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Digitized assessment of mammographic breast density – Effects of continuous combined hormone therapy, tibolone and black cohosh compared to placebo

E. Lundström*, A.L. Hirschberg, G. Söderqvist

Department of Women's and Children's Health, Karolinska Institutet and University Hospital, Stockholm, Sweden

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ABSTRACT

Objectives: To determine the effects of continuous combined hormone therapy, tibolone, black cohosh, and placebo on digitized mammographic breast density in postmenopausal women.
Study design: A prospective, double-blind, placebo-controlled study of 154 postmenopausal women randomized to estradiol 2 mg/norethisterone acetate 1 mg (E2/NETA), tibolone 2.5 mg or placebo and a prospective, open, uncontrolled drug safety study, of which 65 postmenopausal women were treated with black cohosh. Mammograms, at baseline and after six months of treatment, were previously classified according to visual quantification scales.
Main outcome measures: Reanalysis of assessable mammograms by digitized quantification of breast density.
Results: Treatment groups were comparable at baseline. During treatment, both E2/NETA and tibolone significantly increased breast density (mean increase 14.3%, $p < 0.001$ and 2.3%, $p < 0.001$, respectively), while black cohosh and placebo did not. Twenty-four out of the 43 women on E2/NETA had an increase in density exceeding 10% and 6 women had an increase of 30% or more. In the tibolone group, only one woman had an increase in density of more than 10%. The difference in increase in breast density between E2/NETA on the one hand and tibolone, black cohosh and placebo on the other was highly significant ($p < 0.0001$).
Conclusions: Digitized mammographic breast density is a highly sensitive method confirming significant increase in density by standard E2/NETA treatment and to a lesser extent by tibolone, whereas black cohosh does not influence mammographic breast density during six months treatment. Digitized assessment also yields data on individual variation and small increases left undetectable by visual classification.

1. Introduction

Mammographic breast density has been defined as a strong and independent risk factor and it has even been suggested as an intermediate phenotype for breast cancer [1–4]. Density in individual women seems to reflect the net influence of endogenous and exogenous hormonal stimuli and their background genetics on the breast during the lifespan [5,6]. During conventional hormone therapy (HT), an increase in mammographic breast density has repeatedly been demonstrated to occur in a significant proportion of postmenopausal women [7–9]. It also seems that the degree of change during HT follows our current perception of breast cancer risk from different treatment principles. In agreement with data from the Women's Health Initiative (WHI) study, most combined estrogen/progestogen treatments will increase density whereas estrogen alone has very little effect on this risk factor [10].

Many different methods have been used to classify and quantify mammographic breast density. Much of the older information on the effects of HT has been based on visual classifications according to different scales, e.g., Wolfe, BI-RADS and percentage scales [8,11,12]. All these traditional visual classifications represent a rather crude measurement where an increase in density of about 20–25% could be required for an upgrading of one class. Apart from these limitations, visual assessment may also be subject to bias and inconsistency. Since mammographic breast density should be regarded a continuous variable, digitized methods allow a more sensitive measurement and are able to quantify more discrete changes than traditional visual classification. Currently, there is a rapid development of computer assisted techniques for individual assessment of density and the monitoring of change

* Corresponding author at: Department of Obstetrics and Gynaecology, Karolinska University Hospital, SE-171 76 Stockholm, Sweden. Tel.: +46 8 517 742 14; fax: +46 8 517 742 52.
E-mail addresses: eva.lundstrom@karolinska.se, g.soderqvist@telia.com (E. Lundström).

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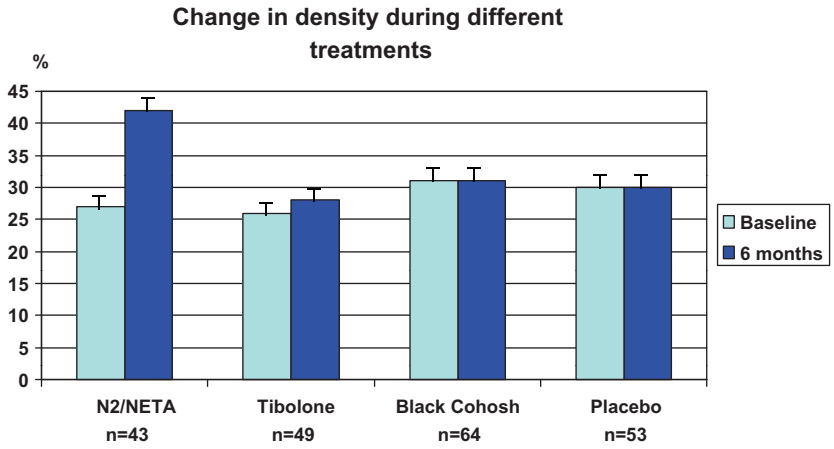

Fig. 1. Mean and SEM values of mammographic breast density at baseline and after 6 months in the four treatment groups. Breast density increased significantly by E2/NETA and tibolone ($p < 0.001$, respectively), but not by black cohosh and placebo.

following interventions [13]. Using visual classifications, we previously reported an increase in density to occur in about 50% of women treated with 2 mg estradiol (E2)/1 mg norethisterone acetate (NETA), but in only 6% of women treated with tibolone [8]. During treatment with black cohosh and placebo none of the women showed any increase [14]. However, in these studies the degree of change in individual women was not quantified.

Therefore, we carried out a reanalysis of the previous material using a highly sensitive digitized technique in order to quantify the individual response and to see if we could confirm the differences between treatments.

2. Materials and methods

2.1. Subjects

Mammograms from a total of 209 postmenopausal women from two earlier prospective studies, where mammographic breast density had been classified according to visual quantification scales, i.e., the Wolfe classification [11] and the percentage scale [8] were reinvestigated by digitized quantification of breast density.

A total of 43 women received E2 2 mg/NETA 1 mg (Kliogest®), 49 women tibolone 2.5 mg (Livial®) and 53 women placebo once daily [8]. In addition, 64 women were treated with black cohosh (Remifemin®) (batch no. 229690), one tablet twice daily. Each tablet contains 0.018–0.026 mL liquid extract of black cohosh rootstock (0.78–1.14:1), corresponding to 20 mg herbal drug (i.e., 2.5 mg dry extract, extraction agent isopropanol 40% [vol/vol]) [14].

All women were postmenopausal women aged 50–70 years with a body mass index (BMI) of 20–30 kg/m^2. Inclusion criteria were last menstrual bleeding 12 or more months before enrolment in the study or follicle stimulating hormone levels greater than 40 IU/L and estradiol levels less than 20 pg/mL. Women who had used HT in the previous 3 months were excluded from the study, as were those with hypertension (systolic blood pressure >170 mm Hg or diastolic blood pressure >105 mm Hg), hyperlipidemia (total cholesterol >8.0 mmol/L or triglycerides >4.0 mmol/L), or type I or II diabetes mellitus. Women with any previous history of breast disease were excluded.

The studies were approved by the independent ethics committee and the Swedish Medical Products Agency; IRB-02-4047 and 151:2002/71141 [14], IRB-98-441 and 151:1965/98 [8], respectively. All women have given their informed consent before participation in the studies.

2.2. Mammographic examinations

Mammograms in both trials were performed at the same site and by the same investigators at baseline and after 6 months of treatment. Mammography examinations comprised the cranio-caudal projection of the left breast [15,16]. Mammography examinations were performed in accordance with the quality control regulations stipulated by the Swedish National Board for Health and Welfare and the Swedish National Radiation Protection Institute.

2.3. Digitized breast density

The identifying data were removed from the films and the operator (E.L.) was unaware of the patient's identity, type and in what order the films were taken. All films were digitized and the dense area of the left cranio-caudal view image was measured by using a computer-assisted program (Cumulus, Sierra plus, Systems Corporation, Medical Imaging, Herndon, VA) [15,16]. In this procedure, the operator establishes "thresholds" for the edge of the breast and the edge of dense tissue. A computer then records the number of pixels in the digitized image that fall within the defined areas. This method of measurement has been shown to give highly reproducible results and details have been given elsewhere [17–19]. In the present study, the intra-assay variation was 8%, as calculated from five repeated measurements in five different mammograms (i.e., a total of 25 mammograms). The mean value of the amount of dense breast tissue (%) was calculated as the mean of three measurements.

2.4. Statistical analyses

The change in individual breast density values during treatment was compared between the four groups by use of the Kruskal–Wallis test. For within group changes the Wilcoxon signed-rank test was used. A p-value <0.05 was considered statistically significant.

3. Results

Mean values for age were 57.1, 57.5, 56.8 and 57.2 years for the E2/NETA, tibolone, black cohosh and placebo group, respectively. The corresponding figures for BMI and years since menopause were

Table 1
The number of women with increase in mammographic breast density according to percentage classes.

Percent increase in density	E2/NETA $n=43$	Tibolone $n=49$	Black cohosh $n=64$	Placebo $n=53$
0–4	16	39	64	53
5–9	3	9		
10–19	9	1		
20–29	9			
30–39	5			
40–49	0			
50–59	0			
60–69	1			

24.8 and 8.2, 24.5 and 7.9, 24.8 and 6.8, 24.4 and 6.6. There were no significant differences between groups at baseline.

3.1. Mammographic breast density according to digitized quantification

Mean and SEM values for the percentage area of dense breast at baseline and after 6 months in the four different groups are illustrated in Fig. 1. There were no significant differences at baseline. During treatment, both E2/NETA and tibolone significantly increased breast density, while black cohosh and placebo did not. Mean values for the increase during 6 months for E2/NETA, tibolone, black cohosh and placebo were 14.3% ($p<0.001$); 2.3% ($p<0.001$); 0% and 0%, respectively. After 6 months of treatment, the difference in increase in breast density between E2/NETA on the one hand and tibolone, black cohosh and placebo on the other was highly significant ($p<0.001$).

An individual increase in the percentage of mammographic density was much more common among women receiving continuous combined HT than among those receiving black cohosh, tibolone and placebo treatment. As illustrated in Table 1, 24 of the 43 women on E2/NETA had an increase in density exceeding 10% and 6 women had an increase of 30% or more. In the tibolone group, only one woman had an increase in density of more than 10% but for 9 women a slight increase of 5–9% was recorded. None of the women using black cohosh and placebo showed any significant change in density.

4. Discussion

In this paper we have re-evaluated mammograms and reclassified breast density by the much more sensitive digitized classification. This method, originally described by Byng et al., has shown a high correlation with visual classification scales and also gives much more detailed information [17–19]. The analysis, although based on a limited number of cases, confirms previous data showing that 2 mg E2/1 mg NETA results in a significantly higher increase in breast density than 2.5 mg of tibolone [8]. The material was obtained from two separate trials, which could imply a potential bias. However, all mammograms were performed at the same site, by the same investigators using the same technical equipment in both trials.

The large individual variation in response to E2/NETA treatment is also in agreement with earlier findings [9]. Previously, according to visual classification about 50% of women showed a response and were upgraded by at least one class [8]. Here, with the digitized method, 24 of the 43 women (56%) on E2/NETA had an increase in density exceeding 10% and 6 of these women had an increase of 30% or more. The digitized method was shown to be significantly better to detect an increase in mammographic density of 10% than the visual percentage classification method $p<0.05$. The results also add new information about individual variation and small increases in density that could not be detected by the previous visual analysis.

The combination of 2 mg E2/1 mg NETA is known to provide effective symptom relief but is also regarded as a high dose therapy similar to the 0.625 CEE/2.5 mg MPA as used in the WHI where a high percentage of mammographic abnormalities were reported [20]. In contrast, using the much lower dose of 0.5 mg E2/0.1 mg NETA, we found no increase at all in breast density after six months of treatment by the same digitized assessment as in the present study [18].

High dose combined estrogen–progestogen treatment has been associated with an increased risk for breast cancer. For tibolone the results are more uncertain. In the Million Women Study, the risk estimate for breast cancer from this compound was clearly lower than for other estrogen/progestogen combinations, but was still significant at RR 1.45 (95% CI 1.25–1.68) [21]. However, this finding was contradicted in a large case control study of a cohort of postmenopausal women from the UKs General Practice Reasearch Database (GRPD) [22]. Here, the corresponding RR for tibolone was 0.86 (95% CI 0.65–1.13) [22]. Furthermore, in the LIFT study, a prospective trial in older women with skeletal fracture as the primary endpoint, there was a significant decrease in the risk of breast cancer (RR 0.32; 95% CI 0.13–0.80) [23]. Recently, in the large multi center LIBERATE trial [24] on the safety of tibolone in breast cancer patients, there was an increased risk of recurrence. Still, the HR of 1.40 (95% CI 1.14–1.70) was lower than the HR 2.4 (95% CI 1.3–4.2) as previously reported for high dose continuous combined treatment in the HABITS trial [25]. In the present study, only one out of the forty-nine investigated women had an increase in density exceeding 10% after treatment with tibolone. Taken together available data suggest that tibolone is a relatively safe alternative for the alleviation of menopausal symptoms in healthy postmenopausal women [26].

The phytoestrogen, black cohosh, has been reported in some women to be an effective non-hormonal alternative for the treatment of climacteric symptoms [27]. The mechanism of action for black cohosh is still incompletely understood, but so far preclinical and clinical data suggest that it has no systemic estrogenic effects. However, black cohosh may possibly act as a natural selective estrogen receptor modulator and may also have a specific activity within the central nervous system [27].

Using visual classification, we have previously reported no increase in density during treatment with black cohosh [14]. Even when density was assessed by the highly sensitive digitized method, black cohosh did not increase mammographic breast density at all. Values did not differ from those of placebo treatment. The high reproducibility of the digitized method was apparent in these two groups where for all individual woman changes in density ranged between only 0 and 4%.

The long-term safety of combined hormonal treatment and especially about the effects on the breast has vividly been discussed over the past decades. There is a strong need to define treatment regimens and alternatives for postmenopausal women that have minimal effects on the breast but still are effective enough to relieve postmenopausal symptoms.

Mammographic density increase is an early event occurring shortly after initiation of therapy (i.e., weeks or months). The increase remains stable and persists during long-term treatment with the same regimen [8,17]. From a clinical perspective, increased breast epithelial proliferation and mammographic density during hormonal treatment should be regarded as unwanted and potentially hazardous side-effects [28]. Changes in density during treatment seem to follow current perception of cancer risk from different doses and regimens. Information on density may improve risk prediction [29]. However, it has to be clarified at what degree of breast density increase a risk of breast cancer may appear in

the individual woman. It seems unlikely that an increase of a few percent in density for a limited duration of time would have any clinical relevance. In addition, it is not yet clear whether an increase in mammographic breast density induced by hormonal treatment reflects the same potential for breast malignancy as a naturally occurring highly dense breast.

Contributors

All authors have contributed to study design, data collection, statistical analyses, writing and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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