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Review

Breast cancer risk in postmenopausal women using testosterone in combination with hormone replacement therapy

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Abstract

Objectives: Testosterone supplementation can be considered as a treatment option for surgically postmenopausal women with a distressful low sexual desire disorder, while on oestrogen therapy with or without progestagens.

The purpose of this study is to review the available clinical data on the impact of exogenous testosterone containing postmenopausal hormone therapy on breast cancer risk.

Methods: A literature search was done in MEDLINE (1969–July 2007) and in addition in EMBASE and Biosis (1990–July 2007) for original reports in English and French. Case reports and studies without a control group were excluded.

Results: No prospective randomized clinical trials were found. The five studies found (two case–control studies, two cohort studies and one retrospective observational study) showed inconsistent results. All studies had severe methodological limitations. Formulations and dosages used could be considered suboptimal.

Conclusion: At present, there are no valid randomized or observational clinical studies that provide evidence that the addition of testosterone to conventional postmenopausal hormone therapy influences breast cancer risk.

Keywords: Postmenopausal patients; Exogenous testosterone; Oestrogen/testosterone combination therapy; Breast cancer risk; Review

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Contents

1. Introduction

In women, the ovaries and the adrenal glands are responsible for the direct production of testosterone (T), while a considerable amount is converted peripherally from androstenedione, which is also produced in ovaries and adrenals. Aging is associated with a progressive decline in androgen levels, but data suggest there is no evidence of a precipitous, perimenopausal decline in androgen production [1]. However, the total serum T concentrations in women >50 years old is approximately half that of women in their 20s [2]. It has been demonstrated that hormonal therapy with oral oestrogens increases the level of sex hormone-binding globulin (SHBG) and suppresses luteinising hormone secretion, decreasing T availability and androgen synthesis in the ovaries [3,4].

During the menopause, the fall in the levels of androgens can be associated with unexplained fatigue, lack of well being, and diminished libido, although these syndromes are not specific for androgens [2,5,6]. In the USA, 43% of women aged 18–59 years experience some form of sexual disorder at some time, with low sexual interest being the most common [7]. Bilateral ovarectomy leads to a dramatic decrease in androgens and the above-mentioned symptoms are frequently more intensive and cause more distress.

Testosterone therapy is seen as a potential means of treating menopause-related sexual dysfunction and considerable numbers of women are currently using T products intended for men. The first T transdermal patch that has been specifically developed for menopausal women was approved in Europe last year for use in surgically menopausal women with low sexual desire disorder (HSDD) on concomitant oestrogen therapy. More T products for use in women are expected in future.

Although there is substantial evidence that prudent testosterone replacement can be effective in relieving both the physical and psychological symptoms of androgen insufficiency, the role of testosterone in breast cancer aetiology is unclear [8]. There are few studies in the literature which have implicitly investigated the role of testosterone in carcinogenesis of the mammary tissue. *In vitro* and *in vivo* studies have demonstrated both proliferative [9,10] and anti-proliferative [11–13] effects of testosterone on cell growth.

One *in vitro* study assessed effects on benign, as well as on cancerous, breast epithelial cells stimulated by stroma-derived growth factors, and found with T (in contrast to certain progestogens), neutral effects in benign but stimulatory effects in cancer cells [14]. This might lead to the speculation that only pre-existing cancer cells proliferate and have serious implications for patients recovering from breast cancer, as this would negate the use of steroidal hormones.

The endpoint 'cell proliferation of normal (benign) cells' has recently been explored *in vivo* in humans, investigation the inhibition of breast cell proliferation during treatment of postmenopausal women with a T patch, using fine needle aspiration (FNA) biopsies and Ki-67 staining [15].

The relationship between endogenous T and breast cancer risk has been reviewed quite extensively by Somboonporn [16], yet in this same paper, exogenous studies are mentioned only briefly. Somboonporn's lack of data on this subject in her review is a reflection of the paucity of information on the relationship between exogenous T and breast cancer risk.

The purpose of this paper is to review the available data on the exogenous use of T in combination with oestrogens. Other substances with androgenic properties like tibolone or progestogens with partial androgenic receptor activity were not included in this

review, because breast cancer risk in women using tibolone had been broadly discussed in other publications [33] and there are no studies dealing with the impact of differences in androgenic potencies of progestogens on breast cancer risk.

2. Methods

In the last two decades only a limited number of papers have been published about the impact of exogenous use of T in combination with oestrogens on breast cancer risk. These papers were identified and they were thoroughly reviewed to provide an overview of the current consensus on this neglected topic. A brief summary of these papers can be seen in Table 1.

With respect to our search strategy, the literature review included a search in MEDLINE since the start of this database in January 1969, which was the prime source for this report. In addition we searched in the databases EMBASE and Biosis for studies since 1990. The search was primarily limited to English-language articles but included also publications in French. To be considered for inclusion, publications had to be original articles. Search terms were breast cancer risk, androgens, exogenous androgens, postmenopausal women, and testosterone. We excluded all case reports and all studies without control groups, as well as studies investigating the relationship between endogenous testosterone and breast cancer risk. *In vitro* and animal studies were primarily excluded by definition of the key words for research. At least two of the authors selected and extracted the studies followed by independent double-checking both the literature searches and data extraction.

We used free text searching as well as MeSH headings to retrieve trials for the following selection:

(1) Observational studies: case/control and cohort studies.
(2) Prospective randomized.
(3) All studies (independent of study design).

We would have liked to proceed with this search according to the 'Jadad criteria' for systematic reviews [17]. For this the most important items are randomization and blinding. However, only two case–control studies, two cohort studies and one retrospective observational study met the criteria for this review.

In addition we found one prospective randomized study investigating a possible breast cancer risk when treating postmenopausal women with exogenous androgens, but the endpoint was not breast cancer incidence but breast cell proliferation. The results and conclusion of this study are also described in the results section and summarized in a separate table.

As we did not find prospective randomized studies, the few available studies were described, trial by trial, with regard to their results, conclusions and limitations. This is followed by a general discussion on the surprisingly limited data, a short summary of possible mechanisms of androgen action and finally a conclusion. Specific examples were used to highlight practically relevant results. Thus, the present review is of a qualitative and empirical nature, no statistical analyses were used to compare the various studies.

2.1. Results and discussion of available studies

This case–control study was published in 1986 and involved 1960 postmenopausal breast cancer cases, and 2258 controls, identified through a nation-wide screening programme [18]. The primary endpoint of the study was evaluation of oestrogen effect on breast cancer risk. The paper states that for 26 women oral methyltestosterone was administered in combination with conjugated equine oestrogen (CEE), but dosing levels were not given.

The study found no significant difference in the relative risk (RR) of breast cancer between the users of the androgen–oestrogen preparation and the control group of non-hormone users [RR 1.18 for most recent use (95% CI, 0.7–2.0) and RR 1.05 for longest usage (95% CI, 0.6–1.8)]. Evaluation of the RR in relation to duration of use of the methyltestosterone/CEE preparation did also not show an increase though the case numbers of use of more than 10 years were small with just 2 cases vs. 4 controls [RR 0.66 for use >10 years and RR 1.12 for use <10 years (23 cases vs. 24 controls)]. Findings on the studys' primary objective of oestrogen effect and breast cancer risk showed no relationship between 'ever' use of menopausal hormones and risk of breast cancer. However, there was a significant trend in risk with increased duration of hormone use in general. Elevations in risk were small, being in the order of 50%, only after 15 years of use. While some further increases were observed after 25 years of use, the maximum rel-

Table 1
Summary of available studies investigating testosterone use and breast cancer risk

Study name	Main study objective	Design	Number of patients	Drug formulation Intervention	Outcome measures	Result	Bias Confounder	Evidence level
Brinton et al. [18]	Evaluate the relationship between post-menopausal oestrogen use and breast cancer risk	Case–control; data collection by home interviews; matched on centre, race, age, duration in program; years of screening	1960 post-menopausal breast cancer cases and 2258 controls identified through nation-wide screening program	Great variability from Premarin 0.3 to Premarin 0,6 to Premarin 1.25; only 26 cases and 27 controls with oestrogen/methyl-testosterone	RR for breast cancer in many subgroups of patients with special focus on oestrogen use in ovarectomized women	No increased risk in T + E users and ever hormone users; increased risk in long-term users of E;	Matching limited number and women from the same screening program; no matching for risk factors	II c
Ewertz [20]	Influence of sex hormones on breast cancer risk	Case Control National Registry; mailed questionnaires	1486 breast cancer cases diagnosed over a 1-year period. 1336 controls from the general population	Estradiol (2.5–5 mg) and T (50–100 mg) administered via intramuscular injections at intervals of 3–7 weeks	RR for breast cancer risk with subgroups with specific brand names	Increased risk for E/androgen combination RR 2.31 (95% CI 1.37–3.88) absolute numbers 56 cases and 21 controls	Low matching number; high dosages; recall bias; biologic implausibility in that E + P + T showed lower risk	II c
Colditz et al. [21]	Relationship between E + P and breast cancer risk	Cohort study Nurses Health Study follow-up until 1992; every 2 years completion of questionnaires about hormone intake and breast cancer	69,566 women followed for 725,550 person-years; 1935 cases of newly diagnosed invasive breast cancer were documented	Conjugated oestrogens (no dosage); other oestrogens (no dosage), oestrogen plus progestins (mainly MPA; no dosage); progestins alone; oestrogen plus androgens	RR for breast cancer in non-users and different user groups	RR for E alone 1.32 (CI 1.14–1.54) RR for E + P mainly MPA 1.41 (CI 1.15–174) Conjug E + T 1.64 (0.53–5.09); time dependent increase	Limitation of cohort study; Dosages; low numbers; assessment of menopausal status; Recall bias	II c

Dimitrakakis et al. [22]	Hypothesis: addition of T diminishes the breast cancer risk in patients with "usual" hormone therapy	Retrospective observational study; T group compared to prevalence in the literature	508 post-menopausal women; mean duration of follow-up 5.8 years	T implants containing 50–150 mg every 5 months, administered alone or in combined with ERT or HRT; oestrogen mainly 0.624 CEE or 1.25 mg of oestrone sulfate; MPA 2.5 mg continuously or MPA 5–10 mg cyclic or NETA 0.3–2.5 mg	Age specific incidence rates of breast cancer in T group compared to ERT and HRT breast cancer risk taken from WHI and Million Women study	238 resp. 293/100,000 breast cancer in T group compared to 380/100,000 in WHI and 520/100,000 in Million Women study of breast cancer cases among E + P users	No real control group; large variety of dosages; duration of use unclear; partially very high dosages	III
Tamimi et al. [8]	Determine the risk of breast cancer in post-menopausal combined use of E plus T	Prospective Cohort study Nurses Health study; follow-up 1978–2002	1,359,323 person-years 4610 incident cases of breast cancer during observation period	Estratest alone or combined with ERT or HRT = PMH; oestrogens mainly CEE; progestogen mainly MPY	RR of never user vs. previous use, current use of E; E + P, and E + T or E + P + T	E alone RR 1.15 (1.05–1.27); E + T RR 1.77 (1.22–2.56) T alone RR 2.52 (0.80–7.94) T comb vs. ERT ($p = 0.007$) vs. HRT ($p = 0.11$); increased risk by year during PMH + T = 17.2% increase/year	Limitation of Cohort study; Esterified E as pharma-cological intervention No control of P effect; lack of biologic plausibility; no previous exposure data; no current exposure data	II C

E, oestrogen; P, progesterone/progestins; T, testosterone.

ative risk (RR) only reached 1.7 for hormone use in general. These findings suggest that if hormone use increases breast cancer risk, the risk is limited to long-term users, and is small compared to oestrogen-related endometrial cancer [18,19]. This study had only a small sample size for the subgroup analysis for androgen application. The significant methodological limitations of the study along with the fact that T therapy and breast cancer risk was not the primary endpoint renders the results inconclusive.

This population-based, case–control study was published in 1988 and involved 1486 breast cancer cases diagnosed over a 1-year period. The control group was an age-stratified random sample of 1336 women from the general population [20]. Data on risk factors were collected from self-administered, mailed, questionnaires and the primary endpoint was the influence of sex hormones on breast cancer risk. Estradiol (2.5–5 mg) and T (50–100 mg) were administered via intramuscular injections at intervals of 3–7 weeks. This study reported an increased breast cancer risk for an oestrogen/androgen combination [RR of 2.31 (95% CI, 1.37–3.88)], with 56 and 21 incident cases of breast cancer in the case and control cohort, respectively.

There are several limitations in this study including the small sample size and the uncertainty regarding the menopausal status of the enrolled patients. The T doses in this study were also very high—far surpassing those which are deemed to be acceptable today. The oestrogen–androgen combinations were also unbalanced and there was a variable regimen interval between injections, confounding the results of this study still further. Also, approximately 24% of women did not state on their questionnaires what brand of hormones they were taking and the authors state: "the results on hormones should therefore be interpreted rather cautiously."

This investigation was an extension of the Nurses Health Study Questionnaire conducted from 1992 and published in 1995 [21]. The women who participated in the study were asked to complete questionnaires every two years to update information on their menopausal status, use of oestrogen and progestin preparations and any diagnosis of breast cancer. During 725,550 person-years of follow-up, 1935 cases of newly diagnosed invasive breast cancer were documented. This study reported a time dependent increase in relative risk of breast cancer in women taking conjugated oestrogen alone [1.32 (CI 1.14–1.54)], oestrogen and progestins [1.41 (CI 1.15–174)] or oestrogen and T [1.64 (CI 0.53–5.09)].

The primary endpoint of the study was the effect of adding progestins to oestrogen therapy on breast cancer risk in postmenopausal women, an investigation of the use of T therapy and breast cancer risk was not considered to be of primary importance. The sample size for the subgroup analysis of oestrogen plus T was small at only 810 person-years and four cases of breast cancer. This limits the validity of the calculated RR. *In situ* breast cancers were not included in the analyses and doses of hormone are unknown. The sample size for the results regarding the use of oestrogen and T was too small to allow the formation of robust conclusions.

This was a retrospective, observational study, published in 2004, that followed 508 postmenopausal women [22]. The mean oestrogen replacement therapy (ERT) exposure time was eight years while the mean T exposure was 5–6 years. T was administered via implants containing 50–150 mg every 5 months (initial dose mostly 100 mg) and was given alone or combined with ERT (oestrogen only) or hormone replacement therapy (HRT). ERT was mostly CEE at 0.625 mg daily or 1.25 mg daily of oestrone sulphate. In those women with a uterus, progestin was administered as medroxyprogesterone acetate (MPA) 2.5–5 mg daily continuously or MPA 5–10 mg cyclically or norethisterone (NET) (0.3–2.5 mg daily).

Within the observation period of this study, seven invasive breast cancer cases were diagnosed among these women, resulting in an incidence of 238 per 100,000 woman years for the combined (E/T and E/P/T) groups. Notably, six of the seven cases and the only death occurred in the E/P/T group, which translates as 293 cases per 100,000 woman-years. This was compared to the incidence of breast cancer cases among E + P users reported in the WHI study and Million Women study—380/100,000 and 520/100,000, respectively.

In this study, the authors conclude that the addition of T to conventional hormone therapy for postmenopausal women does not increase and may indeed reduce the hormone therapy-associated breast cancer risk, thereby returning the incidence to the normal rates observed in the general, untreated population.

The authors' presumptions, based on their own *in vitro* studies, and those of others, may have influenced this observational study. The authors presume that androgens can be protective against breast cancer. *In vitro* studies can be used to investigate mechanisms of action and other facets of pharmacology, but they cannot be used as an indicator of risk evaluation, nor can they replace clinical studies.

Generally, the number of breast cancer cases in each subgroup was small and there was no real control group. The extrapolation of control breast cancer rates was population-based and drawn from different populations. The risk analyses in this study did not take into account prior hormone use, with respect to type, dose and duration of ERT, HRT and T. The study also lacked clear-cut exposure data for current T use. In the publication, "current use" equated to the whole 2-year follow-up intervals. The T implants used in the study were equivalent to pharmacological intervention due to high, non-physiological dosages. The investigation failed to adjust for independent progesterone effects, which may have a large influence on the breast cancer risk. There was a 5–10-fold variation in the NET dosages which were administered to the patients. The study is also flawed because the information on current use of different hormone regimens is lacking—were they administered continuously or cyclically? Woman years such as 100,000 are often used because they conceal small patient numbers. The real incidence would be much more meaningful.

This was a prospective, cohort study conducted from 1978 to 2002, with the results published in 2006 [8]. Every 2 years, information on menopause status, hormone use and breast cancer diagnosis was collected. Over this time period, 4610 cases of invasive breast cancer were reported. The risk of breast cancer in current users of oestrogen plus T was nearly 2.5 times higher than in those patients who had never used postmenopausal hormones. Combined T and oestrogen therapy was associated with a significantly higher risk of breast cancer than oestrogen therapy alone. The authors of the study conclude: "Consistent with the elevation in risk for endogenous T levels, women using oestrogen and T therapies have a significantly increased risk of breast cancer."

This study reported a relative risk of breast cancer of 1.15 (1.05–1.27), 1.77 (1.22–2.56) and 2.52 (0.80–7.94) for oestrogen alone, oestrogen + T and T alone, respectively. This analysis showed that the risk of breast cancer associated with current use of oestrogen and T was significantly greater than oestrogen alone (P for heterogeneity = 0.007) and marginally greater than oestrogen and progesterone therapy (P for heterogeneity = 0.11). The authors conclude their results by stating that: "women receiving PMHs with T had a 17.2% (CI, 6.7–28.7%) increased risk of breast cancer per year of use."

The authors' background presumptions may have been over-simplistic, which may have influenced this observational study. There were no clear-cut results in the hitherto existing human studies due to problems with T assays, failure to adjust for independent ERT and HRT effects, insufficient patient numbers, conflicting results on the effect of endogenous T and controversial results on the effect of exogenous T preparations.

There were several limitations in this study, outlined here. There was a failure to adjust for independent progesterone effects, which may have a large influence on the breast cancer risk. The methyltestosterone administered was equivalent to pharmacological intervention, but there were no data on T and no data with physiological doses. In current users receiving T only, there were only three cases of breast cancer which equates to 360 person-years. Esterified oestrogens were equivalent to pharmacological intervention, but, again, there were no data on T and no data with physiological doses. The risk analyses did not take into account prior hormone use regarding type, dose and duration of ERT, HRT and T preparations; of the ERT + T users in the analysis, 97.6% had received ERT/HRT previously. Again, there were no clear-cut exposure data for current T use—"current use" equated to the whole 2-year follow-up intervals. There were differences in the basic characteristics between patients on ERT vs. ERT + T. There was no information on which formulation of Estratest® was used and it was not stated whether the hormone regimens were administered continuously or cyclically. There were no data on current use of hormone dose and the data from 1988 to 1998 for ERT + T was not explicitly assessed. Data on number-to-harm compared with number-to-treat is also lacking.

This was a prospective randomized, double blind, placebo controlled trial conducted over 6 months in 99 postmenopausal women [15]. The primary objective of this study was to assess the effects of the

300 μg/24 h T patch on breast cell proliferation, compared to the effects of continuous HRT (E_2/NETA) alone in naturally menopausal women. Percutaneous fine needle aspiration biopsies were performed before and after 6 months of treatment. The cells were then quantified and immunostained for the nuclear antigen Ki-67.

Of the patients assigned to treatment, 88 (89%) completed the study. The authors found a marked increase ($p < 0.001$) in breast cell proliferation after 6 months of treatment with E_2/NETA (median value 1.1–6.2%) This was apparent in both epithelial and stromal cells. However, when the T patch was added in women receiving the E_2/NETA treatment, no significant increase in breast cell proliferation was observed (median value 1.6% vs. 2.0%).

This was the first prospective randomized study on the effects of T on breast cell proliferation in postmenopausal women. Previous studies using the FNA biopsy technique for assessment of proliferation by the Ki-67 antibody have repeatedly found a three- to fivefold increase in breast cell proliferation during combined oestrogen/progestogen hormone therapy [23–25]. The same increase seen in this study was apparent in the placebo group. Although, there are currently no data available on breast cell proliferation for a longer period of follow-up than 6 months, the authors have previously shown that an increase in breast density associated with increased cell proliferation, is fully established during the first few months and will not increase further during prolonged treatment with the same regimen [21].

The authors concluded that the addition of T to a regimen of oestrogen/progestogen has the potential to modulate the stimulatory effects of hormones on breast cell proliferation, but further research is needed to elucidate whether T alone in postmenopausal women not receiving EPT will cause the same anti-proliferative effect. A brief summary of this paper can be seen in Table 2.

3. General discussion and conclusion

The available literature with regard to clinical studies addressing the research question of the present study does not provide solid answers. The results of these studies are not consistent and the studies themselves

Table 2
Summary of Hofling et al. [15]

Study name	Study objective	Design	Number of patients	Drug formulation Intervention	Outcome measures	Result	Bias Confounder	Evidence level
Hofling et al. [15]	To study the effects of testosterone addition on breast cell proliferation during postmenopausal oestrogen/progestogen therapy	6 month prospective randomized double-blind placebo-controlled study	99 postmenopausal women	Continuous combined estradiol 2 mg norethisterone acetate 1 mg administered with either a testosterone patch (300 mg/24 h) or a placebo patch	FNA biopsies taken at baseline and after 6 months. Main outcome measure: the percentage of proliferating breast cells positively stained by a Ki-67/MIB-1 antibody	More than a fivefold increase ($P < 0.001$) in the placebo group in total breast cell proliferation from baseline to 6 months. No significant increase was seen after testosterone addition	Numbers are very constrained, 47 in active treatment, 41 in the placebo group will not enable statistical analysis	I A

have serious limitations. In general, sample seizes are very small and the majority of studies have a different primary endpoint.

Preclinical studies indicate androgens may act as a natural endogenous protector of the breast from carcinogenesis [26–29,12]. The review by Liao and Dickson [30] states that androgens have both inhibitory and stimulatory effects on the growth of mammary gland and breast cancer. The authors claim these functions can be attributed to at least six mechanisms:

- "Androgens serve as oestrogen precursors and are converted to oestrogens."
- "Androgens exert oestrogenic effects by directly binding to oestrogen receptor-α (ERα); adrenal androgens have higher affinities for ER-α than T and dihydrotestosterone, and are therefore more potent in this function."
- "Androgens exert androgenic effects by directly binding to androgen receptors."
- "Androgens may bind to progesterone receptors and may exert progestational effects."
- "Androgens may stimulate the expression of prolactin receptors, playing the function of prolactin."
- "In the case of BRCA1 carriers, androgens may act via androgen receptor–BRCA1 complex to inhibit the development of breast cancer; this mechanism, if it really exists, is affected by the length of the CAG repeat in the AR gene."

Another possible interaction of testosterone with breast cancer cells is via DHT as active metabolite of T that cannot be converted by aromatase to oestrogen and acts as a local aromatase inhibitor thus exerting a local anti-oestrogenic effect [12,34].

Knowledge of the role of androgens in the growth and differentiation of breast cells and their possible involvement in breast cancer is compounded by our tenuous understanding of other risk factors. When all known risk factors and characteristics are added together, including genetics and family history, as much as 50% of breast cancer cases remain unexplained [31]. Environmental pollutants, pharmaceuticals, alcohol and light levels are all purported to be risk factors for carcinogenesis in the breast [32].

Until we gain an increased understanding of the complex interactions between these variables it is impossible to attribute the manifestation of breast cancer to a single factor. More long-term studies are needed to fully investigate the role of T in mammary cell growth.

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