Menopausal Hormone Therapy and Breast Cancer: Evidence from Randomized Clinical Trials

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GRADUATE THESIS

Zagreb, 2024

This graduate thesis was made at the Department of Gynecology and Obstetrics at the University Hospital Center Zagreb, mentored by Professor Dinka Pavičić Baldani, MD PhD. and was submitted for evaluation in the academic year 2023/2024.

ABBREVIATIONS:

HRT – Hormone Replacement Therapy

ER – Estrogen Receptor

PR – Progesterone Receptor

HER – Human Epidermal Growth Factor Receptor 2

CV – Cardiovascular

HM – Healthy Menopause

FSH – Follicle Stimulating Hormone

TSH – Thyroid Stimulating Hormone

MHT – Menopausal Hormone Therapy

CEE – Conjugated Equine Estrogen

MPA – Medroxyprogesterone Acetate

IM – Intramuscular

MI – Myocardial Infarction

VTE – Venous Thromboembolism

HDL – High Density Lipoprotein cholesterol

LDL – Low Density Lipoprotein cholesterol

AUB – Abnormal Uterine Bleeding

BRCA1 – Breast Cancer Gene 1

BRCA2 – Breast Cancer Gene 2

TP53 – Tumor Protein p53

PTEN – Phosphatase and Tensin homolog

TNM – Tumor, Node, Metastasis

Ki67 – Antigen Kiel 67

SERM – Selective Estrogen Receptor Modulators

IUD – Intrauterine Device

DHEA – Dehydroepiandrosterone

Tis – Tumor in situ

WHI – Women's Health Initiative

CHD – Coronary Heart Disease

PMD – Percent Mammographic Density

BMI – Body Mass Index

CVD – Cardiovascular Disease

E2 - Estradiol

NETA – Norethisterone

PEPI – Postmenopausal Estrogen/Progestin Intervention trial

ERA – Estrogen Replacement and Atherosclerosis

WEST – Women's Estrogen for Stroke Trial

TIA – Transient Ischemic Attack

DOPS – Danish Osteoporosis Prevention Study

ESPRIT – Estrogen for the Prevention of Re-Infarction Trial

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1. SUMMARY

Title: Menopausal Hormone Therapy and Breast Cancer: Evidence from Randomized Clinical Trials

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The use of menopausal hormone therapy (MHT) significantly declined after 2002, primarily due to the Women's Health Initiative's (WHI) report suggesting that the combination of conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) increased breast cancer risk and did not enhance quality of life. More recently, WHI publications have recognized MHT as the most effective treatment for managing menopausal vasomotor symptoms, reporting that CEE alone decreases the risk of breast cancer by 23% and reduces breast cancer mortality by 40%. The only remaining concern is a slight increase in breast cancer incidence with CEE and MPA (1 per 1,000 women per year), but with no increased risk of breast cancer mortality. Recently, reanalyses of the WHI trial have been published that dispute even this claim about breast cancer risk, pointing out the WHI's presentation of insignificant results as if they were conclusive, misinterpretation of its own data, and the false claim that the WHI findings, and consequent decline in the use of MHT, led to a reduction in breast cancer incidence in the United States. As a result, a generation of women has been largely deprived of MHT due to this widely publicized misinterpretation of the data. Furthermore, clinical trials performed on other types of progesterone do not agree with the results obtained from the WHI study, which analyzed the use of one specific type of progesterone - medroxyprogesterone acetate. This article aims to present and interpret relevant randomized trials, with the intention of assisting patients and physicians in making informed decisions about the use of MHT.

Keywords: Menopausal Hormone Therapy, Breast cancer, Randomized Clinical Trials

2. SAŽETAK

Naslov: Hormonsko nadomjesno liječenje i karcinom dojke: Dokazi iz randomiziranih kliničkih ispitivanja

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Korištenje hormonskog nadomjesnog liječenja (HNL) značajno se smanjilo nakon 2002. godine, prvenstveno zbog izvješća Inicijative za zdravlje žena (WHI) koje sugerira da kombinacija konjugiranog konjskog estrogena (CEE) i medroksiprogesteron acetate (MPA) povećava rizik od raka dojke, a ne poboljšava kvalitetu života. Nedavno objavljene reanalize WHI ispitivanja prepoznale su HNL kao najučinkovitije liječenje za vazomotornih menopauzalnih tegoba, izvješćujući da primjenjena samih CEE smanjuje rizik od raka dojke za 23% te smrtnost od raka dojke za 40%. Jedina preostala zabrinutost je blagi porast incidencije raka dojke uz korištenje kombinacija CEE i MPA (1 na 1000 žena godišnje), ali bez povećanog rizika od smrtnosti od raka dojke. Nedavno su objavljene ponovne analize WHI ispitivanja koje osporavaju čak i ovu tvrdnju o riziku od raka dojke, ističući predstavljanje beznačajnih rezultata WHI ispitivanja kao da su konačnih, pogrešno tumačenje vlastitih podataka i lažnu tvrdnju da su nalazi WHI-a i posljedični pad korištenja HNL, doveli do smanjenje incidencije raka dojke u Sjedinjenim Državama. Kao rezultat toga, zbog ovog objavljenog pogrešnog tumačenja podataka, jednoj generaciji žena je uskraćeno korištenje HNL-a. Nadalje, klinička ispitivanja provedena sa drugim vrstama progesterona bila u suglasnoti s rezultatima dobivenim iz studije WHI, koja je analizirala upotrebu jedne specifične vrste progesterona - medroksiprogesteron acetata. Cilj ovog članka je predstaviti i protumačiti relevantna randomizirana ispitivanja, s namjerom da pomogne pacijentima i liječnicima u donošenju informiranih odluka o upotrebi HNL-a.

Ključne riječi: hormonsko nadomjesno liječenje, karcinom dojke, randomizirana klinička ispitivanja

3. INTRODUCTION

The menopausal transition is generally defined as the time between the onset of irregular menstrual cycles (usually accompanied by some menopausal symptoms) and menopause. Menopause is a retrospective diagnosis and is said to have happened when menstrual activity has ceased for at least 12 consecutive months in the absence of any other physiological or pathological explanation. (1) Menopausal transition involves a set of physical, cardiovascular, endocrine, and psychological changes. Symptoms associated with the menopausal transition occur in up to 85% of women. Every woman's experience of the menopausal transition is unique, and a one-size-fits-all approach to the management of symptoms does not work. It's crucial to consider the pathophysiology and intensity of menopausal symptoms, as well as weigh the advantages and drawbacks of hormonal and non-hormonal treatments when tailoring treatment plans for menopausal symptoms.(2) Vasomotor symptoms which include hot flushes and night sweats are the most common and they affect more than 80% of women during the transition. Other common symptoms include disturbed sleep, tiredness, depressed mood, brain fogging, low libido, and heightened anxiety. There is considerable variation in severity and duration of menopausal symptoms among women. (3) The average duration of menopausal symptoms has been reported to be 7 years, with many cases going beyond that. (4)

Menopausal hormone therapy previously called hormone replacement therapy (HRT) is supplementing women with hormones that are lost during the menopausal transition. To relieve the symptoms associated with menopause, conventional MHT includes an estrogen and progesterone component to mimic hormones secreted in the normal menstrual cycle. (5)Estrogen therapies are numerous, and include those originating in the ovaries, for example, estradiol and estriol. The only role for progestogens in menopausal therapy is to protect against endometrial hyperplasia and endometrial cancer. Accordingly, progestogens are not indicated for women who have had a hysterectomy. All clinical trials and most observational studies that have evaluated multiple progestogens have found important differences in their metabolic effects and in disease

outcomes. Progesterone can provide symptom relief from sleep disturbances and mood instability, and increasing evidence support that it offers protection to the breast tissue.

Breast Carcinoma is the most common malignancy of women globally (excluding nonmelanoma skin cancer) and causes the majority of cancer deaths in women. Almost all breast malignancies are adenocarcinomas and they are divided into three major groups: ER positive (HER2 negative), HER2 positive (ER positive or negative) and Triple negative (ER, PR, HER2 negative). Some important risk factors are age and gender, family history of breast cancer, race/ethnicity, reproductive history, ionizing radiation, postmenopausal obesity, mammographic density etc. (6)

There is strong evidence that estrogen is highly effective in treating menopausal vasomotor symptoms and the genitourinary syndrome of menopause. (7,8) When started before age 60 (or within about 10 years of menopause), MHT effectively reduces all-cause mortality. (9) In this age group, MHT also prevents coronary heart disease (CHD). (10,11) Additionally, MHT helps prevent hip fractures, a significant yet often overlooked cause of morbidity and mortality in postmenopausal women. Despite these benefits, the use of MHT plummeted between 2001 and 2008, after the initial publication of the Women's Health Initiative (WHI) trial on conjugated equine estrogens (CEE) combined with medroxyprogesterone acetate (MPA) in 2002, decreasing from around 43% to 11% among women aged 45–74 years. (12) Fear of MHT, misunderstandings about its risks and benefits, and a lack of proper education among healthcare providers have led to its underutilization, causing unnecessary suffering and increased chronic disease and mortality in postmenopausal women over the past two decades. This disconnect remain so widespread and persistent probably because when asked to prioritize health concerns, both women and healthcare providers consistently rank breast cancer at the top. The use of MHT has shown both decreases and increases in breast cancer risk, depending on the form of MHT, dosage, and duration of use. Initial reports from the largest clinical trial on this topic, the Women's Health Initiative (WHI), indicated that CEE-alone reduced breast cancer risk, although the results were not statistically significant, while the combination of CEE and MPA was associated with an increased risk that was also not statistically significant. Later analyses found significant reductions in invasive breast cancer among women adhering to CEE-alone therapy. The most recent analysis by the WHI group reported that CEE-alone significantly reduced breast cancer risk by 23%, whereas CEE combined

with MPA increased the risk by 28% in unadjusted analyses. Importantly, in the only analysis adjusted for covariates, the increased breast cancer risk with CEE and MPA was not statistically significant. Even if the breast cancer association for CEE and MPA had been significant, it was rare, affecting approximately one woman per 1200 woman-years of treatment. These differences are also reflected in large observational studies using estradiol and various progestogens, where micronized progesterone showed no risk, while synthetic progestins showed varying elevated risks depending on the class.

The aim of this thesis is to present and interpret relevant clinical randomized trials that investigated the association of MHT use and breast cancer risk. with the intention of assisting patients and physicians in making informed decisions about the use of MHT. For the purposes of this paper, the Pubmed database was searched using the keywords MHT, breast carcinoma, randomized trials

4. MENOPAUSE

After a year of amenorrhea due to irreversible loss of ovarian function, menopause is identified. It is often called the last menstruation of a women's life and the time after it is called post menopause. Perimenopause, a time of changing ovarian function, precedes the final menses by several years. The onset of menopause leads to diminished estrogen exposure, resulting in a high morbidity burden related to menopausal symptoms. some of the most serious symptoms are related to cardiovascular (CV) health and lower bone density due to falling levels of estrogen. The next hormone declining is progesterone which has consequences such as abnormal uterine bleeding, cognitive disabilities, migraines etc. The third hormone in declining levels is testosterone which leads to symptoms such as decreased libido. In contrast the rising FSH levels due to the loss of steroid and inhibin feedback at the pituitary gland level are observed. The most common conditions with which women present to healthcare professionals are concerns about worsening vasomotor symptoms, sleep disturbance, mood swings, joint pains, osteoporosis or premature ovarian failure. (3) Assessment should include detailing symptoms and their impact on the quality of life, menstrual history like age and type of menopause (natural or iatrogenic) and information about contraception. (4) Also, family and personal history should include that of malignancies, especially

breast, ovarian and endometrial cancer, colon cancer, venous thromboembolism, migraines and risk factors for osteoporosis, diabetes, hypertension, heart disease and stroke. Physical examination should include recording of weight, height, waist-hip ratio and blood pressure.(13) In women over 45 years irregular or absent menstruation especially in the presence of vasomotor symptoms is diagnostic of the menopause and in the majority of women no further investigations are required.(14) In younger women with suspected premature ovarian failure or early menopause serial FSH measurements should be taken. In menstruating women, measurement of FSH should be performed at the beginning of the follicular phase (days 2–5 of the cycle) to avoid ovulation induced elevations of FSH. Measurement of TSH and prolactin are also helpful in investigating menstrual irregularity. (13) Given that women's lifespans are increasing in developed nations, the menopause can now be viewed as a mid-life occurrence . It is predicted that there will be 1.1 billion postmenopausal women in the world by 2025. The high frequency of hot flushes, vaginal atrophy, which can last for many years, and osteoporosis make caring for ageing women a critical issue for health professionals, even though not all women will experience short- or long-term menopausal problems. The practice is to find a therapy that best suits women's needs and ongoing symptoms, with keeping in mind the possible side effects and complications of such therapy. The goal is to create a framework of a Healthy Menopause. HM is defined as a dynamic state, following the permanent loss of ovarian function and is characterized by a woman's self-perceived satisfactory physical, psychological, and social functioning and incorporating disease and disability as well as a woman's desired ability to adapt. Therefore, HM incorporates resources in order to maintain, adjust if needed, recover and improve that dynamic balance. (15)

4.1 Menopausal Hormone Therapy

MHT's main idea is to replace estrogen. A variety of hormonal preparations are available, each with its own indications, advantages and disadvantages. (3) Menopausal hormone therapy offers numerous solutions for postmenopausal women, such as prevention of osteoporosis, which is induced by alterations in calcium balance and bone density, and the alleviation of vasomotor symptoms. There is evidence MHT also decreases incidence of CV disorders by acting beneficially

on lipoprotein levels, increasing the level of HDL and decreasing the level of LDL.(8) There are many treatment regimens of MHT, the most common being continuous estrogen (daily or at least 21 days of the month), continuous estrogen with cyclic addition of progestin for 10-15 days and continuous daily estrogen and progestin. Alleviation of genitourinary symptoms, cognitive disturbances, sexual function and urinary tract disturbances has all been achieved through these regimens of MHT.(3)

4.2 Types of MHT

For most women needing MHT to relief menopausal symptoms, the harms of hormone replacement therapy are exaggerated while the benefits are often overlooked. There has been extensive evidence that estrogen is an effective treatment of vasomotor symptoms and the genitourinary syndrome of menopause. (7,8) When started before the age of 60 years (or estimate within 10 years of menopause), MHT is effective in reducing all-cause mortality in postmenopausal women.(7–9,16–19) In that given age range, MHT prevents coronary heart disease. (10,11,19) Additionally, HRT prevents hip fractures, which is an important and often ignored cause of morbidity and mortality in postmenopausal women. (20) Different regiments according to estrogens and progestin types, route of administration and the dose are given below in Table 1. (21)

Regimen	Route of administration	Dose
Estrogens		
17beta - Estradiol	Tablet, patch, gel, vaginal cream, ring	Standard dose: 2-4 mg(oral)/ 50-100 µg (transdermal)
CEE	Oral tablet	Standard dose: 0.625-1.25 mg
Estriol	Vaginal gel, cream or ring	0.5-1 mg cream
Promestriene	Vaginal cream or tablets	10 mg
Progestogens		
Micronized progesterone	Oral or vaginal tablet	100-200 mg orally/vaginally
Levonorgestrel	Oral tablet/Transdermal patch or IUD	30-40 µg orally/ 150 µg/ 14-20 mg IUD
Dydrogesterone	Oral tablet	2.5-20 mg
Norethisterone acetate	Oral tablet or patch	0.5-1 mg orally/ 125-250 µg
Drospirenone	Oral tablet	1-2 mg
MPA	Oral tablet	2.5-10 mg
Chlormadionone acetate	Oral tablet	2 mg
Nomegestrol acetate	Oral tablet	2.5-5 mg
Promegestone	Oral tablet	2.5-10 mg
Trimegestone	Oral tablet	0.25 mg
Norgestimate	Oral tablet	0.5 mg
Dienogest	Oral tablet	3-4 mg
Norgestrel	Oral tablet	0.25-0.5 mg
Other		
Ospemifene (SERM)	Oral tablet	60 mg
Bazedoxifene (SERM)	Oral tablet or combined with CEE	10 mg/ 20 mg/ 40 mg and 0.45 mg/0.625 mg
Tibolone	Oral tablet	1.25-2.5 mg
DHEA (prasterone)	Vaginal tablet	6.5 mg

Table 1. Menopausal hormone therapy regimens, A Care Pathway from the European Menopause. And Andropause Society (21)

4.3 Estrogen therapy

Estrogen exerts significant metabolic effects across various major organ systems. It regulates the vaginal and urethral environment, enhances arterial blood flow, and mitigates subclinical atherosclerosis. (19,22–25)Additionally, estrogen fosters a non-atherogenic lipid profile, lowers the incidence of diabetes mellitus, helps prevent bone loss and may facilitate bone restoration, curbs excessive osteoclastic activity, and supports neuronal health in the central nervous system and spine by promoting growth and reducing inflammation. The decline in menopausal hormone therapy (MHT) has led to a rise in the use of individual medications to address the consequences of reduced endogenous estrogen. (19,26–28)Indications for menopausal estrogen therapy are many, starting from treating common symptoms like hot flushes, night sweats, vaginal dryness and atrophy to decreasing risks of cardiovascular disease, osteoporosis, cognitive disturbances and according to newer studies decreasing the risks of breast cancer. (29)CEEs, synthetic esterified estrogens, ethinyl estradiol-containing preparations, or pure 17-beta estradiol-containing products are the estrogens commonly used in MHT preparations in standard or low-dose formulations. (30) In women with average age at menopause, the lowest effective dose should be prescribed, by the same principle as with any medication.(31) Older women usually require lower doses of estrogen to control their symptoms while on other hand, women with premature ovarian insufficiency will generally require higher estrogen doses. (32)

The form of administration of estrogen depends on the specific symptoms the treatment is targeting and associated comorbidities and age of the patient. Both oral and transdermal estrogens provide symptom relief for menopausal symptoms and have bone-sparing effects with equal efficacy. (33) There has been an expansion in the forms and regimens available for MHT. The most significant shift has been the transition from oral estrogens to transdermal products. Within the transdermal category, there has been an increase in the use of compounded 'bioidentical estrogens' that are not approved by regulatory authorities. These compounded products may include estrone and estriol, in addition to estradiol, and often combine progestogens and androgens.(34) Pharmaceutical-grade, FDA-approved 'bioidentical' transdermal estradiol is available as a patch or gel. Notably, transdermal estrogens are exclusively estradiol, whereas oral preparations include a variety of formulations, with the most extensive data available for conjugated equine estrogens (CEE), which

predominantly contain estrone sulfate and equilin sulfate, with minimal estradiol content. Other commonly used oral estrogen preparations include estradiol, esterified estrogens, and estropipate. Unlike estradiol and other oral estrogen preparations, the metabolic conversion of the mixed estrogens in CEE results in the circulation of estrogens with varying receptor affinities, exhibiting both estrogen receptor agonism and antagonism, or selective estrogen receptor modulator-like activity. The increasing use of transdermal estrogens was initially driven by observational data suggesting a lower risk of thrombotic complications. (19,35) Transdermal preparations can also be more convenient for patients, as patches are applied once or twice weekly, while oral estrogen must be taken daily. Combination patches with estrogen and progestogen are available on some formularies. However, for women with a uterus, this convenience is reduced since a progestogen must still be taken daily. Transdermal gels and creams also require daily administration and are less popular than patches. Transdermal estrogen preparations were associated with a lower incidence of cardiovascular complications (including CHD, angina, heart failure, stroke, transient ischemic attacks, pulmonary embolism, and other venous thromboembolisms) compared with oral estrogen preparations. Two large case–control studies in the UK found that oral HRT regimens were associated with increased venous thromboembolism while transdermal use was not.(36,37) Symptoms such as vaginal atrophy, dryness, dyspareunia would be best solved with vaginal, local administration of estrogen. Transdermal estrogen in the form of creams and gels is dosed in numbers of pumps and put anywhere on the body, avoiding the breast area. Oral route of administration of estrogens requires a higher dose since its bioavailability is changed due to the first-pass metabolism in the liver and also the consequences of use of estrogens on the liver should be paid attention to and monitored. (38)Formulations with estrogenic activity according to the type and routes of administration are given below in Figure 1. (39)

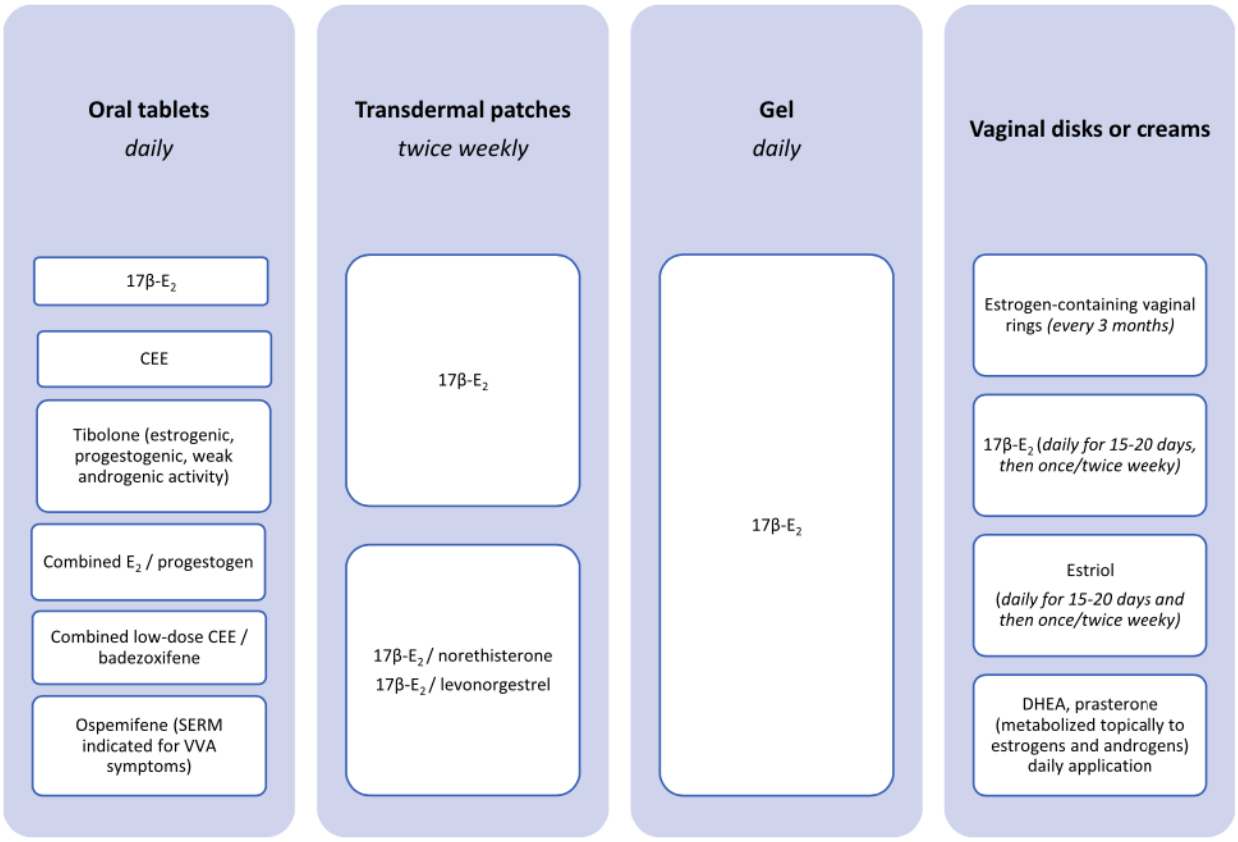

Figure 1. Formulations with estrogenic activity according to the type and routes of administration(39)

4.4 Progesterone therapy

The main reason for using progestogens in menopausal hormone therapy (MHT) is to offer protection against endometrial hyperplasia and consequent endometrial cancer abnormal vaginal bleeding. Progestins related to progesterone are classified into two groups. The one is the 17 alpha-hydroxyprogesterone derivatives (pregnanes) and the other is 19-norprogesterone derivatives (norpregnanes). The class of pregnane derivatives include the non-acetylated pregnanes which are dydrogesterone and medrogestone, and the acetylated pregnane derivatives which are medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate and cyproterone acetate. The norpregnane derivatives include nomegestrol acetate, nesterone, demegestone, promegestone, and trimegestone. The properties of the 17 alpha-hydroxyprogesterone derivatives include more peripheral effect in the body, moderate inhibition of gonadotropins, antiandrogenic effects and

good tolerability. Synthetic progestogens structurally related to testosterone are divided into ethinylated compounds and non-ethinylated compounds such as dienogest and drospirenone. Ethylated derivatives are classified into 19- nor-testosterone derivatives (estranes) norethyndronel, ethynodiol diacetate and lynestrenol, and in 13-ethylgonanes, like levonorgestrel, desogestrel, norgestimate and gestodene. Some properties of 19-nortestosterone derivatives include high bioavailability when given at least 15 days per cycle. They also have a mild androgenic action and rapid absorption, while ethylgonans are more powerful than estranes with less androgenic action. (40,41) Synthetic progestins, which encompass all forms of synthetic progesterone, possess a chemical structure distinct from that of naturally occurring progesterone produced by females. Although synthetic progestins mimic certain actions of natural progesterone, they interact differently with progesterone receptors. Despite having structural similarities to natural progesterone, synthetic progestins exhibit different potencies and pharmacokinetic properties. Consequently, the physiological effects of various progestins depend not only on their intrinsic properties but also on their binding affinity to their receptors. Additionally, there is a statistically significant association between the use of combined hormone therapy and the occurrence of tumors that are positive for both estrogen receptors (ER+) and progesterone receptors (PR+). (42–44) Most progestogens are administered *per os*. Progesterone can, also, be administered directly to the endometrium via intrauterine device, an intrauterine hormone release formulation (LNG-IUS). Levonorgestrel and norethisterone are available in the form of transdermal patches combined with estradiol. Lastly, the levonorgestrel-releasing intrauterine device provides both contraception and protection against endometrial hyperplasia; its activity lasts up to 5 years. (45,46) At recommended doses, higher plasma levels of progesterone and the synthetic progestins are attained after intramuscular (IM) or intravaginal administration than after oral administration. Also, because of the existence of different metabolic pathways depending on the route of administration, a different set of progesterone metabolites can be produced, some of which may be physiologically active and induce sedative-like effects. (47)

In Figure 2 we can see different progestogens used in HRT, according to route and mode of administration. (39)

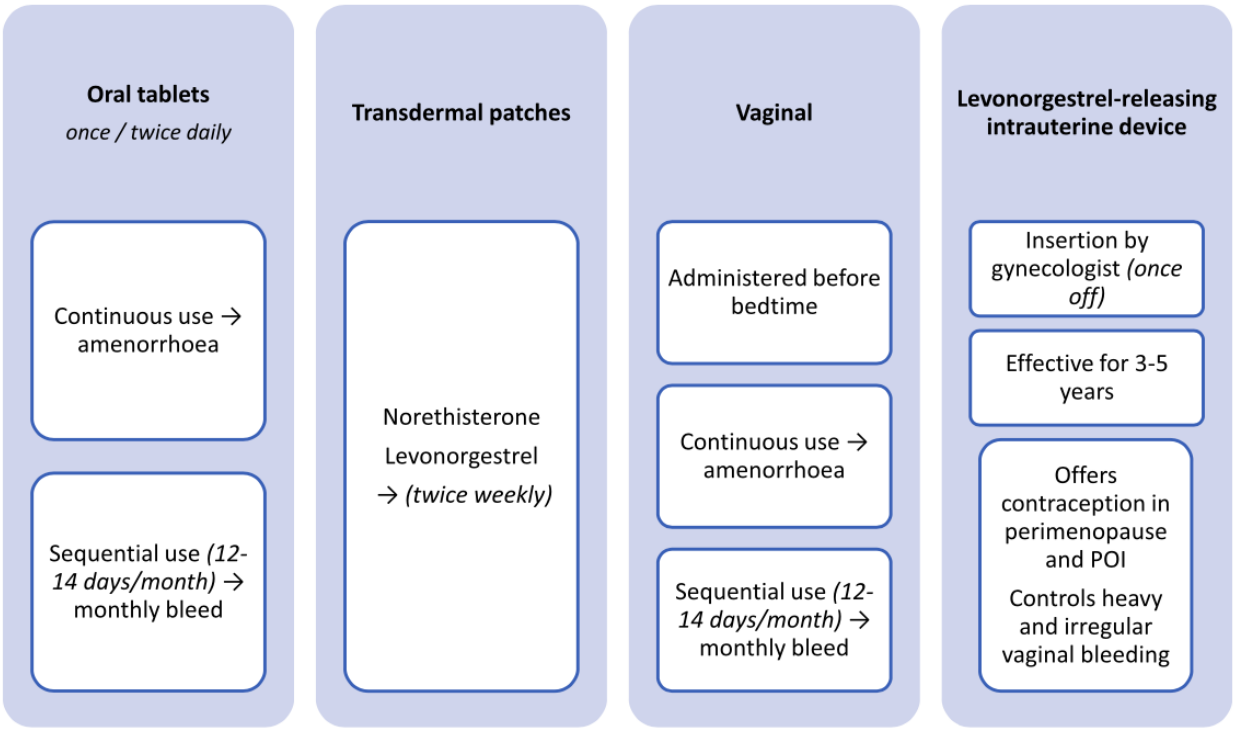

Figure 2. Different progestogens used in HRT, according to route and mode of administration(39)

4.5 Contraindications and side effects of MHT

Contraindications for oral and transdermal hormone therapy include unexplained vaginal bleeding, liver disease, personal history of estrogen receptor positive cancer (including breast cancer), prior coronary heart disease, stroke, MI, or VTE or personal history and inherited high risk of thromboembolic disease. Potential risks of HRT for women aged younger than 60 include the rare risk of breast cancer with estrogen-progesterone therapy, endometrial hyperplasia with consequent endometrial cancer when there is inadequately opposed estrogen, VTE and gallbladder disease. (48) Side effects include most commonly nausea, bloating, weight gain and fluid retention (decreased diuresis), mood swings that are related to the progestogen component of therapy, AUB (breakthrough bleeding), headaches and breast tenderness. From this we can conclude that the best way to go in prescribing HRT is to choose the lowest possible, effective dose of therapy that is consistent with treatment goals while providing benefits and minimizing risks and possible side effects. (49)

4.6 Duration of use of MHT

Hormone replacement therapy remains the first-line and most effective treatment for menopausal symptoms. (50) In symptomatic postmenopausal women, quality of life and sexuality are improved by HRT and, in the presence of symptoms of androgen deficiency, by additional androgen administration. (51) It is impossible to predict whether individual women will still be symptomatic or not when they stop systemic MHT. The limited evidence available does not indicate whether it is better to slowly lower the dosages or to stop abruptly, and equally there is no evidence regarding the length of time for which either systemic or low-dose vaginal MHT should be taken. Therefore, any limits on duration of use are subjective, and therapy should continue for as long as the woman feels the benefits outweigh the risks for her, and decisions must be made on an individual basis. (52)

4.7 Progesterones and Carcinogenesis

Several risk factors contribute to the development of breast cancer. These include advancing age, female gender (with women being significantly more prone to breast cancer than men), race (higher incidence among individuals of white ethnicity), elevated estrogen levels (associated with factors such as estrogen-dependent tumors, early onset of menarche before age 12, late menopause after age 55, nulliparity, postmenopausal obesity, delayed age of first pregnancy, inadequate breastfeeding duration, and habits like alcohol consumption and smoking also history of benign breast disease (especially when histologic atypia is present), increased breast density (women with denser mammographic breasts having a 4-5 times higher risk), family history of breast cancer among first-degree relatives (especially if occurring at a young age and among multiple female relatives), personal history of breast cancer, and rare inherited mutations predisposing to breast cancer. (53–55)

Mammary gland differentiation, crucial for inhibiting cell proliferation and modulating estrogen/progesterone receptor (ER/PR) regulation, plays a pivotal role in breast cancer prevention. Breast cancer typically arises in breasts with suboptimal differentiation. Progesterone, acting in sync with estrogen via specific receptors (PR) on breast epithelial cells, contributes to

breast development regulation. The role of progestogens in mammary carcinogenesis remains less elucidated, with varying effects observed in cell culture and various animal studies—ranging from proliferative to neutral or antiproliferative outcomes. (50,56,57) Molecular and cellular studies have provided significant insights into the role of progesterone in breast cancer development and progression. These studies explore the interactions of progesterone and its receptors at the cellular level, the influence of progesterone on gene expression, and its effects on breast cancer cell proliferation, apoptosis, and metastasis. The studies have shown that breast cancer cells often express progesterone receptors (PRs), which are activated by progesterone. PRs exist in two main isoforms, PR-A and PR-B, which can have different and sometimes opposing effects on gene expression and cell behavior. PRs are often co-expressed with estrogen receptors (ERs), and estrogen can regulate PR expression. The interaction between ER and PR pathways is crucial in understanding the hormonal regulation of breast cancer cells. Upon binding to progesterone, PRs translocate to the cell nucleus and act as transcription factors, regulating the expression of genes involved in cell proliferation, differentiation, and apoptosis. PR activation can also interact with various signaling pathways, including the MAPK and PI3K/Akt pathways, influencing cell survival and growth. (58) Progestogen actions on breast tissue involve complex mechanisms including interactions with steroid receptors, growth factors, oncogenes, and estrogen-metabolizing enzymes. (50) Notably, therapy is not linked to increased breast cancer risk. One hypothesis suggests that progesterone may augment estrogen's proliferative effect on breast tissue, this needing further investigation. Due to the diverse chemical structures, metabolism, pharmacokinetics, and potencies of progestogens, their effects on breast tissue can vary widely.(50) Response differences are influenced by factors such as co-administered hormones, treatment duration, and dosage concentration. Mitotic and nuclear antigen cell indices decrease with estrogen/natural progesterone but significantly increase with conjugated equine estrogen/medroxyprogesterone acetate (MPA) therapy. (56,59) *In vitro* evidence suggests MPA's proliferative impact on breast tissue. The safety of natural micronized progesterone is preferred over MPA regarding the context of the menopausal breast gland. The effect of progestins on breast cancer tumorigenesis may vary depending on the specific type of progesterone used in hormone replacement therapy. The interaction between hormone use and breast cancer progression may be influenced by progestin-mediated effects. (60–62)

4.8 Estrogens and Breast Cancer

There is a plausible biological hypothesis in regard to estrogen and mammary tumor growth. Especially estrogen influence on estrogen- induces apoptosis and the effect estrogen has in regard to the withdrawal of tumor growth. Finally, there is evidence in which the use of estradiol(6mg/d) is used as therapy for postmenopausal, advanced aromatase-inhibitor resistant, hormone-receptor positive breast cancer is supported. Keeping in mind the much higher dose of estradiol taken compared to the recommended dose of estradiol for vasomotor symptom management (0.5-1 mg/d). The critical factor for inducing apoptosis with estrogen is selecting breast cancer cell populations that are resistant to long-term estrogen deprivation. However, these cells can develop estrogen-independent growth. (63–66)

5. BREAST CANCER

5.1 Epidemiology and Risk Factors

Breast cancer is the most common occurring malignancy in women worldwide. Breast cancer is highly heterogeneous in its pathological characteristics, some cases showing slow growth with an excellent prognosis, while others being aggressive tumors. Current predictions and statistics suggest that worldwide the incidence of breast cancer and related mortality are on the rise. (67)Two aspects are believed to contribute to this, first being implementing country screening programs that benefit in early detection of the disease, and the second thought to being social changes that increase breast cancer risk such as delayed childbearing, fewer pregnancies and reduced breastfeeding. Some risk factors are age and gender, family history of breast cancer, especially positive family history for Breast Cancer Gene (BRCA) mutations, reproductive history, postmenopausal obesity (due to estrogen conversion in fat tissue), mammographic density, ionizing radiation, alcohol consumption, geographical factors. (68)

5.2 Pathogenesis

Almost all breast malignancies are adenocarcinomas and we can divide them into three major groups: ER positive (HER2 negative), HER2 positive (ER positive or negative) and Triple negative

(ER, PR, HER2 negative). The three major subtypes arise through distinct pathways that involve the acquisition of driver mutations in the epithelial cells of the duct/lobular system. (6) Genetic factors that contribute to development of breast cancer happen through driver mutations in cancer genes which can be inherited and acquired.(69) The major germline mutations affecting susceptibility to breast cancer affect genes that are responsible for regulating genomic stability or that are involved in signaling pathways. BRCA1 and BRCA2 are tumor suppressor genes and cancer will arise only when both alleles are defective or inactive. Other mutated genes that are related to breast cancer are Tumor Protein p53 (TP53) and Phosphatase and Tensin Homolog (PTEN). Another important driver mutation happening in breast cancer is the amplification of HER2. (6)

5.3 Symptoms

Most symptomatic women with breast cancer have relatively short intervals from presenting with the problem to diagnosis but a minority still experiences delays in diagnosis. (70) Detection of a breast mass is the most common reason for which women will seek professional medical help. In 90% of cases breast masses are benign. Also, a breast mass can be detected in the yearly screening program women undergo. Breast pain is another presenting problem, it is rarely attributed to breast cancer and more to fibrocystic changes in premenopausal and postmenopausal women taking MHT. (71) Erythema, edema and retraction of skin are often associated with malignancy. A very tell-tale sign is the “peau d’orange” (orange peel) appearance of the breast due to chronic edema that produces such an induration. (6) Another common presenting problem is nipple discharge, which can be attributable to benign lesions and hormonal fluctuations, but unilateral, spontaneous and bloody discharges should be red flags for further investigation into a possible diagnosis of breast carcinoma. (72)

5.4 Diagnosis and Treatment

The basis for diagnosis of breast cancer is standard pathomorphological diagnostics. Parts of the histopathological diagnostics encompass the histological type of the tumor, its degree of histological malignancy, the degree of advancement according to the TNM classification, infiltration by cancer cells in vessels and also the expression of steroid receptors—estrogen and

progesterone, HER-2 receptor, and cellular proliferation index Kiel 67 (Ki67). (73) The materials used for such tests are taken by a coarse needle biopsy, intra- or postoperative material. (74) Fine needle biopsy is also an option but by this procedure no information about whether the cancer is infiltrating or pre-invasive as well as to assess the HER2 status. The stage of breast cancer according to TNM classification remains the most important prognostic factor. All individual features of the TNM classification have prognostic importance (T – tumor size, N – lymph node involvement, M – distant metastases). The expression of positivity of steroid receptors—estrogen and progesterone are also important due to the favorable value of prognosis and predictive value for hormonal treatment. This expression is assessed by immunohistochemical method of tissue material. HER2 expression also gives insight into targeted receptor treatment. (68) Basic diagnostic imaging incorporates mammography, ultrasound or Magnetic Resonance Imaging (MRI). Mammography remains the main diagnostic and screening tool in breast cancer detection. The types of surgical treatment are tumor excision, mastectomy, radical mastectomy, excision of sentinel node or excision of the armpit lymphatic system. Regarding therapy, there are possibilities of radiation, neo-adjuvant therapy, adjuvant therapy, targeted and biologic therapy for receptor positive and HER2 positive cancers. (75) Clinical staging of breast cancer from the American Joint Commission on Cancer Guidelines is given in Table 2. (76)

Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T0 T1	N1mi N1mi	M0 M0
Stage IIA	T0 T1 T2	N1 N1 N0	M0 M0 Mo
Stage IIB	T2 T3	N1 N0	M0 M0
Stage IIIA	T0 T1 T2 T3 T3	N2 N2 N2 N1 N2	M0 M0 M0 M0 M0
Stage IIIB	T4 T4 T4	N0 N1 N2	M0 M0 M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Table 2. Clinical staging-American Joint Commission on Cancer guidelines. (76)

5.5 Hormone Receptor Positive Breast Cancer

Most breast cancers show overexpression of estrogen and progesterone receptors. Information about receptor positivity is extremely important to predictive factor for endocrine therapy. ER and PR assays should be performed in all invasive breast cancers.(75)Development of Selective Estrogen Modulator (SERM) medication has made a huge step in the positive direction treatment of these hormone – specific cancers. (77) The current recommended definition of ER or PR positivity is if 1% or more of cells taken stain positively. The scale that is used to determine the

hormonal status of tumors is called the Allrad Scale, in which the percentage of stained nuclei of cancer cells and the strength of coloration is assessed. The total value is the sum of both parameters, with the maximal score of 8. In practice, only the percentage of stained nuclei of cancer cells is considered. (75,78) Every patient with a positive result of receptor positive breast cancer should undergo hormone therapy regardless of age, the lymph node involvement or additional indications for chemotherapy due to the efficacy of complementary treatment with SERM medication and aromatase inhibitors. (68,79,80)

6. MENOPAUSAL HORMONE THERAPY AND BREAST CANCER- RANDOMIZED TRIALS

The use of menopausal hormone therapy radically declined after 2002, mostly due to the Women's Health Initiative's report that indicated that the combination of conjugated equine estrogen and medroxyprogesterone acetate increased breast cancer risk and did not enhance quality of life. However, more recent WHI publications recognize HT as the most effective treatment for menopausal vasomotor symptoms and report that CEE alone reduces breast cancer risk by 23% and decreases breast cancer mortality by 40%. The only remaining concern is a slight increase in breast cancer incidence with combination therapy of CEE+MPA (1 per 1,000 women per year), but with no associated increase in breast cancer mortality. (81)Breast cancer is the most commonly diagnosed cancer in women worldwide, so an understanding of the potential effect of hormone replacement therapy on breast cancer risk is very important. (82) There were also 5 smaller randomized trials ongoing in the 1990s period with different information on breast cancer incidence and mortality. Details of relevance will be presented here. Two of the trials, the PEPI (Postmenopausal Estrogen/Progestin Intervention trial) and the ERA (Estrogen Replacement and Atherosclerosis tral) trials reported only 3 cases of breast cancer. However, they were designed to provide information on concepts regarding estrogen-alone influence on cardiovascular disease and other clinical outcomes in 1990-1996.

6.1 Women's Health Initiative

The National Institute for Health (NIH) conducted a number of randomized clinical trials starting in 1991. for the purpose to investigate the main health problems that cause morbidity and mortality in postmenopausal women.

The motivation for WHI initiation was a lack of comprehensive research and large-scale studies investigating the effects of various interventions and prevention of chronic conditions such as CVD, osteoporosis and cancer. The WHI consisted of three randomized trials and one observational study:

1. Hormone Therapy Trials (PHT): These trials evaluated the effects of estrogen-alone therapy (for women who had undergone a hysterectomy) and combined estrogen-plus-progestin therapy (for women with an intact uterus).

Primary Endpoints:

1. Combined Estrogen-Progestin Therapy:
    - Primary Endpoint: Incidence of coronary heart disease (CHD), which includes nonfatal myocardial infarction (heart attack) and CHD death.
2. Estrogen-Alone Therapy:
    - Primary Endpoint: Incidence of coronary heart disease (CHD).

Secondary Endpoints:

- Incidence of invasive breast cancer.
- Incidence of stroke.
- Incidence of pulmonary embolism (blood clots in the lungs).
- Incidence of colorectal cancer.
- Incidence of hip fractures and other osteoporotic fractures.
- All-cause mortality.
- Quality of life and menopausal symptoms.

2. Dietary Modification Trial (DM): This trial assessed the impact of a low-fat diet

Primary Endpoints:

- Incidence of invasive breast cancer.

- Incidence of colorectal cancer.

Secondary Endpoints:

- Incidence of coronary heart disease (CHD).
- Stroke.
- Total cardiovascular disease.
- All-cause mortality.
- Other site-specific cancers.

3. Calcium and Vitamin D Supplementation Trial (CaD): This trial investigated the role of supplements

Primary Endpoints:

- Incidence of hip fractures.

Secondary Endpoints:

- Incidence of total fractures.
- Incidence of colorectal cancer.
- Incidence of other cancers.
- Cardiovascular outcomes.
- All-cause mortality.

4. Observational Study (OS): This component followed a large cohort of women to identify risk factors for disease and to understand the natural history of health issues in postmenopausal women

Primary Endpoints:

- Identifying risk factors for major chronic diseases, including coronary heart disease, cancer (especially breast and colorectal), and osteoporotic fractures.
- Understanding the natural history and course of these diseases in postmenopausal women.

Secondary Endpoints:

- Developing risk prediction models for various diseases.
- Studying the impact of lifestyle factors, diet, and genetic factors on disease risk.

- Understanding the effects of different hormone regimens in the real world.

Figure 3 shows the outcomes for each arm of the WHI Clinical Trial. (83)

Outcome	PHT	DM	CaD	OS
Cardiovascular				
Coronary heart disease	1°	2°	x	x
Stroke	2°	2°	x	x
Congestive heart failure	2°	2°	x	x
Angina	2°	2°	x	x
Peripheral vascular disease	2°	2°	x	x
Coronary revascularization	2°	2°	x	x
Venous thromboembolic disease				
Pulmonary embolism	2°	x	x	x
Deep vein thrombosis	2°	x	x	x
Total cardiovascular	2°	2°	x	x
Cancer				
Breast	2°	1°	2°	x
Colorectal	x	1°	2°	x
Endometrial	2°	2°	x	x
Ovarian	2°	2°	x	x
Total cancers	2°	2°	2°	x
Fractures				
Hip	2°	x	1°	x
Other fractures	2°	x	2°	x
Total fractures	2°	x	2°	x
Other				
Diabetes mellitus requiring therapy	x	2°	x	x
Death from any cause	2°	2°	2°	x

"1°" indicates primary outcome; "2°" secondary or safety outcomes; "x" ascertained.

Figure 3. Outcomes for each arm of the WHI Clinical Trial(83)

The Hormone Therapy trials of the Women's Health Initiative trial , started with the hypothesis that MHT use in postmenopause can decrease the risk of cardiovascular disease. The primary protocol-defined monitoring of the trial was testing the connection between CVD and MHT, not the hypothesis of menopausal hormone therapy and breast cancer. All test subjects were randomized and categorized according to risk factors for coronary artery disease (CAD), not breast cancer risk factors. So, the CAD was the primary endpoint, while breast cancer was the secondary endpoint. This is important to emphasize as it is well-known that secondary endpoints are additional events of interest, but for which the study is not specifically powered to assess. So as the design of the study is not based around secondary endpoints, the analyzes of secondary endpoints need to be viewed with caution.

The WHI-CEE+MPA trial cohort was comprised of women who were on average 63.3 years old and 12 years since menopause, with an average body mass index (BMI) of 28.5 kg/m^2 (overweight) in which 34.1% had a BMI >30 kg/m^2 (obese), the mayority were smokers, 10-15% had a history of cardiovascular disease and 40% had hypertension, 1,906 women at enrollment were taking medication to lower cholesterol blood levels, 5,988 were on medication to lower their blood pressure, 296 had prior heart attacks, 472 had a history of angina, and 138 had a history of prior strokes. It is important to note the fact that 73% of the women in the WHI study with a uterus had never previously received any form of MHT. Only 33% of the WHI women who were given CEE/MPA were <60 years. (84)As such, a major critical issue with the WHI-CEE-MPA trial has always been the overgeneralization of data generated from overweight-obese women more than a decade since menopause to the typical population of women near the time of menopause when initiating MHT. The WHI MHT trials were not intended to evaluate the common clinical use of MHT initiated near menopause and not statistically powered to do so; only 10% of the WHI cohort was 50–54 years of age when started on randomized MHT(85) So the WHI's PHT randomized, placebo-controlled trials using long-term follow-up data enrolled 27,347 healthy postmenopausal women (mammogram clearance before entry) aged 50-79 years at 40 US clinical centers during 1993-1998, including 10,739 post-hysterectomy patients in a trial consisting of conjugated equine estrogen and 16,608 participants with a uterus in the trial of CEE plus medroxyprogesterone acetate. Patients were randomized for receiving either CCE or placebo in estrogen-only trial, while in estrogen plus progestin trial patients were randomized for reciveing CEE+MPA or placebo.

Participants were contacted at 6-month intervals regarding clinical outcomes through 2005, and then annually. (86)Both trials were terminated early, with median intervention periods of 5.6 years for the CEE + MPA trial and 7.2 years for the CEE-alone trial. Participants were instructed by e-mail to discontinue the study drugs immediately upon the publication of the study results (February 2004, for CEE alone and July 2002, for CEE+MPA). Surveys conducted 8 to 12 months post-intervention indicated limited use of nonprotocol hormone therapy, with 4.3% in the CEE+MPA group and 4.5% in the CEE-alone group. Subsequent annual assessments from 2005 to 2010 found that less than 4% of women reported personal use of hormone therapy during the first extension phase. During the second extension phase (2011-2012), personal use of hormone therapy remained low at less than 4%. (86–88) Although participants were similar in age, CEE-alone trial participants were more likely to be African-American, obese (increased BMI), reporting prior hormone therapy use, and had a bilateral oophorectomy procedure than women in the CEE- plus-MPA trial. (86) The trial had an 18-year-follow up period and the results were the following: use of CEE alone compared with placebo among 10 739 women with a prior hysterectomy was associated with significantly lower breast cancer incidence with 238 cases vs 296 cases in the control group. Furthermore, the use of CEE alone was associated with lower breast cancer mortality with 30 deaths vs 46 deaths in the placebo group. The results were non-significant for combined estrogen – progestin therapy (CEE plus MPA), but there was increased breast cancer risk and reduced endometrial cancer risk. The use of CEE plus MPA compared with placebo among 16 608 women with a uterus was associated with statistically higher breast cancer incidence with 584 cases vs 447 cases in the control group and no significant difference in breast cancer mortality with 71 deaths in the CE plus MPA arm vs 53 deaths in the placebo arm. (86)As detailed in the July 17, 2002 issue of JAMA (84) and an NIH news release(89), the Data and Safety Monitoring Board (DSMB) recommended halting the hormone therapy (HT) versus placebo trial due to the risks surpassing the benefits. (90) The collected statistical data revealed an increase in breast cancer cases, and the global index statistic further indicated that the overall risks outweighed the benefits. The global index, which combines monitored outcomes such as coronary heart disease (CHD), stroke, pulmonary embolism, colorectal cancer, endometrial cancer, hip fracture, and death from other causes, has not been independently validated. (90)The WHI researchers had hoped that the global index would show an overall benefit despite expecting an increase in breast cancer cases.

However, this was not the outcome. There were no differences in mortality or cause of death between the treatment and placebo groups. (84) In the 18 years follow up period, CEE alone therapy breast cancer mortality was reduced by 45%. (91) The CEE+MPA data shows that the breast cancer risk elevation was affected while mortality wasn't significantly affected. ((86,92) This misleading conclusion contradicts WHI data that showed no increased breast cancer risk with CEE+MPA treatment among women who had not taken hormone therapy before entering the WHI trial. (93,94) Additionally, there was no increased risk with this therapy in women with a positive family history of breast cancer and for the CEE+MPA group with statistical adjustments per protocol for this secondary outcome. (93,95) There were various limitations in this study, firstly breast cancer mortality analyses were not mentioned in the protocol, which means the study had constraints and breast cancer death is the most relevant clinical outcome. Secondly, the clinical trials evaluated these MHT regimens with only 1 dosage, 1 route of administration and formulation of the trial drugs so these findings are not generalizable to other formulations, routes and preparations. Lastly, as this trial was discontinued, participants stopped using their chosen therapy. (86) Another interesting fact that did elevate the ratio of cancer in the CEE+MPA group was the extremely low incidence of breast cancer that was in the placebo group, taking into account this is the most common malignancy in women one can conclude that the result in the drug trial group was also artificially elevated. When women who had used hormone therapy (HT) before joining the CEE+MPA trial were excluded from the analyses, thereby reflecting the typical experience of most women who start hormone therapy during perimenopause - the unusually low incidence of breast cancer observed in the placebo group returned to its expected level, and the increased hazard ratio disappeared. The critical point is that the reason for the low incidence rate in the placebo group is irrelevant. Whether it is due to prior hormone therapy use, unequal covariates, or other factors, this low incidence rate in the placebo group artificially elevates the hazard ratio, which the Women's Health Initiative (WHI) misinterprets as an increase in breast cancer risk. (81,96,97) This is shown in Figure 4. (81)

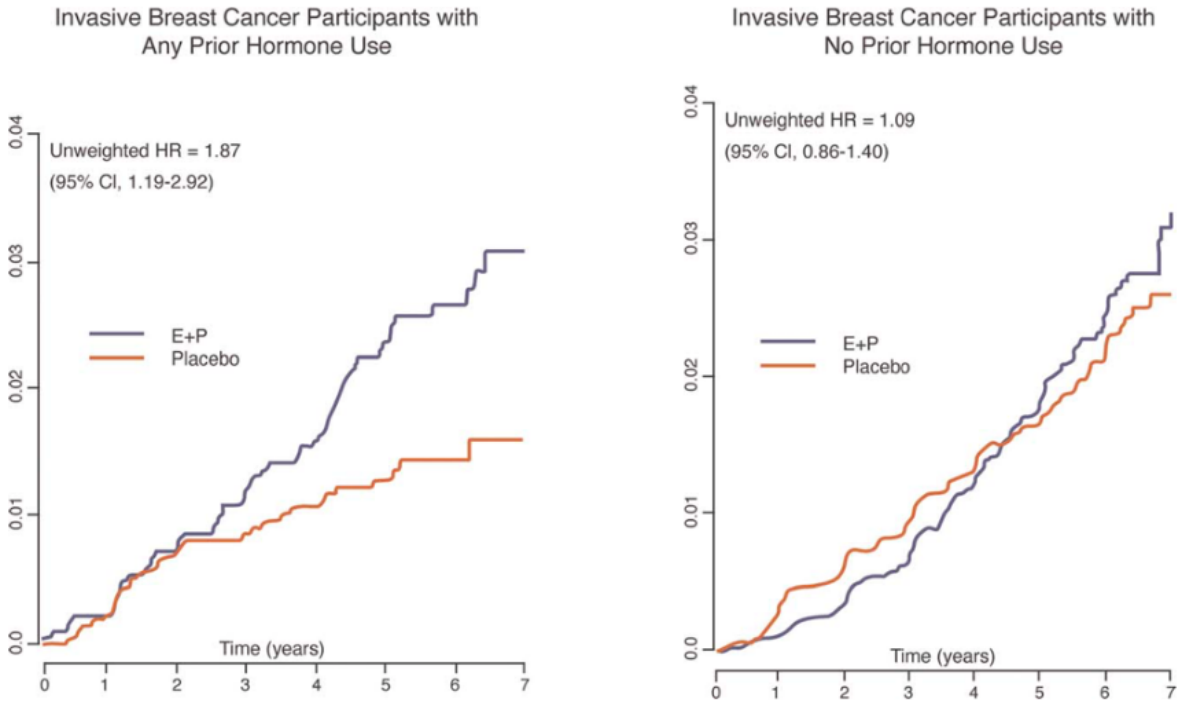

Figure 4. Risk of invasive breast cancer with and without prior hormone use(81)

6.2 PEPI Trial

The Postmenopausal Estrogen/Progestin Intervention (PEPI) trial, initiated in 1989, enrolled 875 healthy postmenopausal women aged 45 to 64, regardless of whether they had a uterus. There was no hormone therapy contraindication. Participants were randomly assigned to receive either a placebo, CEE alone, or CEE combined with one of three different progestin regimens. The primary outcomes measured were circulatory markers or mediators associated with CVD. (98)The positive influence on study endpoints, reported in 1995, suggested a CVD benefit. Regarding breast cancer, breast cancer was reported in 1 of 175 patients in the placebo group and 1 of 174 patients in the CEE-alone group.(99)

6.3 ERA Trial

The Estrogen Replacement and Atherosclerosis (ERA) trial, initiated in 1995, involved 309 women aged 55 and older with coronary disease confirmed by angiography. The primary endpoint was the change in coronary artery diameter. Participants were randomly assigned to receive either

a placebo, CEE alone, or CEE with MPA. Since 44% of the women with a uterus were randomized to the CEE alone group, concerns about the risk of endometrial cancer remained unresolved at those times. (99) The main finding of the ERA study was that MHT did not significantly slow the progression of atherosclerosis in women with existing heart disease.(100) So, ERA study did not primarily focus on breast cancer incidence but rather on the impact of MHT on atherosclerosis progression in postmenopausal women with established coronary artery disease. However, some secondary outcomes, including adverse effects such as breast cancer, were monitored. According to the results of the ERA study, there was no statistically significant increase in breast cancer incidence among hormone replacement therapy users compared to the placebo group during the study period.

6.4 WEST Trial

The Women's Estrogen for Stroke (WEST) trial, initiated in 1993, enrolled postmenopausal women who had experienced a stroke or transient ischemic attack within the past 90 days. The primary outcomes were overall mortality and nonfatal stroke. The participants had a mean age of 71 years and were randomized to receive either a placebo or estradiol 17β. Breast cancer was recognized as a potential adverse effect; thus, women with a history of breast cancer were excluded from the trial. Clinical breast examinations and mammograms were required before entry, and annual mammograms were conducted. During the trial, 5 out of 327 women in the placebo group and 5 out of 337 women in the estradiol 17β group developed breast cancer. (99) Therefore, WEST found that estrogen use did not significantly affect the risk of breast cancer. Specifically, the findings indicated no significant increase or decrease in the incidence of breast cancer among women who were taking estrogen compared to those who were not. This trial provided important insights into the safety profile of estrogen use, particularly concerning breast cancer risk. (99)The study, concluded in 2001, found that estradiol 17β did not reduce mortality or the recurrence of stroke. (101)

6.5 DOPS Trial

The Danish Osteoporosis Prevention Study (DOPS) trial, started in 1990, randomized healthy women who were recently postmenopausal or had perimenopausal symptoms with supportive serum FSH values to no therapy or estradiol-alone if they had a hysterectomy or estradiol plus norethisterone if the women had a uterus.(102) History of breast cancer was an exclusion criterion while no mammography was provided. The breast cancer incidence was the following: there were 9 of 97 breast cancers in the placebo group vs. 6 of 95 breast cancers in the estradiol group. Additionally there was a statistically significant reduction in breast cancer mortality in the estradiol only group. (99)

6.6 ESPRIT Trial

The oEStrogen in the Prevention of Reinfarction (ESPRIT) trial had 1017 women with or without a uterus, surviving their first myocardial infarction, aged 50 to 69 years (mean, 63 years) in it. They were randomized to receive estradiol valerate or placebo for 2 years. (103)Previous breast cancer was an exclusion. The primary outcomes tested for the 14-year follow-up were death and any cancer incidence. There were 15 in 504 breast cancers in the placebo group versus 7 in 513 breast cancers in the estradiol valerate group and the conclusion was that "unopposed estrogen may be used safely by women with an intact uterus surviving a first MI." This is a conclusion that current findings on estrogen therapy and endometrial cancer do not support. (99)

6.7 Discussion

When results of the "smaller" randomized trial are added together, it can be concluded that there were 17 cases of breast cancer in 1220 women undergoing the trials in the estrogen groups and 28 breast cancers in 1206 women that were in the placebo/no therapy groups. Looking at these 5 "smaller trials", and comparing the results to the results of the larger, WHI randomized control trial it can be concluded that the results of the lower breast cancer incidence and mortality in the CEE-alone group are not accidental and an isolated outcome. This can be additional reassurance for women with a prior hysterectomy, considering starting estrogen therapy in menopausal symptom management. Furthermore, for this group of women, a 35% lower risk for coronary heart

disease was reported, all cancers, excluding nonmelanoma skin cancers were 20% lower and all-cause mortality was 21% lower.(9,48,86,92) All of these findings are statistically significant. Some study limitations were different primary outcomes in the studies and different estrogens evaluated. (99) A research project was started as a prospective study aiming at identifying differences in the expression profile of genes by taking core needle biopsies taken from the breasts of postmenopausal women. Breast tissue samples were taken from 33 healthy postmenopausal women both before and after treatment with either 2mg micronized estradiol, 2mg micronized estradiol and 1mg norethisterone acetate (E2+NETA), 2.5mg tibolone or no MHT. Knowing the gene expression patterns in breast cancer tumors that exist may be used to identify risk factors in the breast tissue of healthy women. The effects of estradiol, estradiol together with progestins, or tibolone on gene expression related to breast cancer in normal breast tissue was taken from postmenopausal women and may be used to identify the gene expression profile in healthy women that may be associated with a higher risk of getting breast cancer. The results conferred that the treatment of women with micronized estradiol+norethisterone acetate yielded the highest number of differentially regulated genes. The mammary cells triggered by this hormone formulation differentiated by steroidogenic up-regulation through down regulation of the estrogen-receptor pathway. Micronized estradiol alone and tibolone were associated to a lesser extent and not at all with a change in expression of genes involved in breast cancer. This study was conducted on only 33 women, randomly assigned to hormone formulations except for the hysterectomized women which received only micronized estrogen and can provide the basis for genome analysis to identify different markers involved in increased risk of getting breast cancer in the future. (104)

Many facts from women's personal histories contribute to the possible increased risk of getting breast cancer while using MHT. Starting from positive family history for BRCA1 and BRCA2 genes. Women with a BRCA1 mutation have a 55-65% chance of developing breast cancer by age 70, while women with a BRCA2 mutation have about a 45-55% chance of developing breast cancer by age 70. By comparison, the average woman in the general population has about a 12% (or 1 in 8) risk of developing breast cancer over her lifetime. (105) A relatively short time of usage of MHT did not increase the risk of developing breast cancer in BRCA1 positive women.(106) BRCA-associated breast cancer tend to be hormone receptor negative, there is a biologically

backed theory that MHT would likely not impact breast cancer risk in this group of women. (107) Another risk factor that is checked yearly through mammography screening programs is breast density. Radiographic appearance of breast tissue on mammography exhibits variability among women. On mammography, fat appears dark while connective tissue and epithelial tissue are denser and appear white. This is the basis for determining mammographic density, and the proportion of dense vs lucent breast areas (PMD). It has been proven that characteristics of breast density strongly correlate with the chance of developing breast cancer. There is a four-to-six fold gradient in risk among women with a PMD of over 75% compared to those with a PMD of 10% or less. (108) While menopause and the decline that happens in estrogen production and blood level estrogen level result in a decrease in breast density, the opposite may happen in women who use MHT. (109) There have been studies that show a slight increase in PMD over the years while using MHT (1.6% change in 1 year of usage). (110) Recommending a close mammographic monitoring to detect alterations in breast density after the initiation of MHT is highly recommended and encouraged. On note, relevant further data on dosages, hormone combinations and routes of delivery is still missing. (111) Two issues in the WHI's statistical analyses underscore the lack of significant correlation between CEE-MPA and breast cancer. First, the WHI continues to report "nominal" rates as the primary statistic. This term refers to a "simple, unadjusted" analysis that does not account for factors that could lead to a false-positive result. The initial 2002 claims of increased breast cancer risk were based on this method, yielding a nominal, unadjusted hazard ratio (HR) of 1.26 for breast cancer in participants randomized to CEE+MPA, which "almost reached nominal statistical significance" (95% CI, 1.00-1.59). However, per-protocol adjustments for multiple outcomes and repeated data analysis reported in the same article found a 95% CI of 0.83 to 1.92. (112) The WHI's 2003 article, which also examined the link between CEE+MPA and breast cancer, reported an HR of 1.24, claiming statistical significance with a nominal 95% CI of 1.01 to 1.50. (113) Yet, with minimal adjustment for sequential monitoring (as required for this secondary outcome), it was not statistically significant (95% CI, 0.97-1.59). Moreover, the WHI protocol required multivariate adjusted analyses for secondary outcomes, including breast cancer in the HT trials. An analysis adjusting for breast cancer risk factors—such as age, ethnicity, body mass index, physical activity, smoking, alcohol use, parity, age at first birth, oral contraceptive use, family history of breast cancer, and mammography use—was published in 2006 by Anderson

et al. (93) This per-protocol adjustment showed that the association between CEE+MPA and breast cancer was not statistically significant (HR, 1.20; 95% CI, 0.94-1.53). (114) These findings indicate that the apparent difference between CEE+MPA and placebo was due to an imbalance of baseline risk factors. Indeed, whenever the WHI has reported adjustments for sequential monitoring, multiple outcomes, or risk factors, the results have not shown a statistically significant association between CEE+MPA and breast cancer.

Prescribing MHT should be done in an individualized manner for each patient while always keeping in mind if the benefits of such therapy outweigh the risks. Further analysis of the WHI randomized controlled trial concluded that for women who weren't on MHT prior randomization to WHI, the breast cancer incidence rate was not affected by CEE + MPA therapy relative to placebo. It actually had a null effect on them and this is the typical subgroup of women receiving MHT, who are MHT naive before receiving menopausal MHT. The present scientific evidence indicates that MHT may or may not cause breast cancer but the totality of data neither establish nor refute this possibility. Further, any association that may exist between HRT and breast cancer appears to be rare and isn't more dangerous than using any other medication commonly prescribed in clinical medicine. Although different combinations and types of estrogen and progestogen, doses, timing of initiation, duration of therapy, and individual characteristics of patients all play a possible role in the effect of HRT on breast tissue, all conclusions that HRT causes breast cancer have not been definitely proved by anyone, including the WHI. The subgroup of women on CEE+MPA therapy were on average 63.3 years old (12 years since start of menopause) and had an increased BMI putting them in the overweight/obese group, this brings up the problem of overgeneralization where the test subjects were obese women that have spent more than a decade in menopause already contrary to women first getting prescribed HRT in perimenopause or in the early postmenopause period, which are the most common patients. When observing the CEE+MPA group, there was an increase of 8 breast cancer cases/10 000 women/year, which gives an increased but rare absolute risk of breast cancer that can be attributed to other commonly used medication such as lipid- lowering medication and anti-hypertensives. (85,91,93,115,116)As the 2 subgroups in the CEE+MPA group of women were not clearly differentiated, valuable information came from understanding that in the HRT naive group (no prior use of hormone

therapy) breast cancer incidence was not affected when compared to placebo after 5.6 and 11 years follow- up, while in the subgroup of women with prior use of hormone therapy, the breast cancer incidence was increased compared to placebo after 5.6 years and 11 years follow-up. (85,93,115) Smaller randomized trials have shown similar reductions in breast cancer with only estrogen therapy to the WHI trial with long-term use of up to 10 years and total follow-up for 16years. Also, smaller randomized trials have shown non-significant effects on breast cancer with combination of estrogen plus progestogen therapy to the WHI CEE+MPA trial with combination of hormone therapy for up to 10 years and total follow-up for 16 years. (93,117,118) Current recommendations indicate the use of HRT for vasomotor symptoms, prevention of bone loss, premature hypoestrogenism, genitourinary symptoms. For healthy women who are within 10 years of the menopause transition and who have significant and bothering menopause symptoms, the benefits of hormone therapy (whether estrogen therapy or estrogen-progestin therapy) outweigh the risks, with fewer CVD events in younger versus older women. (86,119) The risk of breast cancer in women (mean age, 63 years) randomized in the group of CEE+MPA therapy in the WHI trial is less than one additional case of breast cancer diagnosed per 1000 users of HRT yearly. (86) A risk that is slightly greater than that observed with one daily glass of wine, less than with two glasses a day, and similar to the risk with obesity and low physical activity. (116,120)When compared to placebo or women not using hormone therapy, there appears to be no additional effect of hormone therapy with age or increased personal breast cancer risk. The relative risk for breast cancer is similar in women at average or increased risk, in reality, the actual risk will be greater in women with an increased underlying risk. (121) Studies also suggest there is no increase in relative risk with taking HRT in women with a family history of breast cancer, for BRCA1 and BRCA2 positive women after undergoing an oophorectomy and women with a history of benign breast biopsies. (122–129) Hormone therapy for women that had breast cancer is not advised, and 2 randomized controlled trials showed conflicting evidence with one showing an elevated risk for recurrence and the other showing no effect on breast cancer recurrence in hormone therapy users compared to women not using it. With severe symptoms of estrogen deficiency that are unresponsive to nonhormone options, women can, with consulting an oncologist, choose HRT. (119,130–136)The safety of using HRT is most favorable when therapy is started in healthy women younger than 60 years/ within 10 years of menopause onset while initiation of therapy in women that are older

should be done with caution and weighing the risk-benefit ratio. No large RCTs have assessed the effect of long duration of hormone therapy use. Long-term continuation of therapy should take into account the ongoing symptoms a woman has, the risk for CVD or breast cancer and the existence of alternative therapies that are appropriate. There is no need to routinely discontinue HRT in women that are 65+ years old as already mentioned, a more preferred way is an individual approach and individual assessment of risks and benefits. (119) Any association of potential breast cancer risk with HRT is rare and no greater than other endogenous and lifestyle risks factors. (91) Had the WHI transparently reported their breast cancer findings in 2002, emphasizing the lack of statistical significance in breast cancer risk in the per-protocol adjusted statistics, promptly followed up with a per-protocol analysis adjusting for baseline breast cancer risk factors, and clarifiying that their findings did not apply to women initiating hormone therapy during perimenopause or early postmenopause, there would have been minimal controversy and confusion. Consequently, women's health would not have suffered as dramatically in the following decades. (81) A positive attitude should be maintained regarding HRT but with caution, considering the whole range of risks and benefits coming with it. (137)

7. CONCLUSION

Menopause is a significant event in every woman's life, bringing with it various symptoms and increased risks such as cardiovascular disease, osteoporosis, genitourinary issues, vasomotor symptoms, and cognitive disturbances. Most women will experience one or more of these issues during the perimenopausal and postmenopausal periods. Hormonal preparations are often prescribed to address these symptoms, with treatment options including estrogen-only regimens for hysterectomized women and combined estrogen-progestogen regimens. The routes of administration are numerous and tailored individually to best address each woman's specific complaints. It is always recommended to start with the lowest effective dose and monitor patients over time. Since the inception of menopausal hormone therapy, there has been ongoing debate about its potential link to breast cancer. Breast cancer is the most common malignancy among

women worldwide, with risk factors ranging from genetic predisposition and family history to environmental and social factors. Given that some breast cancers are estrogen receptor-positive and progesterone receptor-positive, concerns have arisen that MHT might increase breast cancer risk by upregulating these receptors through exogenous hormone administration. Although numerous studies have been conducted to address this concern, definitive answers remain elusive. The Women's Health Initiative (WHI) randomized controlled trial was the largest study to investigate the potential risks of menopausal hormone therapy. It found that estrogen-only therapy in hysterectomized women might offer a protective effect against breast cancer, whereas combined estrogen-progestogen therapy was associated with an increased incidence of breast cancer. The WHI study's subjects, averaging 63.3 years old and in menopause for over a decade, were predominantly overweight or obese. This demographic differs significantly from typical patients who begin MHT in perimenopause or early postmenopause. The WHI reported an increased but rare absolute risk of breast cancer (8 additional cases per 10,000 women per year) attributable to CEE + MPA therapy, a risk comparable to that of other medications like lipid-lowering drugs and antihypertensives. In women with no prior hormone therapy, breast cancer incidence was unaffected by CEE + MPA compared to placebo after 5.6 and 11 years. Smaller randomized trials have shown similar reductions in breast cancer with estrogen-only therapy and non-significant effects with combined estrogen-progestogen therapy over long-term use. Therefore, several factors must be considered when interpreting the influence of MHT on breast cancer incidence, including participants' age, weight, compliance, the limitation of using only one dose and type of progestogen, and the route of administration.

Current guidelines recommend an individualized and tailored approach to each patient, weighing the symptoms, risks, and benefits of starting or continuing MHT Any potential association between MHT and breast cancer appears to be rare and is not more dangerous than the use of other commonly prescribed medications., moderate alcohol consumption, obesity and low physical activity,

Various cohort and observational studies have examined large groups of women, and combining their results with those from the WHI and other randomized controlled trials may help determine

the potential risk of developing breast cancer while on MHT. For now, further research and updated guidelines are needed to clarify the possible connection between MHT and breast cancer.

Current guidelines recommend HRT for vasomotor symptoms, bone loss prevention, premature hypoestrogenism, and genitourinary symptoms. For healthy women within 10 years of menopause onset, MHT benefits outweigh the risks, with fewer CVD events noted in younger women. The increased breast cancer risk for women on CEE + MPA therapy is less than one additional case per 1,000 users annually, a risk similar to moderate alcohol consumption, obesity, and low physical activity. Had the WHI clearly reported the lack of statistical significance in breast cancer risk with per-protocol adjustments and clarified their findings did not apply to women starting MHT during perimenopause or early postmenopause, much of the ensuing controversy and confusion could have been avoided, benefiting women's health. A positive yet cautious attitude toward MHT is essential, weighing all associated risks and benefits.

8. ACKNOWLODGMENTS

I would like to thank my incredible mentor, Professor Dinka Pavicic Baldani, MD PhD for her support, encouragement and patience throughout my studies and especially in this last year and in writing this graduate thesis. I value the knowledge she gave me and look forward to many more valuable lessons in the future.

I would like to thank my family for their love, support and always believing in me throughout my university years, especially to my mother, without her I wouldn’t have been where I am now. It meant the world to me.

I am grateful for my friends for always listening to me and providing me the encouragement I needed to push through the hard times and cherish the good ones.

Thank you for being part of my journey through all these years and helping me finish my MD degree.

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10. BIOGRAPHY

I was born on the 9th of May in 1996 in Zagreb, Croatia. After living abroad, I started my education at "Jabukovac" Primary School. After that, I enrolled into the Accredited Private Classical High School in Zagreb. Upon completing my high school education, I pursued my lifelong dream of studying medicine, and enrolled into the University of Zagreb, Medical School in English program. During my years there, I participated in International and Croatian student congresses, both actively and passively. I am both Croatian and Canadian, so I am a native speaker of English also.