E+P treatment.

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Table 1. Baseline characteristics (mean±SD, or N (%)) of breast cancer cases and non-cases in the estrogen plus progestin and estrogen alone trials

	Estrogen plus Progestin Trial			Estrogen Alone Trial		
Variable	**Incident Breast Cancer Cases (N= 960)**	**Non-Cases (N= 15518)**	**P-value***	**Incident Breast Cancer Cases (N= 500)**	**Non-Cases (N= 10135)**	**P-value***
Age (years)	63.2 ±6.8	63.3 ±7.1	0.86	63.6 ±7.0	63.6 ±7.3	0.95
Weight (kg)	77.3 ±16.2	74.5 ±16.6	<0.0001	83.1 ±18.3	78.3 ±17.2	<0.0001
Race			0.002			0.11
White	847 (88.41)	13035 (84.2)		396 (79.2)	7638 (75.51)	
Black or African American	53 (5.53)	1047 (6.76)		69 (13.8)	1521 (15.04)	
Other	58 (6.05)	1399 (9.04)		35 (7.0)	956 (9.45)	
Age at menarche (years)			0.12			0.0007
≤10 years	64 (6.8)	957 (6.2)		47 (9.4)	798 (7.9)	
11-14 years	808 (84.2)	12801 (82.5)		424 (84.8)	8211 (81.0)	
≥15 years	88 (9.2)	1760 (11.3)		29 (5.8)	1126 (11.1)	
Age at menopause (years)	50.3 ±4.6	49.9 ±4.8	0.02	44.6 ±7.7	44.4 ±7.6	0.74
Smoking			0.01			0.06
Never	463 (48.7)	7650 (49.9)		254 (51.2)	5123 (51.1)	
Past	411 (43.3)	6070 (39.6)		205 (41.3)	3842 (38.3)	
Current	76 (8.0)	1626 (10.6)		37 (7.5)	1065 (10.6)	
Alcohol drinking			0.59			0.46
Never	110 (11.6)	1768 (11.5)		61 (12.4)	1371 (13.6)	
Past	146 (15.4)	2639 (17.1)		118 (24.0)	2410 (24.0)	
<1 drink per month	126 (13.3)	2150 (14.0)		77 (15.7)	1447 (14.4)	
<1 drink per week	185 (19.6)	3016 (19.6)		87 (17.7)	1940 (19.3)	
1-7 drinks per week	244 (25.8)	3878 (25.2)		95 (19.3)	2010 (20.0)	
>7 drinks per week	135 (14.3)	1949 (12.7)		54 (11.0)	872 (8.7)	
Female hormone use			0.54			0.26
Never	560 (60.6)	9039 (60.5)		186 (39.1)	3729 (38.2)	
Past	299 (32.4)	4977 (33.3)		220 (46.2)	4804 (46.2)	
Current	65 (7.0)	925 (6.2)		70 (14.7)	1219 (12.5)	
Family history of breast cancer in first degree relative	191 (20.8)	2258 (15.8)	<0.0001	113 (24.0)	1634 (17.2)	0.0001
History of breast biopsy	173 (20.0)	2347 (16.5)	0.01	109 (24.2)	1872 (20.5)	0.06
History of needle aspiration	134 (15.5)	1392 (9.8)	<0.0001	72 (16.0)	972 (10.6)	0.0004
History of hysterectomy	0 (0.0)	1 (0.01)	0.80	499 (99.8)	10122 (99.9)	0.67
History of bilateral oophorectomy	5 (0.5)	48 (0.3)	0.16	161 (32.2)	3865 (38.1)	0.03
Gail risk score >1.7%	386 (40.2)	5406 (34.8)	0.0007	192 (38.4)	3135 (30.9)	0.0004
Hot flash status			0.3			0.98
None	660 (68.8)	10880 (70.1)		331 (66.2)	6676 (65.9)	
Mild	197 (20.5)	3197 (20.6)		107 (21.4)	2178 (21.5)	
Moderate/Severe	103 (10.7)	1441 (9.3)		62 (12.4)	1281 (12.6)	

*T-test used to calculate p-values for continuous variables and Pearson's chi square test for categorical variables

Table 2. Effect of hot flash status on risk of breast cancer in the placebo arms of the Estrogen plus Progestin and Estrogen alone trials

	Total Number (Number of Cases)	**Unadjusted Analysis Hazard Ratio (95% CI)**	**Adjusted Analysis* Hazard Ratio (95% CI)**
Hot Flash Status			
None	9148 (467)	1.00	1.00
Mild	2850 (152)	0.99 (0.83-1.19)	1.05 (0.85-1.29)
Moderate/Severe	1403 (73)	1.00 (0.78-1.28)	1.17 (0.89-1.53)
P-trend		0.98	0.28

*Adjusted for age, race, age at menarche, history of needle aspiration, family history of breast cancer and weight

Table 3. Effect of estrogen plus progestin therapy on risk of breast cancer by baseline hot flash status

	Estrogen plus Progestin Trial				
	Total Number (Number of Cases)	**Unadjusted Analysis**		**Adjusted Analysis***	
		HR (95% CI)	**P-Interaction****	**HR (95% CI)**	**P-Interaction****
Hot Flash Status					
None	11540 (660)	1.22 (1.05-1.42)		1.16 (0.98-1.37)	
Mild	3394 (197)	1.20 (0.91-1.59)	0.94	1.19 (0.88-1.63)	0.87
Moderate/Severe	1544 (103)	1.48 (0.99-2.20)	0.37	1.46 (0.96-2.24)	0.32

*Adjusted for age, race, age at menarche, history of needle aspiration, family history of breast cancer and weight
**P-value for hormone therapy assignment and hot flash status interaction

Tables 4. Effect of estrogen alone therapy on risk of breast cancer by baseline hot flash status

	Estrogen Alone Trial				
	Total Number (Number of Cases)	**Unadjusted Analysis**		**Adjusted Analysis***	
		HR (95% CI)	**P-Interaction****	**HR (95% CI)**	**P-Interaction****
Hot Flash Status					
None	7007 (331)	0.88 (0.71-1.09)		0.89 (0.70-1.12)	
Mild	2285 (107)	0.68 (0.46-1.00)	0.26	0.59 (0.38-0.92)	0.12
Moderate/Severe	1343 (62)	0.81 (0.49-1.34)	0.78	0.72 (0.43-1.23)	0.49

* Adjusted for age, race, age at menarche, history of needle aspiration, family history of breast cancer and weight
**P-value for hormone therapy assignment and hot flash status interaction

Chapter 4: Discussion and Recommendations

Using data from the WHI Hormone Therapy Clinical Trials, we conducted an analysis with the intent to look at the following two aims: 1) To investigate the association of baseline hot flash status with the subsequent development of invasive breast cancer, and 2) To assess whether baseline hot flash status modifies the effect of E+P and E-alone therapy on invasive breast cancer risk. In this prospective study, we observed that in postmenopausal women who were not on E+P or E-alone therapy, baseline hot flash status was not associated with risk of invasive breast cancer. In addition, hot flash status did not modify the effect of PHT, both the E+P and E-alone formulations, on breast cancer risk.

Regarding the Aim 1, there was a 5% increased risk in invasive breast cancer among women who experience mild hot flashes compared to women with no symptoms. A 17% increased risk in invasive breast cancer was seen in women with moderate/severe symptoms compared to women with no symptoms. The trend across hot flash status was not statistically significant ($p=0.28$), and we fail to reject the null hypothesis that among women in the placebo arms of both trials, the risk of breast cancer does not differ between hot flash status. Our results regarding the association of hot flashes with breast cancer risk were consistent with the Australian Longitudinal Study of Women's Health (ALSWH) which found no association between hot flashes and incident breast cancer (HR=1.09, 95% CI: 0.84-1.33) [20]. Two other studies found significant associations between hot flash symptoms, but also both differed in study design [18, 19]. The Two Sister Study, a controlled case-control study, focused solely on early-onset breast cancer [19]. The other population-based case control study, looked at all menopausal symptoms,

not only hot flashes, and used a referent group of never experiencing menopausal symptoms during their analysis. Cases were also based on women diagnosed with invasive breast cancer, with the possibility of both cases and controls having current or past use of PHT [18].

Regarding Aim 2, no other study has investigated the effect of hot flashes on the association of hormone therapy with invasive breast cancer risk, to our knowledge. Ancillary analysis from the WHI E+P trial looked into endogenous hormone levels to determine if pretreatment levels of endogenous sex hormones modified the effects of the E+P therapy on breast cancer incidence. It was found that there was an increased risk of breast cancer with E+P therapy for women in the lowest quartiles of endogenous estrogen [23]. Given the association of hot flashes with lower levels of estradiol [51] as well as an increased risk of breast cancer among women prescribed E+P with low levels of endogenous estrogen, we expected the risk of breast cancer to vary by hot flash status within each of the hormone therapy trials. While the overall interaction between hot flash status and E+P was not statistically significant, there was a hint of a trend where the effect of E+P on breast cancer risk was found to increase with higher severity of hot flashes. In women who experienced moderate/severe symptoms, E+P was associated with a 46% increased risk in breast cancer compared to placebo; corresponding estimates in women who experienced mild hot flashes and in those with no symptoms were 19% and 16% respectively.

Among women in the E-alone trial, the interaction between hot flash status and E-alone was not statistically significant. For women not experiencing hot flashes, E-alone had a 11% decreased risk in breast cancer compared to placebo. For women who experienced mild symptoms, E-alone had a 41% decreased risk compared to placebo and E-alone had a 28% decreased risk in breast

cancer for women experiencing moderate/severe symptoms compared to the placebo. While the associations for no symptoms and moderate/severe symptoms were not statistically significant, the association for mild symptoms with invasive breast cancer was statistically significant (95% CI 0.38-0.92).

These findings are consistent with what we expected if hot flashes are associated with lower levels of estradiol, and the risk for breast cancer among women taking E+P increases with lower levels of endogenous estrogen. Further research is needed to confirm these results, but early findings have the potential for significant clinical importance. Hot flash symptoms could help advise women whether or not to be put on E+P based on their risk for breast cancer. If confirmed, these findings suggest that E+P should not be recommended for women experiencing moderate to severe hot flash symptoms, as these women could be at an increased risk to develop invasive breast cancer. Women experiencing mild hot flash symptoms should be cautious of beginning E+P as well, but the risk is not as high for these women.

Strengths of this study include a large, ethnically diverse study population randomized by the study design of a double-blind, placebo-controlled trial. This prospective study also has the longest follow-up time and largest sample size of any study investigating hot flashes and breast cancer risk. The WHI is a nationwide study conducted to address the major health issues facing postmenopausal women. Before the WHI, few studies had been conducted to address women's health, yet alone the health of postmenopausal women. This further influenced the WHI to investigate strategies for prevention and control of common diseases among women, more specifically: cardiovascular diseases, cancer and osteoporosis [50]. The WHI Hormone Therapy

trials enrolled 10,739 women with prior hysterectomy into the E-alone trial and 16,608 women with an intact uterus into the E+P trial, assessing coronary heart disease as the primary outcome and breast cancer as one of the secondary adverse outcomes [52].

Limitations of this study include not having baseline endogenous hormone measurements. Results from the study are also not generalizable due to the racial composition of study participants, with the majority of women being white. It is also important to take into consideration that women in this study were well into menopause, compared to the other studies investigating this topic. Therefore, hot flash incidence in the WHI may be less common than in other studies to allow a detectable effect on risk or PHT effects.

Further research is needed to confirm these findings, but early findings have the potential for significant clinical importance in the future.

Conclusion

Among participants in the placebo group of both trials, hot flashes did not predict invasive breast cancer risk in postmenopausal women. No statistically significant difference in breast cancer risk was seen between hot flash status. Hot flash status also did not modify the effect of E+P or E-alone therapy on breast cancer risk, although further studies are needed to evaluate the possibility that women with moderate/severe symptoms have a higher risk for breast cancer with E+P treatment.

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