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Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole: A prospective, observational study

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ABSTRACT

Objectives: There is evidence that androgens are breast protective and that testosterone therapy treats many symptoms of hormone deficiency in both pre and postmenopausal patients. However, unlike estrogen and progestins, there is a paucity of data regarding the incidence of breast cancer in women treated with testosterone therapy. This study was designed to investigate the incidence of breast cancer in women treated with subcutaneous testosterone therapy in the absence of systemic estrogen therapy.
Study design: This is a 5-year interim analysis of a 10-year, prospective, observational, IRB approved study investigating the incidence of breast cancer in women presenting with symptoms of hormone deficiency treated with subcutaneous testosterone (T) implants or, T combined with the aromatase inhibitor anastrozole (A), i.e., T + A implants. Breast cancer incidence was compared with that of historical controls reported in the literature, age specific Surveillance Epidemiology and End Results (SEER) incidence rates, and a representative, similar age group of our patients used as a 'control' group. The effect of adherence to T therapy was also evaluated.
Results: Since March 2008, 1268 pre and post menopausal women have been enrolled in the study and eligible for analysis. As of March 2013, there have been 8 cases of invasive breast cancer diagnosed in 5642 person-years of follow up for an incidence of 142 cases per 100 000 person-years, substantially less than the age-specific SEER incidence rates (293/100 000), placebo arm of Women's Health Initiative Study (300/100 000), never users of hormone therapy from the Million Women Study (325/100 000) and our control group (390/100 000). Unlike adherence to estrogen therapy, adherence to T therapy further decreased the incidence of breast cancer (73/100 000).
Conclusion: T and/or T + A, delivered subcutaneously as a pellet implant, reduced the incidence of breast cancer in pre and postmenopausal women. Evidence supports that breast cancer is preventable by maintaining a T to estrogen ratio in favor of T and, in particular, by the use of continuous T or, when indicated, T + A. This hormone therapy should be further investigated for the prevention and treatment of breast cancer.

1. Introduction

Excluding skin cancer, breast cancer is the most common cancer among women, with a lifetime risk of 1 in 8. It is well recognized that estrogen and progestin therapy stimulates breast tissue and increases the incidence of breast cancer. However, the long-term effect of T therapy on the incidence of breast cancer has not been previously documented in a prospective study. This is becoming increasingly important as more research is being performed, and more studies are being published on the benefits of T therapy in women [1–7].

There is some concern about T and breast cancer risk. Although some epidemiological studies have shown an increased incidence of breast cancer associated with endogenous T levels [8–12], others have not [13–16]. In addition, some studies have found T levels to be protective [17,18]. Overall, the evidence from epidemiological studies is conflicting. Furthermore, there are methodological

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limitations, both for these studies, and for T assays, which have been shown to be inaccurate in women.

Another concern is that T is the major substrate for estradiol and therefore has a secondary 'stimulatory' effect at the estrogen receptor (ER). Anastrozole (A), combined with T in a pellet implant, has been shown to prevent aromatization and provides adequate levels of T without elevating estradiol in breast cancer survivors [19,20]. Also, T has been shown to safely relieve side effects of aromatase inhibitor therapy in cancer survivors [21,22].

T therapy is being increasingly prescribed, and its long-term effect in the breast need to be further elucidated. The Testosterone Implant Breast Cancer Prevention Study, i.e., 'Dayton study', is a prospective, observational study that was specifically designed to investigate the incidence of breast cancer in women treated with subcutaneous T implants for symptoms of hormone deficiency. This 5-year interim analysis addresses the incidence of breast cancer in women treated with subcutaneous T, or T + A without concurrent use of systemic estrogen or synthetic progestins.

2. Methods

2.1. Study design, setting, and participants

All patients enrolled in the study are part of an ongoing, 10-year observational, longitudinal prospective IRB approved study, investigating the incidence of breast cancer in women treated with subcutaneous T implants. The study was approved in March of 2008 at which time recruitment was initiated. An interim analysis was planned for year 5, March of 2013.

Pre and post menopausal patients participating in the study were either self-referred or referred by their physician to the clinic (RG) at the Millennium Wellness Center in Dayton, Ohio for symptoms of relative androgen deficiency including hot flashes, sweating, sleep disturbance, heart discomfort, depressive mood, irritability, anxiety, pre-menstrual syndrome, fatigue, memory loss, menstrual or migraine headaches, vaginal dryness, sexual problems, urinary symptoms including incontinence, musculoskeletal pain and bone loss. Female patients with no personal history of breast cancer were asked to participate in this study. Study size was not predetermined. All patients continuing T therapy were invited to participate in the study. No patient was excluded from participation based on age, prior hormone use, oral contraceptive use, endometrial pathology, breast density, increased cancer risk, menopausal status or body mass index (BMI). Mammography and clinical breast exam were not protocol determined. Screening mammograms were recommended, but not required, prior to enrollment. As predetermined, patients with a single T pellet insertion were not included in this analysis. Patients who had received T implants prior to the IRB approval date were not excluded from participation and were recruited to the study beginning March 2008. An IRB approved, written informed consent was obtained on all patients enrolled in the study. As per IRB protocol, the incidence of breast cancer in our study population was to be compared to historical controls as well as age specific Surveillance Epidemiology and End Results (SEER) data.

Although a control group was not part of the original IRB approved protocol, it was predetermined that patients receiving only one pellet implant, i.e., 3 month of therapy, would not be eligible for analysis. Such short-term hormone use would not have a long-term affect on the incidence of breast cancer. This group of 119 patients, enrolled and treated prior to 2010, was followed prospectively as a 'control' group. As of January 2010, only patients who continued T therapy (>1 insert) were enrolled in the study.

Table 1
Indications for aromatase inhibitor therapy in female patients.

History of breast cancer
Increased risk for breast cancer
Atypical ductal hyperplasia
Strong family history
Lobular carcinoma in situ
Severe fibrocystic breast tissue, breast pain
Endometriosis, uterine fibroids, dysfunctional uterine bleeding
Weight gain, increased abdominal obesity/fat
Insulin resistance, metabolic syndrome with elevated estradiol
Menstrual or migraine headaches
PMS, anxiety, irritability, aggression, fluid retention, bloating

Adapted from the 9th European Congress on Menopause and Andropause [10].

2.2. Subcutaneous implants, the evolution of testosterone therapy in clinical practice, testosterone combined with anastrozole

The T and T + A implants used in this clinical practice (RG) are compounded by a pharmacy in Cincinnati, Ohio. They are composed of non-micronized USP testosterone (T) and steric acid, or non-micronized USP T, steric acid and USP anastrozole (A); compressed with 2000 pounds of pressure using a standard pellet press into 3.1 mm (diameter) cylinders; sealed in glass ampoules, and sterilized at 20–25 psi of pressure at 121 °C (250 F) for 20–30 min. The sterile implants are inserted into the subcutaneous tissue of the upper gluteal area or lower abdomen through a 5 mm incision using a disposable trocar kit.

This clinical practice (RG) has evolved over the past 6 years. Systemic estrogen therapy was used in the majority of patients through 2008. However, it became evident that subcutaneous T was able to treat symptoms in over 95% of patients, and the routine use of estrogen, including estradiol (E2) implants, was discontinued. In this practice, T implant dosing is weight based with an average starting dose of 2–2.5 mg/kg and is adjusted based on clinical response; the average interval for T pellet insertion is 13.8 ± 3.8 weeks [1,24].

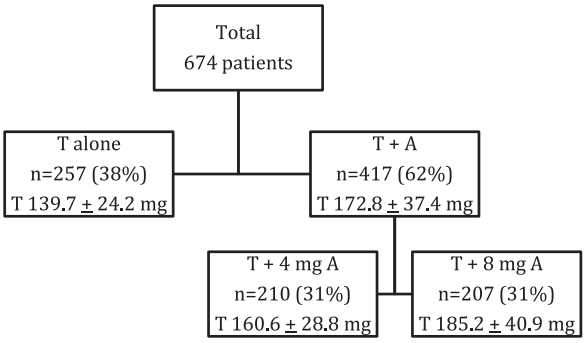
We began using anastrozole (A), an aromatase inhibitor, combined in the pellet implant in 2008; initially, to treat symptoms of hormone deficiency in breast cancer survivors and men on T implant therapy [19,20]. Subsequently, beginning early 2010, women who presented with signs or symptoms of hyperestrogenism, obesity or increased risk for breast cancer were offered A in combination with T as a pellet implant. We have also found that pre-menopausal patients with symptoms of excess estrogen including migraine headaches, dysfunctional uterine bleeding, endometriosis, uterine fibroids, breast pain or severe premenstrual syndrome, also benefit from the 'low dose' (compared to 1 mg/day oral) A delivered subcutaneously with T. As previously reported, current indications for aromatase inhibitor (AI) therapy followed in this clinical practice are listed in Table 1 [20].

The amount of A in the pellet implant is 4 mg combined with 60 mg of T, which allows for consistent, simultaneous release of both the T and A. Two implants, a total of 8 mg of A, has been shown to prevent elevation of E2 in breast cancer survivors treated with subcutaneous T [19]. We have subsequently found that 4 mg of A, one T + A pellet, is able to prevent symptoms of excess estrogen in many women without breast cancer. The T and T + A dosing is based on clinical history, symptoms, clinical observation, weight, amount of fatty tissue, T dose and laboratory evaluation. Often, heavier, more obese patients require higher doses of both T and A, Table 2.

2.3. Data analytics, patient follow up

From March of 2008 through February of 2010, all data were entered into an excel format. In February 2010, a custom web-based application using Microsoft Active Server Pages with a MySQL

Table 2
Current use and dosing of T alone, T + 4 mg A, T + 8 mg A: female patients treated 1 December 2012–1 March 2013.



database backend system was custom developed to prospectively follow and track patients along with 'person-days' of therapy. Date of the first T implant insertion, dose, and date of each subsequent insertion along with patient identifiers were entered. The computer program was programmed to identify women who had not returned for therapy within a pre-set time frame of 240 days, the maximum duration or exposure from T implant therapy as previously determined, i.e., 2.5 times the average interval of insertion of 96 days [1]. Weekly 'follow-up' phone calls were performed by designated research personnel. Any participant who was not seen for 240 days was contacted and breast cancer status was documented. All patients no longer receiving therapy have agreed to contact the office in the future for any subsequent diagnosis of breast cancer. In addition, approaching year 5, additional phone calls were made to patients no longer on T therapy.

2.4. Statistical methods

The incidence rates of breast cancer are reported as an unadjusted, un-weighted value of newly diagnosed cases divided by the sum of person-time of observation of the 'at risk' populations (ITT, adherent and control).

Person-days of observation were calculated from the date of first T pellet insertion for each participant up to the date of cancer registration, the date of death, or the set date of 31 March 2013, whichever came first. The computer program accurately and continually tracks the number of person-days for patient and calculates a running sum (cumulative total) across the group. Person-years (p-y) are calculated by dividing person-days by 365.

The incidence of breast cancer was calculated per 100 000 p-y so that our results could be compared to the incidence of breast cancer in published historical controls, as has been done in other studies [25], as well as age-group SEER published breast cancer incidence rates for 2000–2009. Also, using SEER incidence rates, the expected breast cancer incidence rate for our ITT group was calculated from the age distribution of enrolled patients as of 31 March 2013. The 'expected incidence' is a weighted sum of the SEER incidence rates with the weights corresponding to the proportion of the Dayton study patients in each of the SEER age groups on March 31, 2013.

In order to investigate the effect of non-adherence on the incidence of breast cancer, the computer was programmed to calculate person-days of compliance or adherence to therapy. Non-adherence was defined as, and calculated at, 240 days after the last T pellet insertion (per individual patient) at which time the person-days no longer accrued. The effect of non-adherence was conducted by recalculating adherent p-y of therapy (as described above) and incidence of breast cancer after censoring events that occurred in women who had inconsistent T insertions at >8 month intervals or events that happened within the first 8 months after starting therapy.

In addition, bootstrapping, a method of estimating the sampling distribution of the estimates, was used to confirm the significance of these results [26]. Bootstrap sampling distributions of the ITT group and the adherent group were constructed to determine if there were important differences in breast cancer incidence rates between the two groups. In addition, bootstrapping distribution was performed on our 'control' group of patients not receiving T therapy. Bootstrap calculations were performed using the Bootstrap R (S-plus) functions (boot), R package version 1.3-9.

Table 3
Patient demographics Dayton study, 31 March 2013. One patient, inadvertently accrued to the study at first implant, was not eligible for analysis.

Participants accrued (*N*)	1388
Eligible for analysis, ITT	1268
Controls	119
Age at first implant, y	
ITT	52.2 ± 8.7
Control	53.5 ± 9.3
Age at analysis, y	
ITT	56.6 ± 8.9
Control	57.8 ± 9.3
Menopausal, %	76.8
Surgical, %	66.2
Natural, %	43.8
Pre/perimenopausal, %	23.2
Family hx breast cancer, %	29.0
1st degree relative, %	13.4
Menarche age, y	12.8 ± 1.6
Age at first birth, y	24.8 ± 5.2
BMI, kg/m^2	26.3 ± 5.5

3. Results

3.1. Patient demographics, accrual

Patient demographics are shown in Table 3. As of 31 March 2013, interim analysis study year 5, 1388 patients were accrued to the Dayton study with 1268 patients having received more than one pellet implant and eligible for analysis, i.e., Intent to treat (ITT) group. There were 119 eligible 'control' patients and one non-eligible patient, being recently inadvertently enrolled at first implant. The mean age at first T pellet insertion was 52.2 ± 8.7 years. The mean age of the 119 'control' patients was 53.5 ± 9.3 years. Patients were not at an increased (or decreased) risk for breast cancer, in regards to risk factors such as family history, or hormonal and reproductive factors, shown in Table 3.

The majority of patients (62%) were accrued to the study within the first year. Over 85% of patients were accrued by study year 2, 90% by year 3, and 96% by year 4. Only 4% of patients were accrued between 1 March 2012 and 1 March 2013. The mean number of years since first T implant was 4.6 ± 1.3 years; the median was 4.7 ± 1.7 years. The maximum duration of T therapy was 7.36 years, which includes women who received their first T implant prior to study accrual. Since the study remained open for accrual through 31 March 2013, the minimum duration of therapy was 0.28 years.

The percent of female patients (without breast cancer) treated with the combination T + A implant has increased from approximately 11% in 2010, to 30% January through July 2011, and to 62% 1 December 2012 through 1 March 2013; with half of those patients currently being treated with the 4 mg A dose, Table 2.

Table 4
Breast cancer cases in women using testosterone (T) or T with anastrozole (A) without estrogen compared with major studies using estrogen (E), progestin (P) therapy, E/P/T, E/T, past users, never users and SEER incidence rates.

	N patients	Age, years	Cases/100 000 person-years	Years observed
WHI (E/P)	8506	63.2	380	5.2
Placebo	8102	63.3	300	
MWS current users	394,697	55.1	501	14
Past users	221,056	56.7	337	
Never users	513,272	57.7	325	
Adelaide (E/P/T, E/T)	508	56.4	238	5.9
Dayton (T, T/A)	1268	52.2[a] 56.6[b.]		5.0
ITT			142	
Adherent			73	
SEER incidence rates		50–54	234	
		55–59	293	
		60–64	358	

[a] Mean age at first T insertion.
[b] Age at time of analysis, for comparison to SEER data.

3.2. Person-years: Intent to treat, adherence to therapy, 'control'

As of 31 March 2013, there were 2,059,144 days of follow up, equating to 5642 p-y of observation in the ITT group of patients. There have been 1,508,476 days, or 4133 p-y of adherent follow up, as defined above. In addition, there have been 187,365 days, or 513 p-y of follow up in the 119 patients followed as 'controls'.

3.3. Breast cancer incidence and characteristics

Breast cancers were verified by obtaining the pathology report from biopsy and definitive surgery. As of 31 March 2013, 8 cases of invasive breast cancer have been diagnosed in 1268 women (0.63%) treated with T implant therapy in 5642 p-y of follow-up, resulting in an incidence of 142 cases per 100 000 p-y, Table 4.

Table 5
Patient data and tumor characteristics, eight patients treated with T or T + A implants (no 'current' systemic estrogen) diagnosed with invasive breast cancer March 2008 through March 2013.

Pt.	Age at dx.	BMI 1st insert	Menopausal status	Prior estrogen use	Date of diagnosis	Diagnosis	Receptor status	T (alone) implant therapy	Comments
1	49	19.2	Surgical TAH	E2/T implants 1/07, 7/07	3/2010	T1b, N0 Gd 2 IDC Stage 1	ER+, PR+ HER2 neg	4 implants 3/08–8/09	History of severe fibrocystic breast with multiple aspirations/bxs. Adherent
2	58	33.3	Post	E2/T implants 2/06–7/07	9/2010	T3, N2 Gd 3 IDC Stage 3	ER−, PR− HER2 neg	4 implants over 3 years 12/07–2/10	Non-adherent
3	52	19.2	Pre	OCP Until 2009	10/2011	T1c, N0 Gd 1 IDC	ER+, PR+ HER2 neg	1 implant 12/08 3 implants 3/11–9/11	Diagnosed 7 months after (re) starting therapy. Severe fibrocystic disease. Non-adherent/Adherent 7 m
4	70	24.7	Surgical TAH BSO	CEE > 20 yrs	11/2011	T1c, N0 Gd 1 IDC Stage 1	ER+, PR+ HER2 neg	7 implants 4/09–6/11	Weaned CEE after starting T therapy Adherent T therapy
5	48	21.5	Surgical TAH		3/2012	T1c, N0 G1, IDC (tubular) Stage 1	ER+, PR− HER2 neg	3 implants 4/09–4/10 Implant 2/12	Mother and aunt BCA. Diagnosed 1 month after a single insert following 2-year absence. Non-adherent
6	52	20.5	Surgical TAH	Oral, unknown type	4/2012	T1a, N0 Gd 1 IDC Stage 1	ER+, PR+ HER2 pos	8 implants 5/09–5/11	Not seen 11 months prior to diagnosis Non-adherent
7	66	27.4	Post	Estrace 5-yrs until 2008	1/2013	T1b, N0 Gd 1, IDC (tubular) Stage 1	ER+, PR+ HER2 neg	3 T implants 4/10–10/10	Mother and sister BCA Non-adherent
8	55	24.5	Surgical TAH BSO	E2 implants 2006–07	3/2013	T1b, N1a Gd 2 ILC Stage 2	ER+, PR− HER2 neg	Implants since 3/06 T + A 8/10	FCC, dense breast Adherent T or T + A therapy since 9/2007

Abbreviations: Pt. (patient), Dx. (diagnosis), BMI (body mass index), E2 (estradiol), OCP (oral contraceptive pill), CEE (conjugated equine estrogens), IDC (infiltrating ductal carcinoma), ILC (infiltrating lobular carcinoma), T (tumor size), a (<0.5 cm), b (>0.5, <1 cm), c (>1, <2 cm), T2 (20–50 mm), T3 (>50 mm), N (node status); N0 (no nodes positive), N1a (single node < 5 mm), N2 (4–9 nodes positive), Gd (tumor grade: 1, low grade; 2, intermediate grade; 3, high grade), ER (estrogen receptor), PR (progesterone receptor), HER2 (human epidermal growth factor 2).

Patient data, prior therapy and tumor characteristics are presented in Table 5. The mean age at diagnosis was 56.3 ± 8.0 years. Seven patients were postmenopausal with 5 of these 7 having surgical menopause. Seven of 8 patients had been on systemic estrogen in the past and 3 of those 7 had been treated with E2 implants. Although this study is reporting on the incidence of breast cancer in patients treated with T alone, these patients were included in the results as they had been off estrogen therapy for at least 1 year. Interestingly, the four patients age 52 years of age and younger, had a lower BMI (20.1 ± 1.1) compared to the four patients 55 years of age and older (27.5 ± 4.1). Baseline serum T levels were available in 5 of the 8 patients diagnosed with cancer, and were lower compared to baseline T in our general population (13.6 ± 6.3 vs. 19.0 ± 12.8 ng/dl). A 6th patient had a salivary T level below the lower limit for females. Patient 2 had been on E2 and T implants by another physician since 2005 and screening labs were not available.

Three patients (no. 1, 4 and 8) diagnosed with breast cancer had been treated consistently with T implants for at least 8 months prior to diagnosis, i.e., adherent to therapy. Only one patient (no. 8) had been consistently treated with T + A combination therapy prior to diagnosis. Patient 6 had been consistently treated for 2 years, but was non-adherent for 11 months prior to the diagnosis of a 4 mm invasive cancer. She has continued to be treated with T + A implant therapy along with oral anastrozole. Patient 3 (premenopausal) was treated with T implants for fewer than 8 months prior to her diagnosis. She has continued therapy with T + A combination implants, initially with Tamoxifen, which was changed to anastrozole after menopause. Patient 2 had been non-adherent to T therapy and was diagnosed with an aggressive hormone receptor negative tumor. Patient 5 had a single T implant prior to being diagnosed with breast cancer one month later. Patient 7 had received 3 implants between April and September 2010 and had been off T therapy for over 2 years prior to diagnosis.

One additional patient, a 55 year-old postmenopausal female, has been diagnosed with an 8 mm non-high grade (non-invasive) ductal carcinoma in situ, which was treated with a lumpectomy alone. The patient continued T therapy and is currently treated with the combination T + A implants.

3.4. Tumor characteristics

Six of 8 tumors were Stage 1, small (<2 cm), node negative, estrogen receptor (ER) positive infiltrating ductal carcinomas; five of these 6 were HER2 negative. One ER positive patient (no. 8) who had lymph node involvement, had a small, <0.5 mm infiltrating lobular carcinoma. One tumor was an aggressive, 5 cm, node positive, triple negative (i.e., ER negative, PR negative, HER2 negative) invasive cancer, Table 5.

3.5. Effect of adherence to T therapy

3 breast cancers (events) were diagnosed in women (patients 1, 4, and 8) who were adherent to therapy (predetermined as consistent therapy at ≤8 month intervals, event diagnosed >8 months after initiating T therapy) in 4133 p-y, which equates to an incidence of 73 per 100 000 p-y. Although patient 6 had been off therapy for 11 months prior to diagnosis, if she were re-classified as 'adherent', the incidence would be 97 per 100 000 person-years, still significantly less than the ITT group, 142 per 100 000.

There have been 2 cases of breast cancers diagnosed in the 119 'control' patients, both diagnosed 18 months or longer after their single T pellet insert. This equates to an incidence of 390/100 000 p-y, which is substantially greater than the 142/100 000 for the ITT. The percent of breast cancer cases diagnosed was significantly higher in the 'control' group (1.68%) compared to ITT group (0.63%).

Comparison of bootstrap sampling distribution of the incidence of breast cancer revealed a significantly ($P < 0.001$) lower incidence of breast cancer in the T adherent population compared to the ITT group as well as the calculated expected SEER incidence rate based on the age distribution, further supporting that T and T + A therapy reduces the incidence of breast cancer, Fig. 1. In addition, breast cancer occurrence for the 'control' group was significantly higher than either the adherent group or the ITT group ($P < 0.001$).

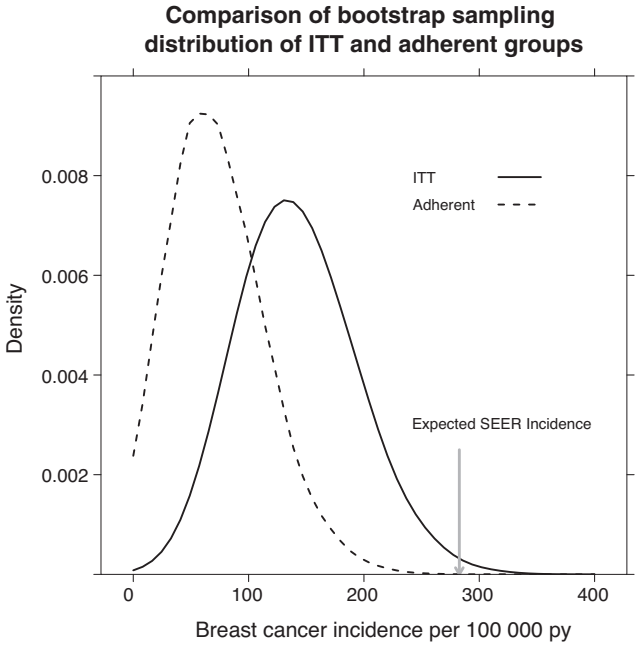


Fig. 1. Bootstrap sampling confirmed a significantly lower incidence of breast cancer, in the T adherent population compared to ITT group ($P < 0.001$). Expected SEER, incidence rate for age group comparison is shown.

3.6. Adverse drug events, side effects of therapy

We have previously measured T levels in a representative subgroup of patients from this population and have shown that pharmacologic doses of subcutaneous T (55–240 mg), as evidenced by serum levels on therapy, are necessary to produce a physiologic effect. Serum T levels measured at 'week 4' (299.36 ± 107.34 ng/dl), and when symptoms returned (171.43 ± 73.01 ng/dl), were several-fold higher compared to levels of endogenous T. Despite pharmacologic serum levels, there have been no reported adverse drug events attributed to T therapy other than expected androgenic side effects, which are reversible with lowering T dose. As previously reported, the majority of patients studied (92%) reported a slight or moderate increase in facial hair; however, no patient discontinued therapy because of this. Interestingly, 63% of patients who reported scalp hair thinning prior to T therapy, reported hair re-growth on therapy [23]. 51% of patients reported a mild or moderate increase in acne, and 52% (including patients with an increase in acne) reported improved appearance of the skin. In addition, there have been no adverse drug events related to subcutaneous A. The amount of A released over an average of 100 days is approximately 0.04–0.08 mg per day, compared to 1 mg orally per day. The only reported side effect from the 8 mg dose of A has been hot flashes, which resolve with lowering the dose of A to 4 mg (one implant) or treating the patient with T alone, no A.

4. Discussion

In the past 7 years, over 16 000 T pellet insertions have been performed in 1388 pre and postmenopausal women followed on protocol since 1 March 2008. We have previously reported on the

benefits and safety of T therapy, T dosing and levels on therapy, as well as efficacy of A combined with T [1,2,19,20,23]. Our current data evidence that an average T dose of 2.0–2.5 mg/kg (range 55–240 mg), delivered subcutaneously with or without 4.0–8.0 mg of A at approximately 3 month intervals, has resulted in a reduction in the incidence of breast cancer.

The 5-year interim analysis of the Dayton study resulted in a ratio of 142 breast cancer events per 100 000 p-y, which is remarkably lower when compared to all previous studies concerning hormonal treatments, Table 4. Even in comparison to the placebo group of the Women's Health Initiative Randomized Trial (300/100 000) and never users from Million Women Study (325/100 000), there was a reduced incidence of breast cancer with T therapy used in the Dayton study [27–29].

We also demonstrated a reduced incidence of breast cancer with T and T + A in comparison to the Adelaide Study (238/100 000), which previously reported a reduced incidence of breast cancer with T-use in combination with conventional hormone therapy [25].

In addition, the Dayton study showed a reduced incidence of breast cancer compared with age-specific SEER incidence rates for both the younger age group, i.e., 50–54 y (234/100 000) and the current age comparable (at time of analysis) group, i.e., 55–59 y (292/100 000) [30,31]. Notably, the incidence rate for our ITT group (142/100 000) was approximately half of the calculated expected SEER incidence rate (276 ± 6.81/100 000) based on the age distribution of Dayton study participants. Even more significant, women who were adherent to T or T + A therapy had over a 3.5-fold reduction in the expected incidence rate of breast cancer.

These outcomes, which indicate a beneficial effect of T and T + A in the breast, were not unexpected. There is sufficient biological and clinical data indicating that androgens have a protective role in breast tissue. Although some epidemiological studies show an association between increased serum androgen levels and higher breast cancer incidence, others do not; and many of these studies fail to isolate T from the circulating estrogens or account for local aromatization to estrogens [8–18]. In addition, they do not address the known insulin-inflammation-cancer connection; insulin stimulation of T production; or the insulin stimulated increase in aromatase activity, including locally in the breast; all of which would contribute to increased cancer risk [32–36].

Most importantly, association does not infer causation [37], and a 'cause and effect' interpretation of inconsistent epidemiologic data conflicts with the known biological effect of T in the breast. Although a few studies report both a proliferative and antiproliferative affect of T in breast cancer cell lines [38,39], the abundance of evidence supports that T's direct effect via androgen receptors (AR) is anti-prolific, pro-apoptotic, and inhibits breast cancer cell growth [40–46]. In addition, clinical studies in primates and humans, including long-term studies in female to male transgender patients, as well as clinical observations, support the protective role of T in the breast [47–51]. Androgens, including T pellet implants, have been used to successfully treat breast cancer in the past as well as symptoms of menopause in breast cancer survivors [52–55]. Androgen receptor status in breast cancer has been shown to be prognostic; evidencing smaller tumor sizes, lower histological grade, better prognosis and increased disease free survival [56–61].

A limitation of the study was a lack of a matched control group from the onset of the study. However, we prospectively followed patients receiving a single T insert, i.e., limited, 3-month exposure to therapy, as a 'control' group. Although our 'control' group was small ($n = 119$), these patients were representative of the same population, presented with similar symptoms and were similar in age. We demonstrated a reduced incidence of breast cancer in both our adherent group (73/100 000 p-y) as well as our ITT group (142/100 000 p-y) in comparison to our 'control' group (390/100 000 p-y).

Of note is the discordant effect of 'adherence to estrogen–progestin (E/P) therapy' compared to 'adherence to T therapy' on outcome. We have shown that adherence to T therapy has the converse outcome on breast cancer incidence, i.e., decreased events (signifying a protective effect), compared to adherence with E/P therapy, i.e., increased events [27].

A possible argument regarding the low breast cancer incidence reported in our study is that our patient population was at low risk for breast cancer. Indeed, there was no specific criterion excluding or including high-risk women. In the Dayton study, women enrolled were seeking therapy for symptoms of hormone deficiency; thus, our population was comparable to populations included in the historical hormone therapy trials mentioned above. Our mean age at the time of analysis, 56.6 y, compares with the mean age of other studies at recruitment/screening and evaluation, and age specific SEER data. Although our mean age at the time of recruitment was slightly lower, the younger pre/peri-menopausal women who enrolled in the study presented with symptoms of estrogen excess and androgen deficiency, possibly putting them at an increased risk for breast cancer. Regarding additional risk factors for breast cancer, our incidence of family history was comparable to other studies, as was mean age at menarche, first birth, and menopause [27,29].

Currently, patients at highest risk for increased aromatization are being increasingly identified and treated with A delivered simultaneously with T in the combination implant. We did not begin using the combination implant in this population until early 2010, implying that the reduction in breast cancer reported at year 5, is likely a result of T's effect at the AR rather than primarily aromatase inhibition. With the addition of low dose aromatase inhibitor (AI) therapy in high-risk women, the favorable balance of T to E ratio may be further restored and it is possible that a further reduction in the incidence breast cancer will be demonstrated in the future.

A critique of our practice is the use of low dose, subcutaneous AI in some pre/perimenopausal patients. Although AI are not indicated for therapy in premenopausal breast cancer patients, it is not because they are ineffective; but rather, with oral AI therapy total suppression of estradiol in this subgroup could 'potentially' increase gonadotropin releasing hormone, and secondarily stimulate the ovary to re-produce estrogen via ovarian-pituitary negative feedback mechanism [62]. Although a discussion of AI therapy in pre-menopausal patients is beyond the scope of this paper, our interventions were based on the previously successful use of AI to treat breast and gynecologic diseases where pathological tissues overexpress aromatase and increase local production of estrogens [63]. We have found that low dose A (0.04–0.08 mg/d) combined with T, delivered subcutaneously, effectively treats these conditions without adverse effects, or alteration of menstrual cycles.

The favorable effect of T in the breast is further supported by the lack of recurrence in our breast cancer survivors treated with subcutaneous T or T + A [19]. This observation represents the preliminary result of an ongoing trial designed to assess T treatment role in breast cancer survivors. We are also examining the effect of neoadjuvant, intra-mammary (peritumoral) T + A implants in invasive breast cancers, and demonstrating a rapid clinical response of tumors to this therapy (report under publication).

It is possible that continuous, subcutaneous T + A could help prevent breast cancer in high-risk women, and recurrences in breast cancer survivors. In addition to providing consistent therapeutic levels of T and A, the subcutaneous delivery route also allows both T and A to bypass the liver, avoiding the 'first pass effect' and thus eliminating the increased risk of blood clots, pulmonary embolism and deep venous thrombosis. It also increases efficacy

and decreases side effects, including gastrointestinal side effects of oral therapy.

Testosterone is critical to both physical and mental health in women. However, patients differ in their ability to aromatize T to E2. Caution should be used in treating patients with clinical evidence increased aromatase activity and consideration should be given to the addition of AI therapy.

5. Conclusion

Continuous T and T + A, delivered as a subcutaneous implant, seems to represent safe and effective therapy in treating hormonal symptoms in both pre and postmenopausal women. In this study, safety is verified by the significant decline in breast cancer incidence. We demonstrated that subcutaneous T, and subsequently, T + A, has a protective effect in the breast, and prevented cancer occurrence in some cases. Our findings are consistent with the known favorable biological effect of T on the breast tissue via the AR, as well as data from previously reported preclinical and clinical studies. Our results refute the 'cause and effect interpretation' of epidemiological studies demonstrating an association of endogenous testosterone levels with breast cancer. Further studies should be done on subcutaneous T, and the combination of T + A, for the possible prevention and therapy of breast cancer.

Study details

Glaser, R (PI) and Dimitrakakis, C (PI). Testosterone Implants and the Incidence of Breast Cancer: TIBCaP 0108 (Testosterone Implant Breast Cancer Prevention Trial 0108). IRB Approved 21 March 2008, continued through March 2013. Atrium Medical Center, Premier Health Partners, One Medical Center Drive, Middletown, Ohio 45005. Registered through the Office for Human Research Protections (OHRP).

As of March 2013, no additional patients will be accrued to this study. Patients may continue to be accrued under a central institutional review board (Wright State University IRB) study, SC# 509, 'Testosterone Implants and the Incidence of Breast Cancer', Number: FWA00002427.

Contributors

RG and CD contributed equally to the research, design of the study, analyzing the data, writing and editing the manuscript. RG recruited participants. Both authors approved the final manuscript.

Competing interest

Neither author (RG, CD) has any competing interests.

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