A Novel Approach to Breast-Cancer Prevention: Reducing Excessive Ovarian Androgen Production in Elderly Women

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Abstract

Background: Minimizing endogenous estrogen production and activity in women at high risk for breast cancer is a prominent approach to prevention of the disease. This is currently accomplished either by administration of aromatase inhibitors, which decrease the synthesis of estrogen from its androgen precursors in peripheral tissues, or by selective-estrogen-receptor-modulators, which block the binding of estrogen to its cellular receptors in breast epithelium. A number of clinical trials have shown that this approach does indeed reduce the incidence of breast cancer. Unfortunately, these drugs often produce adverse effects on the quality of life, with musculoskeletal, vasomotor, and gynecological symptom resulting from the acutely decreased levels of estrogens. As a consequence, they have been poorly accepted by many women, even those that are at high risk for breast cancer according to risk-prediction models.

Discussion: We propose a novel alternative approach to decreasing estrogen production at a biochemically earlier stage: suppression of ovarian production of the androgen precursors of estrogens by administration of long-acting gonadotropin-releasing hormone (GnRH) analogues, which inhibit the synthesis of gonadotropins and thereby suppress ovarian function. The optimal subjects for this approach would be women with high blood levels of testosterone, marker of excessive ovarian androgen production and recognized factor of risk for breast cancer. The specific target population would be elderly postmenopausal women, since breast cancer incidence increases with age and reaches its maximum around the age of seventy years .

Summary: anti-estrogens counter increased estrogen formation and activity, which are consequences of excessive androgen production. We propose to counter the source of androgen excess in women with ovarian stromal hyperplasia, thus reducing the substrate for estrogen formation without completely inhibiting estrogen synthesis. Testosterone levels are measured at baseline to identify women at risk and during the follow-up to evaluate the effectiveness of therapy. Available evidence indicates that GnRH analogues can be safely used for breast cancer prevention in postmenopausal women.

Keywords: Breast cancer prevention, postmenopausal women, postmenopausal ovary, androgens, androgen excess, testosterone levels, medical oophorectomy, GnRH analogues, ovarian stromal hyperplasia.

Background

A large body of evidence points to estrogens as promoters of breast cancer development. This has led to attempts at cancer prevention through diminution of the impact of estrogen on the breast, either by administration of aromatase inhibitors, such as anastrozole or letrozole, which inhibit the synthesis of estrogens from their androgen precursors, or by administration of selective estrogen-receptor modulators (SERMs), such as tamoxifen or raloxifen, which block the binding of estrogens to their cellular receptors. Clinical trials have shown that applying this approach to women at high risk for breast cancer does indeed reduce the incidence of the disease [3-7]. Unfortunately, these drugs often produce adverse effects on the quality of life, with musculoskeletal, vasomotor, and gynecological symptoms resulting from acutely decreased levels of estrogen [3-6]. As a consequence, they have been poorly accepted by many women [8-10], even those that are at high risk for breast cancer according to risk-prediction models [11-14].

In the present paper, we propose a novel alternative approach to breast cancer prevention that is virtually devoid of severe adverse events, namely suppression of ovarian production of the androgen precursors of estrogen by administration of long-acting gonadotropin-releasing hormone (GnRH) analogues, which inhibit the secretion of luteinizing hormone (LH), the necessary stimulus to ovarian androgen production. That this might be an effective approach was suggested by the extensive evidence that women with breast cancer often have interstitial-cell hyperplasia of the ovaries, with resulting increased synthesis of androgens, which is often reflected in elevated blood testosterone levels [20]. Determining the blood testosterone levels of potential patients would delineate the women who would be optimal candidates for our proposed approach. The prime target population would be elderly postmenopausal women, since breast cancer incidence increases with age and reaches its maximum around the age of seventy years [21, 22].

In the next section we describe the statement of our proposal and discuss the role of androgen excess in breast cancer, the endocrine function of the postmenopausal ovary, the identification of women at increased risk, and the tolerability and side-effects of GnRH analogues.

Discussion

Statement of our proposal

Our proposal develops from the evidence that: aromatization of androgen precursors, androstenedione and testosterone, is an obligatory step in the synthesis of estrogens, estrone and estradiol, respectively [15]; high circulating androgen levels are a known factor of risk for postmenopausal breast cancer that can stimulate cancer growth by conversion into estrogens [16-19]; and the postmenopausal ovary is an important source of excessive androgen production which

originates from the ovarian interstitial-cell hyperplasia frequently present in breast cancer patients [20].

We assume that anti-estrogens are effective in countering increased estrogen formation and activity, which are consequences of excessive androgen production, and suggest directly countering the source of androgen excess in women with ovarian stromal hyperplasia, thus reducing the substrate for estrogen formation without completely inhibiting estrogen synthesis and function.

Inhibition of ovarian androgen production can be accomplished by administering long-acting GnRH analogues, which induce medical castration by inhibiting the synthesis of gonadotropins. The target population includes healthy women in natural menopause, with intact ovaries, 60 years of age or older, at increased risk of breast cancer according to one of the validated risk-prediction models, and with high serum testosterone levels. Elevated serum testosterone levels are a good marker of excessive ovarian androgen production [20] and a recognized factor of risk for breast cancer [16-19]. The availability of a risk factor that is powerful and that can be corrected, is useful to identify a distinct subset of women at risk, to evaluate the effectiveness of treatment, and to personalize the treatment schedule according to the necessities of each subject.

Circulating testosterone levels should be regularly checked every six to twelve months in women to whom GnRH analogues are administered; the treatment should be interrupted when blood testosterone levels return to normal and should be resumed when they rise again after the initial decrease.

The role of androgen excess in breast cancer

The androgen-excess theory, developed in studies by our group over the last 45 years [20], suggests a central role for androgens in breast cancer development. Androgen excess can stimulate breast cancer growth by three principal mechanisms: increased conversion into estrogens, which directly stimulate estrogen receptor (ER)-positive tumors; direct tumor stimulation by binding to androgen receptors (AR) in ER-negative/AR-positive tumors; and increased synthesis of epidermal growth factor, which stimulates cancer growth by binding to its own receptor in the ER-negative/AR-negative tumors [23, 24].

A series of prospective studies in healthy postmenopausal women have shown that elevated blood androgen levels are associated with increased risk of breast cancer [16-19]. In these same studies, increased risk of breast cancer was also found associated with high serum levels of estrogens, a finding consistent with increased formation of estrogens by women with high levels of their androgen precursors. Evidence that the effect of increased androgen levels in favoring the development of estrogen-dependent breast cancers is a direct one is provided by the finding of a

strong relationship between blood testosterone levels and the ER content of tumors in contrast to the weak relationship of blood estradiol levels to ER content [25, 26].

Endocrine function of the postmenopausal ovary

About 50% of circulating testosterone is produced in equal amounts by the ovary and the adrenals; the remaining 50% is produced in peripheral tissues by conversion from androstenedione [27, 28]. Androstenedione is synthesized in the ovary and the adrenals, and in peripheral tissues by conversion from adrenal androgen precursors, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEAS) [27, 28].

During a woman's lifespan, blood testosterone levels decrease with age from the twenties till just before menopause [29, 30], do not change during the menopausal transition [30-32] and then increase slightly in the late postmenopausal years [32-34]. In contrast, the blood levels of DHEA and DHEAS decline steadily with age from the early twenties regardless of menopausal status [30, 31].

The postmenopausal ovary continues to secrete large amounts of testosterone and moderate amounts of androstenedione and DHEA well into old age [28, 32, 33, 35, 36]; the androgen production occurs in the interstitial cells of the ovarian stroma under the stimulus of LH [35, 37, 38]. Ovarian stromal hyperplasia is a characteristic feature of breast cancer patients, as was reported for the first time by Sommers and Teloh in 1952 [39]. Our early studies in the 1970s [20] showed that such hyperplasia was constantly present in the ovaries of breast cancer patients with supranormal urinary testosterone excretion who underwent oophorectomy, either prophylactically, to prevent disease progression, or therapeutically, for treatment of metastases. In those patients, independent of their menopausal status, testosterone levels usually reverted to normal after the ovaries were removed, and metastases often regressed [20]. Different degrees of stromal hyperplasia, mild, medium, and severe, have been frequently found in postmenopausal women oophorectomized for gynecological disorders and the concentrations of testosterone and androstenedione in the ovarian veins were found to be positively associated with the degree of stromal hyperplasia [40-42]. A significant reduction of testosterone and androstenedione circulating levels has been constantly reported after oophorectomy [32, 33, 35, 36, 40-42].

A study of BRCA1 and BRCA2 mutation carriers showed a highly significant reduction of breast cancer risk in women who had oophorectomy after natural menopause, whereas women with natural menopause who were not oophorectomized showed no such effect [43]; the authors suggested that the reduction of circulating testosterone levels after oophorectomy might account for its protective effect [43]. In a recent study [44], blood levels of androgens and estrogens were measured in a

group of women who underwent natural menopause without oophorectomy and in two groups of women who underwent oophorectomy either at premenopausal age or after natural menopause. Significantly lower testosterone levels were found in both oophorectomized groups than in women with intact ovaries, while the levels of estradiol, estrone, estrone-sulfate and DHEAS did not differ among the three groups [44].

It appears, therefore, that the postmenopausal ovary is an endocrine organ that produces androgens well into old age; that ovarian stromal hyperplasia increases androgen production; that blood testosterone level is a good marker of ovarian androgen production; and that oophorectomy after the natural menopause significantly reduces the blood testosterone levels and may be protective against breast cancer.

Identification of women at increased risk for breast cancer

Several breast cancer risk-prediction models are available to identify women at high risk. In these models, risk is calculated by awarding a score to known risk factors including reproductive and family history, previous breast biopsies and others [11-14]. Although effective in predicting absolute risk, these models have limited ability to discriminate between women who will develop breast cancer and those who will not [12]. Tworoger et al. [45] have recently shown that the inclusion of blood levels of sex steroids in the models improves risk prediction, a finding that supports our proposal to evaluate serum testosterone levels for identifying a subset of women at increased risk.

An estimate of the number of cancers that could be prevented by correcting androgen excess in otherwise healthy women can be obtained by data of our studies in a cohort of 534 postmenopausal breast cancer patients [26]. High testosterone levels were found in about half of ER-positive patients and in about 6% of ER-negative patients of our cohort [23, 46]. It has been reported [47] that a single measurement of testosterone can predict breast cancer risk for up to twenty years in healthy women, therefore we can assume that the high testosterone levels detected in patients of our cohort were present before the diagnosis of cancer and contributed to the development of about 50% of ER-positive tumors and of a small percentage of ER-negative tumors.

Testosterone levels are commonly measured by radioimmunoassay (RIA), a method that may be appropriate for identifying women with elevated serum testosterone values in prospective studies but that has been criticized for its lack of the accuracy and sensitivity required to measure the low blood testosterone levels commonly present in normal women and children [49-52]. This problem stimulated the development of mass spectrometry techniques for such measurements. Currently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the preferred method for

measurement of low testosterone and estradiol concentrations [52] and may be preferable to RIA also because of its ability to simultaneously quantitate several steroids in a single run
Prospective studies in healthy postmenopausal women have reported an association of elevated levels of estrogens and androgens with increased risk of breast cancer, regardless of the method used for steroid quantitation, whether direct RIA, RIA after prior extraction and purification of the sample, or mass spectrometry [Endogenous Hormones and Breast Cancer Collaborative Group 2015]. In our proposal, we suggest selecting normal postmenopausal women with elevated serum testosterone levels for prophylactic treatment with GnRH analogues, and in order to do this we need to determine the best cut-off value between normal testosterone levels and elevated levels. In studies of postmenopausal breast-cancer patients [46, Berrino et al. 2005, Micheli et al. 2007], we measured blood testosterone levels by direct RIA and found that patients with levels above the median value for the group were at higher risk of relapse than patients below the median value. In those studies, carried out at different times and in different groups of breast-cancer patients, the median value of blood testosterone concentration was consistently 0.40 ng/ml, which corresponds to the lower boundary of the uppermost tertile in three hundred healthy postmenopausal women recruited in a previous study [48]. Since that cut-off value distinguished two groups of patients with different outcomes, it seems possible to use it to characterize the women in the general population who have elevated levels of testosterone and are therefore at higher risk for breast cancer.

Endogenous Hormones and Breast Cancer Collaborative Group: **Steroid hormone measurements from different types of assays in relation to body mass index and breast cancer risk in postmenopausal women: Reanalysis of eighteen prospective studies.** *Steroids* 2015, **99** 49–55

Berrino F, Pasanisi P, Bellati C, Venturelli E, Krogh V, Mastroianni A, Berselli E, Muti P, Secreto G:**Serum testosterone levels and breast cancer recurrence.** *Int J Cancer* 2005, **113**:499-502;

Micheli A, Meneghini E, Secreto G, Berrino F, Venturelli E, Cavalleri A, Camerini T, Di Mauro MG, Cavadini E, De Palo G, Veronesi U, Formelli F: **Plasma testosterone and prognosis of postmenopausal breast cancer patients.** *J Clin Oncol* 2007, 25:2685-90.

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Tolerability and side-effects of GnRH analogues

Long-acting GnRH analogues inhibit the pituitary synthesis of the gonadotropins, LH and follicle stimulating hormone (FSH), and thereby deprive the ovaries of the necessary stimulation for androgen and estrogen production. Giving these agents is commonly referred to as “medical oophorectomy”; the effect is reversible when the GnRH analogue treatment is stopped.
The usual use of these agents is in premenopausal women with gynecological disorders such as uterine fibroids or endometriosis, in men with prostate cancer, and in premenopausal early breast cancer patients as adjuvant therapy of ER-positive tumors. They have also been used in

premenopausal women with metastatic breast cancer, where they achieve responses similar to those of surgical oophorectomy, but produce the same estrogen-deprivation side-effects as the surgical procedure. A few studies have examined the effect of the GnRH analogues in unselected postmenopausal women with metastatic breast cancer [54-56]. No significant adverse reactions, in particular no hot flushes, were observed [54-56], but the therapeutic response in these unselected patients was low (16-20%) [55], so treatment with the analogues has not been recommended by these authors. On the other hand, if the GnRH analogue treatment were offered to postmenopausal women with elevated testosterone levels, one might expect a much higher percentage of responders.

Adverse effects of GnRH analogues in premenopausal women are the consequence of premature ovarian failure induced by the therapy. They are generally of low or moderate intensity but a high intensity of one or more menopause-like disturbances has been reported in 3.9% to 16.3% of premenopausal breast cancer patients treated with the analogues [57, 58, 60]. Such negative side effects should not occur in women who are already postmenopausal.

Decrease of bone mineral density and increased risk of osteoporosis are common consequences of ovarian suppression in premenopausal women. FSH stimulates aromatase activity in the ovarian follicles, the principal site of estrogen production in premenopausal women. After the menopause, ovarian follicles disappear and estrogen production occurs only in peripheral tissues, including the adipose tissue that is the main source of estrogens at this age. In the adipose tissue, aromatase activity is unaffected by FSH and is stimulated by glucocorticoids [63-68]. Therefore, inhibition of the synthesis of gonadotropins by GnRH analogues does not influence estrogen production in postmenopausal women and should not adversely affect the bones. Nevertheless, bone mineral density should be evaluated at baseline and regularly checked during the follow-up of a breast cancer prevention study in postmenopausal women.

Injecton-site and allergic reactions of low or moderate intensity were seen in 5% to7% of premenopausal early breast cancer patients treated with GnRH analogues [61]. Severe events were virtually absent (0.5% of allergic reaction of 3rd or 4th degree) [61].

Summary

Inhibition of endogenous estrogen production and activity in healthy women at increased risk of breast cancer is effective in reducing cancer incidence but is associated with negative side-effects. We suggest the following approach: 1. Elderly postmenopausal women at high risk for breast cancer should be screened for elevated blood testosterone levels. 2. Since the source of such elevation is ovarian stromal hyperplasia, the women with high levels should be offered “medical

oophorectomy" with long-acting GnRH analogues. 3. Testosterone levels should be measured during the treatment in order to evaluate its effectiveness and provide a rationale for continuing it. Overall, the available data suggest that GnRH analogues can be used safely and with few side-effects for breast cancer prevention in postmenopausal women, but the paucity of information available about the results of such use mandates caution and close follow-up.

References

1. Clemons M, Goss P: **Estrogen and the risk of breast cancer.** *N Engl J Med* 2001, **344**:276–85.

2. The ATAC (Arimidex, Tamoxifen Alone or in Combination) Trialists' Group: **Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial.** *Lancet* 2002, **359**: 2131–9.

3. Cuzick J, Sestak I, Bonanni B, Costantino JP, Cummings S, DeCensi A, Dowsett M, ForbesJF, Ford L, LaCroix AZ, Mershon J, Mitlak BH, Powles T, Veronesi U, Vogel V, Wickerham DL: **SERM chemoprevention of breast cancer overview group. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data.** *Lancet* 2013, **381**:1827–34.

4. Goss PE, Ingle JN, Alés-Martínez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, McTiernan A, Robbins J, Johnson KC, Martin LW, Winquist E, Sarto GE, Garber JE, Fabian CJ, Pujol P, Maunsell E, Farmer P, Gelmon KA, Tu D, Richardson H; NCIC CTG MAP.3 Study Investigators: **Exemestane for breast-cancer prevention in postmenopausal women.** *N Engl J Med* 2011, **364**:2381–91.

5. Cuzick J, Sestak I, Forbes JF, Dowsett M, Knox J, Cawthorn S, Saunders C, Roche N, Mansel RE, von Minckwitz G, Bonanni B, Palva T, Howell A, on behalf of the IBIS-II investigators: **Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomized placebo-controlled trial.** *Lancet* 2014, **383**: 1041–8.

6. Visvanathan K, Hurley P, Bantug E, Brown P, Col NF, Cuzick J, Davidson NE, Decensi A, Fabian C, Ford L, Garber J, Katapodi M,Kramer B, Morrow M, Parker B, Runowicz C, Vogel VG , Wade JL, Lippman SM: **Use of pharmacologic interventions for breast cancer risk reduction: American Society of Clinical Oncology clinical practice guideline.** *J Clin Oncol* 2013, **31**:2942–62.

7. Advani P, Moreno-Aspitia A: **Current strategies for the prevention of breast cancer**. *Breast Cancer* 2014, **6:**59–71.

8. Lippman SM: **The dilemma and promise of cancer chemoprevention.** *Nature Clinical Practice Oncology* 2006, **3**:523 www.nature.com/clinicalpractice/onc

9. Ropka ME, Keim J, Philbrick JT: **Patient decisions about breast cancer chemoprevention: a systematic review and meta-analysis.** *J Clin Oncol* 2010, **28**: 3090–5.

10. Waters EA, Cronin KA, Graubard BI, Han PK, Freedman AN: **Prevalence of tamoxifen use for breast cancer chemoprevention among U.S. women**. *Cancer Epidemiol Biomarkers Prev* 2010, **19**:443–6.

11. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, Mulvihill JJ: **Projecting individualized probabilities of developing breast cancer for white females who are being examined annually.** *J Natl Cancer Inst* 1989, **81**:1879–86.

12. Rockhill B, Spiegelman D, Byrne C, Hunter DJ, Colditz GA: **Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention.** *J Natl Cancer Inst* 2001, **93**:358–66.

13. Meads C, Ahmed I, Riley RD: **A systematic review of breast cancer incidence risk prediction models with meta-analysis of their performance.** *Breast Cancer Res Treat* 2012,**132**:365–77. doi: 10.1007/s10549-011-1818-2. Epub 2011 Oct 22.

14. Gail MH: **Twenty-five years of breast cancer risk models and their applications**. *J Natl Cancer Inst* 2015, 107:1-6 djv042. doi: 10.1093/jnci/djv042.

15. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC: **Production and actions of estrogens.** *N Engl J Med* 2002, **346**:340–52.

16. Key T, Appleby P, Barnes I, Reeves G: **Endogenous sex hormones and breast cancer in postmenopausal women: Reanalysis of nine prospective studies.** *J Natl Cancer Inst* 2002, **94**:606–16.

17. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE: **Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women.** *J Natl Cancer Inst* 2004, **96**:1856–65.

18. Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, Dossus L, Lukanova A, Bingham S, Khaw KT, Allen NE, Bueno-de-Mesquita HB, van Gils CH, Grobbee D, Boeing H, Lahmann PH, Nagel G, Chang-Claude J, Clavel-Chapelon F, Fournier A, Thiébaut A, González CA, Quirós JR, Tormo MJ, Ardanaz E, Amiano P, Krogh V, Palli D, Panico S, Tumino R, Vineis P, Trichopoulou A, Kalapothaki V, Trichopoulos D, Ferrari P, Norat T, Saracci R and Riboli E: **Postmenopausal serum androgens, oestrogens and breast cancer risk: The European prospective investigation into cancer and nutrition.** *Endocr Relat Cancer* 2005, **12**:1071–82.

19. Cummings SR, Lee JS, Lui LY, Stone K, Ljung BM and Cauleys JA: **Sex hormones, risk factors, and risk of estrogen receptor–positive breast cancer in older women: a long-term prospective study.** *Cancer Epidemiol Biomarkers Prev* 2005, **14:**1047–51.

20. Secreto G: **The androgen-excess theory of breast cancer**. In: *The Androgen-Excess Theory of Breast Cancer.* Edited by Secreto G, Zumoff B. Trivandrum, Kerala, India: Research Signpost; 2012, p. 47-70. http://www.trnres.com/ ebook.php.

21. Yancik R, Wesley MN, Lynn AG, Ries LAG, Havlik RJ, Edwards BK, Yates JW: **Effect of age and comorbidity in postmenopausal breast cancer patients aged 55 years and older.** *JAMA* 2001, **285**:885–92.

22. Anderson WF, Pfeiffer RM, Dores GM, Sherman ME: **Comparison of age distribution patterns for different histopathologic types of breast carcinoma.** *Cancer Epidemiol Biomarkers Prev* 2006, **15**:1899–905.

23. Secreto G: **Endocrine classification of postmenopausal breast cancers**. In: *The Androgen-Excess Theory of Breast Cancer.* Edited by Secreto G, Zumoff B. Trivandrum, Kerala, India: Research Signpost; 2012, p. 80-109. http://www.trnres.com/ ebook.php.

24. Secreto G, Venturelli E, Meneghini E, Carcangiu ML, Paolini B, Agresti R, Pellitteri C, Berrino F, Gion M, Cogliati P, Saragò G, Micheli A: **Androgen receptors and serum testosterone levels identify different subsets of postmenopausal breast cancers.** *BMC Cancer.* 2012, **12**:599. doi: 10.1186/1471-2407-12-599.

25. Secreto G, Venturelli E, Meneghini E, Greco M, Ferraris C, Gion M, Zancan M, Fabricio AS, Berrino F, Cavalleri A, Micheli A: **Testosterone and biological characteristics of breast cancers in postmenopausal women.** *Cancer Epidemiol Biomarkers Prev* 2009, **18**:2942–8.

26. Secreto G, Meneghini E, Venturelli E, Cogliati P, Agresti R, Ferraris C, Gion M, Zancan M, Fabricio AS, Berrino F, Cavalleri A, Micheli A. **Circulating sex hormones and tumor characteristics in postmenopausal breast cancer patients. A cross-sectional study.** *Int J Biol Markers* 2011, **26**:241–6.

27. Burger HG: **Androgen production in women.** *Fertil Steril* 2002, 77 Suppl **4**:S3–5.

28. Kotsopoulos J, Narod SA: **Androgens and breast cancer.** *Steroids* 2012, **77**:1–9. doi: 10.1016/j.steroids.2011.10.002. Epub 2012 May 7

29. Zumoff B, Strain GW, Miller LK, Rosner W: **Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women.** *J Clin Endocrinol Metab* 1995, **80**:1429–30.

30. Davison SL, Bell R, Donath S, Montalto JG, Davis SR: **Androgen levels in adult females: changes with age, menopause, and oophorectomy.** *J Clin Endocrinol Metab* 2005, **90**:3847–53

31. Burger HG, Dudley EC, Cui J, Dennerstein L, Hopper JL: **A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition.** *J Clin Endocrinol Metab* 2000, **85**:2832–8.

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32. Fogle RH, Stanczyk FZ, Zhang X, Paulson RJ: **Ovarian androgen production in postmenopausal women.**
J Clin Endocrinol Metab 2007, **92**:3040–3.

33. Laughlin GA, Barrett-Connor E, Kritz-Silverstein D, von Mühlen D: **Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo study.**
J Clin Endocrinol Metab 2000, **85**:645–51.

34. Sowers MFR, Zheng H, McConnell D, Nan B, Karvonen-Gutierrez CA, Randolph Jr JF: **Testosterone, sex hormone-binding globulin and free androgen index among adult women: chronological and ovarian aging.**
Human Reproduction 2009, **24**:2276–85.

35. Adashi EY: **The climacteric ovary as a functional gonadotropin-driven androgen-producing gland.**
Fertil Steril 1994, **62**:20–7.

36. Jongen VH, Sluijmer AV, Heineman MJ: **The postmenopausal ovary as an androgen-producing gland; hypothesis on the etiology of endometrial cancer**.
Maturitas 2002, **43**:77–85.

37. Rice BF, Savard K: **Steroid hormone formation in the human ovary. IV. Ovarian stromal compartment; formation of radioactive steroids from acetate-1-14C and action of gonadotropins.**
J Clin Endocrinol Metab 1966, **26**:593–609.

38. Stegner HE: **Hormonally related non-neoplastic condition of the ovary.** In: *Ovarian Pathology, Current Topics in Pathology 78*. Edited by Francisco Nogales. Berlin Heidelberg, Germany: Springer-Verlag; 1989, 11-39.

39. Sommers SC, Teloh HA: **Ovarian stromal hyperplasia in breast cancer.**
AMA Arch Pathol 1952, **53**:160–6.

40. Lucisano A, Russo N, Acampora MG, Fabiano A, Fattibene M, Parlati E, Maniccia E, Dell'Acqua S: **Ovarian and peripheral androgen and oestrogen levels in post-menopausal women: correlations with ovarian histology.**
Maturitas 1986, **8**:57–65.

41. Sluijmer AV, Heineman MJ, Koudstaal J, Theunissen PH, de Jong FH, Evers JL: **Relationship between ovarian production of estrone, estradiol, testosterone, and androstenedione and the ovarian degree of stromal hyperplasia in postmenopausal women.**
Menopause 1998, **5**:207–10

42. Jongen VHWM, Holleman H, van der Zee AGJ, Santema JG, Heineman MJ: **Ovarian stromal hyperplasia and ovarian vein steroid levels in relation to endometrioid endometrial cancer.**
Br J Obstet Gynaecol 2003, **110**:690–5.

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43. Kotsopoulos J, Lubinski J, Lynch HT, Kim-Sing C, Neuhausen S, Demsky R, Foulkes WD, Ghadirian P, Tung N, Ainsworth P, Senter L, Karlan B, Eisen A, Eng C, Weitzel J, Gilchrist DM, Blum JL, Zakalik D, Singer C, Fallen T, Ginsburg O, Huzarski T, Sun P, Narod SA: **Oophorectomy after menopause and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers.**
Cancer Epidemiol Biomarkers Prev 2012, **21**:1089–96.
doi: 10.1158/1055-9965.EPI-12-0201. Epub 2012 May 7

44. Kotsopoulos J1, Shafrir AL, Rice M, Hankinson SE, Eliassen AH, Tworoger SS, Narod SA: **The relationship between bilateral oophorectomy and plasma hormone levels in postmenopausal women**.
Horm Cancer 2015, **6**:54–63.

45. Tworoger SS, Zhang X, Eliassen AH, Qian J, Colditz GA, Willett WC, Rosner BA, Kraft P, Hankinson SE: **Inclusion of endogenous hormone levels in risk prediction models of postmenopausal breast cancer.**
J Clin Oncol 2014, **32**:3111–7.

46. Secreto G, Zumoff B: **Role of androgen excess in the development of estrogen receptor positive and estrogen receptor-negative breast cancer.**
Anticancer Research 2012, **32**:3223–8.

47. Zhang X, Tworoger SS, Eliassen AH, Hankinson SE: **Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up.**
Breast Cancer Res Treat 2013, **137**:883–92.

48. Berrino F, Bellati C, Secreto G, Camerini E, Pala V, Panico S, Allegro G, Kaaks R: **Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial.**
Cancer Epidemiol Biomarkers Prev 2001, **10**:25-33.

49. Kushnir MM, Rockwood AL, Roberts WL, Pattison EG, Bunker AM, Fitzgerald RL, Meikle AW: **Performance characteristics of a novel tandem mass spectrometry assay for serum testosterone.**
Clin Chem 2006, **52**:120–8. Epub 2005 Nov 18.

50. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H: **Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement.**
J Clin Endocrinol Metab 2007, **92**:405–13. Epub 2006 Nov 7.

51. Moal V, Mathieu E, Reynier P, Malthièry Y, Gallois Y: **Low serum testosterone assayed by liquid chromatography-tandem mass spectrometry. Comparison with five immunoassay techniques.**
Clin Chim Acta 2007, **386**:12–9. Epub 2007 Jul 19.

52. Ketha H, Kaur S, Grebe SK, Singh RJ: **Clinical applications of LC-MS sex steroid assays: evolution of methodologies in the 21st century.**
Curr Opin Endocrinol Diabetes Obes 2014, **21**:217–26.
doi: 10.1097/MED.0000000000000068

53. Santen R: **Endocrine-Responsive Cancer**. In: *Williams Textbook of Endocrinology*. 10th Edition. Edited by Larsen PR, Kronenberg HM, Melmed S, Polonsky KS. Philadelphia, USA: Saunders (an imprint of Elsevier); 2002, p. 1797-1876.

54. Plowman PN, Nicholson RI, Walker KJ: **Remission of postmenopausal breast cancer during treatment with the luteinising hormone releasing hormone agonist ICI 118630.** *Br J Cancer* 1986, **54**:903–9.

55. Crighton IL, Dowsett M, Lal A, Man A, Smith IE: **Use of luteinising hormone-releasing hormone agonist (leuprorelin) in advanced post-menopausal breast cancer: clinical and endocrine effects.** *Br J Cancer* 1989, **60**:644–8.

56. Dowsett M, Jacobs S, Aherne J, Smith IE: **Clinical and endocrine effects of leuprorelin acetate in pre- and postmenopausal patients with advanced breast cancer.** *Clin Ther* 1992, **14** Suppl A:97–103.

57. Jonat W, Kaufmann M, Sauerbrei W, Blamey R, Cuzick J, Namer M, Fogelman I, de Haes JC, de Matteis A, Stewart A, Eiermann W, Szakolczai I, Palmer M, Schumacher M, Geberth M, Lisboa B; Zoladex Early Breast Cancer Research Association Study: **Goserelin versus cyclophosphamide, methotrexate, and fluorouracil as adjuvant therapy in premenopausal patients with node-positive breast cancer: The Zoladex Early Breast Cancer Research Association Study.** *J Clin Oncol* 2002, **20**:4628–35.

58. International Breast Cancer Study Group (IBCSG), Castiglione-Gertsch M, O'Neill A, Price KN, Goldhirsch A, Coates AS, Colleoni M, Nasi ML, Bonetti M, Gelber RD: **Adjuvant chemotherapy followed by goserelin versus either modality alone for premenopausal lymph node-negative breast cancer: a randomized trial.** *J Natl Cancer Inst* 2003, **95**:1833–46.

59. Davidson NE, O'Neill AM, Vukov AM, Osborne CK, Martino S, White DR, Abeloff MD: **Chemoendocrine therapy for premenopausal women with axillary lymph node-positive, steroid hormone receptor-positive breast cancer: results from INT 0101 (E5188).** *J Clin Oncol* 2005, **23**:5973–82. Epub 2005 Aug 8.

60. Schmid P, Untch M, Kossé V, Bondar G, Vassiljev L, Tarutinov V, Lehmann U, Maubach L, Meurer J, Wallwiener D,Possinger K: **Leuprorelin acetate every-3-months depot versus cyclophosphamide, methotrexate, and fluorouracil as adjuvant treatment in premenopausal patients with node-positive breast cancer: the TABLE study.** *J Clin Oncol* 2007, **25**:2509–15.

61. Pagani O, Regan MM, Francis PA; TEXT and SOFT Investigators; International Breast Cancer Study Group: **Exemestane with ovarian suppression in premenopausal breast cancer.** *N Engl J Med.* 2014, **371**:1358–9. doi: 10.1056/NEJMc1409366.

62. Shiba E, Yamashita H, Kurebayashi J, Noguchi S, Iwase H, Ohashi Y, Sasai K, Fujimoto T: **A randomized controlled study evaluating safety and efficacy of leuprorelin acetate every-3-months depot for 2 versus 3 or more years with tamoxifen for 5 years as adjuvant treatment in premenopausal patients with endocrine-responsive breast cancer.**

Breast Cancer 2015 Feb 6.
DOI 10.1007/s12282-015-0593-z [Epub ahead of print]

63. Folkerd EJ, Jacobs HS, van der Spuy Z, James VH: **Failure of FSH to influence aromatization in human adipose tissue.**
Clin Endocrinol (Oxf) 1982, **16**:621–5.

64. Simpson ER, Cleland WH, Mendelson CR: **Aromatization of androgens by human adipose tissue in vitro.**
J Steroid Biochem 1983, **19**:707–13.

65. Nawata H, Tanaka S, Tanaka S, Takayanagi R, Sakai Y, Yanase T, Ikuyama S, Haji M: **Aromatase in bone cell: association with osteoporosis in postmenopausal women.**
J Steroid Biochem Mol Biol 1995, **53**:165–74.

66. Zhao Y, Nichols JE, Bulun SE, Mendelson CR, Simpson ER: **Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in regulation of the adipose-specific promoter.**
J Biol Chem 1995, **270**:16449–57.

67. Tanaka S, Haji M, Takayanagi R, Tanaka S, Sugioka Y, Nawata H: **1,25-Dihydroxyvitamin D3 enhances the enzymatic activity and expression of the messenger ribonucleic acid for aromatase cytochrome P450 synergistically with dexamethasone depending on the vitamin D receptor level in cultured human osteoblasts.**
Endocrinology 1996, **137**:1860–9.

68. Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, Martin R, Utsunomiya H, Thung S, Gurates B, Tamura M, Langoi D, Deb S: **Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment.**
Pharmacol Rev 2005, **57**:359–83.

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