The Safety of Local Hormonal Treatment for Vulvovaginal Atrophy in Women With Estrogen Receptor-positive Breast Cancer Who Are on Adjuvant Aromatase Inhibitor Therapy: Meta-analysis

R.T. Pavlović,[1] S.M. Janković,[2] J.R. Milovanović,[2] S.M. Stefanović,[1] M.M. Folić,[1] O.Z. Milovanović,[1] C. Mamillapalli,[3] M.N. Milosavljević[1]

Abstract

Atrophic vaginitis is a relatively common adverse effect of aromatase inhibitors used as an adjunctive treatment for breast cancer. Vaginal estrogen therapy is a treatment option, but the safety of its use in estrogen receptor-positive breast cancer remains understudied. The aim of our study was to determine the safety of local hormonal treatment of vulvovaginal atrophy in women treated with aromatase inhibitors. Our meta-analysis was based on a systematic search of the literature and selection of high-quality evidence. The safety of local hormonal therapy of vaginal atrophy in women on aromatase inhibitors were summarized using calculators built by the authors; heterogeneity was assessed by the Cochrane Q test and I^2 values. Several types of bias were assessed; publication bias was calculated by a funnel plot and the Egger regression. Eleven studies fulfilled the inclusion criteria for our study. After 8 weeks of local hormonal treatment, there was no change in the serum levels of luteinizing hormone and estradiol, whereas sex hormone binding globulins were low, and follicle stimulating hormone was almost doubled compared with the baseline. Adverse effect rates of vaginal discharge, facial hair growth, urinary tract or yeast infection, and vaginal or vulvar itching and/or irritation did not show significant changes in the sensitivity analysis, with exception of a single trial. Current evidence suggests that vaginal estrogen administration in postmenopausal women with a history of breast cancer is not associated with systemic absorption of sex hormones and may provide indirect evidence for the safety of their use.

Clinical Breast Cancer, Vol. 19, No. 6, e731-40 © 2019 Elsevier Inc. All rights reserved.
Keywords: Adverse effects rates, Breast cancer survivors, Local hormonal therapy, Serum level of sex hormones, Study heterogeneity

Introduction

The reduction of estrogen concentration has been used for the treatment of hormone-modulated conditions such as breast cancer, ovulation induction, and endometriosis.[1] A widely adopted approach is the use of selective estrogen receptor modulators or aromatase inhibitors (AIs). Trials have shown that AIs improve disease-free survival, overall survival, and the distant metastasis rate in post-menopausal women with hormone receptor-positive breast cancers[2] and decrease breast cancer recurrence rates and contralateral breast cancer after 10 years of treatment.[3] However, extended treatment has been associated with an increased risk of developing cardiovascular diseases,[4] hyperlipidemia,[5] bone fractures,[6] treatment discontinuation for adverse events,[7] deaths without breast cancer recurrence,[8] and musculoskeletal syndrome.[9] The use of any of antiestrogens for hormone-dependent breast cancer, especially AIs, as the first-line adjuvant therapeutic option in postmenopausal patients, can also induce the appearance of unpleasant and severe symptoms of vulvovaginal atrophy (VVA) or may further exacerbate

[1]Department of Pharmacy, Faculty of Medical Sciences
[2]Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[3]Department of Endocrinology, Springfield Clinic, Springfield, IL

Submitted: Apr 25, 2019; Revised: Jul 13, 2019; Accepted: Jul 14, 2019; Epub: Aug 21, 2019

Address for correspondence: O.Z. Milovanović, MD, PhD, Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, Serbia
E-mail contact: olivera.milovanovic09@gmail.com

1526-8209/$ - see frontmatter © 2019 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.clbc.2019.07.007

the symptoms of preexisting VVA, that both require the appropriate treatment.

Atrophic vaginitis or VVA or genitourinary syndrome of menopause is a relatively common complication of endocrine therapy for breast cancer, which can significantly compromise the already impaired health condition of patients. Caused by an additional fall in the circulating level of estrogen, it occurs with almost 20% higher incidence in survivors with breast cancer than in postmenopausal women without breast cancer.[10-12] Treatment of VVA in postmenopausal women includes vaginal moisturizers, lubricants, and/or hormonal substitution. The aim is to alleviate the inconvenient symptoms such as genital dryness, irritation, itching, and painful sexual activity (dyspareunia). The first line of non-hormone therapy includes widely available paraben-free vaginal moisturizers and lubricants (water, mineral or plant oil, or silicone-based), especially in women with mild to moderate symptoms of genitourinary syndrome of menopause.[13] Vaginal estrogen therapy can restore normal, complex physiological functions of estrogens in the female lower urogenital tract[14-17] and is reserved for the treatment of the patients with severe symptoms of VVA.[18] However, this therapeutic modality use in patients with estrogen receptor-positive (ER^+) breast cancer continues to be debated because of concerns regarding long-term safety. Estrogen is a key component in the pathogenesis of breast cancer, and suppression of estrogen therapy using endocrine therapy is used as an adjuvant treatment for estrogen-positive breast cancer. There are concerns with respect to increased systemic absorption of vaginally applied estrogens,[19] even after use of these drugs in low doses, with potential higher risk of breast cancer progression and recurrence.[20,21]

Based on sparse literature evidence, vaginal androgens could be an effective and safe alternative to estrogens in resolving VVA symptoms related to AI therapy in survivors of breast cancer.[22-24] Vaginal testosterone used to treat AI-associated VVA showed significant symptomatic improvement, without raising estradiol or testosterone plasma levels[22] and other relevant adverse effects[23] and could be a potential alternative to vaginal estrogen treatment in women with breast cancer on AIs.[25] Moreover, in a recently published study by Melisco et al, intravaginal testosterone cream demonstrated comparable safety and efficacy to the estradiol-releasing intravaginal ring in patients with early stage breast cancer with AI-associated VVA.[22] Serum estradiol level is affected by estradiol levels at baseline, resulting in significant variance, and will need additional research to account for the variability and the appropriate interpretation of the estradiol levels.

As per the recent statement of American College of Obstetricians and Gynecologists, use of local hormonal therapy to treat AI-induced VVA in survivors of breast cancer should be individualized, based on the patient's risk/benefit ratio and clinical presentation.[26] The safety of AI-induced VVA in postmenopausal females with underlying breast cancer still remains debated, given that the current evidence is based on studies of small sample size and short follow-up. Definitively establishing the safety of treatment of AI-induced VVA in postmenopausal women with breast cancer will guide both physicians and patients with breast cancer in making better informed decisions in the treatment of VVA, which, in turn, will enhance the compliance to therapy and result in a significant improvement of patients' quality of life.

We conducted a meta-analysis to assess the safety of local hormonal treatment of VVA. The primary objective of our study was to evaluate breast cancer outcomes after local estrogen or androgen therapy in women with ER^+ breast cancer treated with AIs as an adjuvant therapeutic option. The secondary objective was to evaluate the systemic absorption and incidence of other hormone-dependent cancers after local estrogen or androgen therapy.

Materials and Methods

Our study was registered with the PROSPERO register of systematic reviews and meta-analyses under the number CRD42018116986 prior to commencement of the research.

We identified the studies using the following criteria: (1) types of studies: clinical trials, observational cohort or cross-sectional studies, and case series; (2) types of participants: women with early estrogen-positive breast cancer who have been treated with AIs after breast surgery, have developed VVA, and have had their VVA treated with local preparations; (3) types of intervention: local estrogen or androgen preparations in various formulations (vaginal cream, rings, or tablets) and at different doses (low or ultra-low doses) or dosage regimens; comparators were non-hormonal local therapy, including placebo. The primary outcome measures were the relapse rate of breast cancer and mortality owing to breast cancer. The secondary outcome measures were serum levels of estrogens, androgens, sex-hormone binding globulins (SHBGs), follicle-stimulating hormone (FH), and luteinizing hormone (LH) after administration of either estrogen or androgen local preparations for treatment of vaginal atrophy, and the incidence of other sex hormone-dependent cancers.

The search methods for identification of studies primarily included electronic databases, and the collection of journal articles and books of the University Library, University of Kragujevac, Kragujevac, Serbia. Electronic searches of the literature were conducted in MEDLINE (PubMed, coverage from 1966 to present), Scopus/Elsevier (coverage from 1966 to present), EBSCO (Discovery Service, coverage from 1944 to present), The Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley Online Library, coverage from 1966 to present), and a registry and results database of clinical studies of human participants at ClinicalTrials.gov up to February 15, 2019. Additional searches were conducted up to March 31, 2019. Electronic databases were searched independently for relevant studies by 6 authors (SS, JM, RP, OM, MF, and MM). The searching strategies are presented in detail for each of the investigators in the Supplemental material (in the online version). The most comprehensive strategy was used by the author MM for the MEDLINE database, which consisted of the following ((“oestrogen”[All Fields] OR “estrogens”[Pharmacological Action] OR “estrogens”[MeSH Terms] OR “estrogens”[All Fields] OR “estrogen”[All Fields]) OR (“oestradiol”[All Fields] OR “estradiol”[MeSH Terms] OR “estradiol”[All Fields]) OR (“oestriol”[All Fields] OR “estriol”[MeSH Terms] OR “estriol”[All Fields]) OR (“testosterone”[MeSH Terms] OR “testosterone”[All Fields]) OR (“lactobacillus acidophilus”[MeSH Terms] OR (“lactobacillus”[All Fields] AND “acidophilus”[All Fields]) OR “lactobacillus acidophilus”[All Fields])) AND ((local[All Fields] AND (“organization and

administration"[MeSH Terms] OR ("organization"[All Fields] AND "administration"[All Fields]) OR "organization and administration"[All Fields] OR "administration"[All Fields])) OR ("vaginal creams, foams, and jellies"[MeSH Terms] OR ("vaginal"[All Fields] AND "creams"[All Fields] AND "foams"[All Fields] AND "jellies"[All Fields]) OR ("vaginal"[All Fields] AND "cream"[All Fields]) OR "vaginal cream"[All Fields])) AND (("breast neoplasms"[MeSH Terms] OR ("breast"[All Fields] AND "neoplasms"[All Fields]) OR "breast neoplasms"[All Fields] OR ("breast"[All Fields] AND "cancer"[All Fields]) OR "breast cancer"[All Fields]) OR ("breast neoplasms"[MeSH Terms] OR ("breast"[All Fields] AND "neoplasms"[All Fields]) OR "breast neoplasms"[All Fields] OR ("breast"[All Fields] AND "neoplasm"[All Fields]) OR "breast neoplasm"[All Fields])) AND ((("aromatase inhibitors"[Pharmacological Action] OR "aromatase inhibitors"[MeSH Terms] OR ("aromatase"[All Fields] AND "inhibitors"[All Fields]) OR "aromatase inhibitors"[All Fields]) AND induced[All Fields] AND ("vagina"[MeSH Terms] OR "vagina"[All Fields] OR "vaginal"[All Fields]) AND ("atrophy"[MeSH Terms] OR "atrophy"[All Fields])) OR (("vagina"[MeSH Terms] OR "vagina"[All Fields] OR "vaginal"[All Fields]) AND ("atrophy"[MeSH Terms] OR "atrophy"[All Fields])))). There were no restrictions on publication date, format, or language in the search strategy. The references of the retrieved articles were searched for further similar studies ("snowball search"). The collection of journal articles and books of the University Library, University of Kragujevac were manually searched for relevant studies by 2 authors independently (JM and MM).

Data Collection and Analysis

The data collection sheet was created and the articles included in the review were assessed for: (1) study ID; (2) report ID; (3) review author initials; (4) citation and contact details; (5) eligibility for review; (6) study design; (7) total study duration; (8) risk of bias (randomization if any, sequence generation, allocation sequence concealment, blinding, other concerns about bias); (9) number of enrolled patients; (10) number of patients who completed the study; (11) gender of the patients; (12) average age of the patients (with variability measures); (13) body mass index of the patients (with variability measures); (14) time elapsed from the breast cancer diagnosis (with variability measures); (15) duration of AI therapy (with variability measures); (16) AI used; (17) type of local hormonal therapy used with dosage regimen; (18) recurrence rate of breast cancer (persons per year/1000); (19) mean baseline, after 2, 4, 8, 12, and 26 weeks estrone serum level (with variability measures); (20) mean baseline, after 2, 4, 8, 12, and 26 weeks estradiol serum level (with variability measures); (21) mean baseline, after 2, 4, 8, 12, and 26 weeks estriol serum level (with variability measures); (22) mean baseline, after 2, 4, 8, 12, and 26 weeks testosterone serum level (with variability measures); (23) mean baseline, after 2, 4, 8, 12, and 26 weeks FSH serum level (with variability measures); (24) mean baseline, after 2, 4, 8, 12, and 26 weeks LH serum level (with variability measures); (25) mean baseline, after 2, 4, 8, 12, and 26 weeks SHBG serum level (with variability measures); (26) rate of adverse events; (27) rate of vaginal discharge; (28) rate of facial hair growth; (29) rate of vaginal or vulvar itching and/or irritation; (30) rate of vaginal odor; (31) rate of urinary tract or yeast infection; and (32) mortality rate. Values provided as percentages were converted into actual patient numbers for analysis, as well as standard errors into standard deviations using number of patients, when reported as such.

Selection of Studies

Based on the search strategy, all titles and abstracts retrieved were independently scanned by 6 authors (SS, JM, RP, OM, MF, and MM). Eligibility of the retrieved articles was assessed at first from the title and the abstract, and if it was not possible, the full text of the articles was retrieved and searched. An article was included for review if all authors (SS, JM, RP, OM, MF, and MM) agreed that eligibility criteria had been met. In the case where the reviewers had different opinions about eligibility of a study for inclusion, the matter was resolved by the senior author (SJ).

Data Extraction and Management

The data were extracted from eligible studies using the data collection sheet described previously (under the "data collection and analysis" subheading). The data collection sheet was constructed in an Excel 2016 worksheet. The data were extracted by 4 investigators independently (JM, OM, MF, and MM), and then collating of the 4 tables was done by the other 3 investigators (SS, RP, and SJ), who produced the final extraction table.

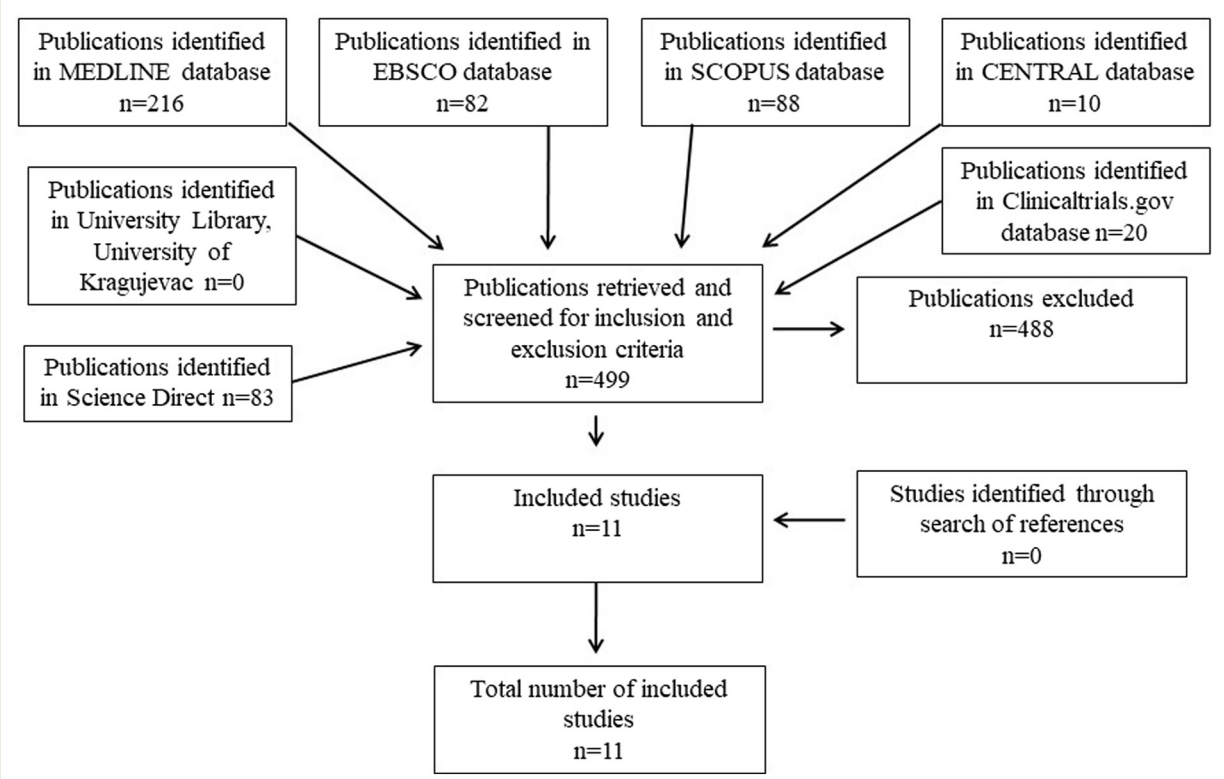
Assessment of Risk of Bias in Included Studies

Risk of bias was assessed by 6 investigators independently (SS, JM, RP, OM, MF, and MM), and collating the assessments was done by the senior investigator (SJ). The following sources of bias were assessed: (1) randomization if any; (2) sequence generation; (3) allocation sequence concealment; (4) blinding; (5) performance bias; (6) detection bias; (7) attrition bias; and (8) reporting bias. None of the studies had high risk of bias, so none was excluded from further analysis.

Measures of Treatment Effect

Continuous variables (estrone, estriol, estradiol, testosterone, FSH, LH, and SHBG) were measured on the same scale in all studies, and the treatment effect was assessed by obtaining baseline mean serum levels and levels at 4, 8, 12, and 26 weeks from the onset of local therapy (hormonal or non-hormonal) of vaginal atrophy (outcomes). The differences in mean serum concentrations of certain abovementioned parameters between the groups with local hormonal and local non-hormonal treatment could not be summarized, because local non-hormonal treatment groups existed in only 3 of the included studies, and serum levels of hormones and SHBG were measured in only 1 of the 3 studies. Serum levels of estrone and testosterone could not be summarized, because they were reported in only 1 of the included studies. The following outcomes were dichotomous: rate of adverse events, rate of vaginal discharge, rate of facial hair growth, rate of vaginal or vulvar itching and/or irritation, rate of vaginal odor, rate of urinary tract or yeast infection, breast cancer recurrence rate, and mortality rate. For these outcomes, the treatment effect could not be summarized by odds ratios, because only 1 of the included studies reported comparison of rates in local hormonal and local non-hormonal treatment groups. Therefore, dichotomous

Figure 1 Selection of the Studies for the Meta-analysis



treatment effects were summarized as mean rates with 95% confidence intervals.

Unit of Analysis Issues

Unit of analysis in clinical trials that were included in this meta-analysis were individual patients. Individual participants were randomized to 1 of 2 parallel intervention groups, and a single measurement for each outcome from each participant was collected and analyzed. Units of analysis in observational studies included in this meta-analysis were also individual patients, and their outcomes were measured once, aggregated, and analyzed.

Dealing with Missing Data

Missing data were requested directly from the original investigators; however, we did not receive additional information. When available, we retrieved missing data from the results presented on ClinicalTrials.gov. The potential impact of missing data on the findings of the meta-analysis is commented on in the Discussion section.

Assessment of Heterogeneity

The presence of heterogeneity between studies was assessed with the Cochrane Q test using a χ^2 distribution (P values $< .10$ were considered significant). The magnitude of heterogeneity was evaluated using the I^2 statistic. If the Q value was equal or less than number of included studies minus one, I^2 was given zero value. The following arbitrary thresholds are used to categorize I^2 values: $< 30\%$ low heterogeneity, 30% to 50% moderate heterogeneity, 50% to 90% substantial heterogeneity, and $> 90\%$ considerable heterogeneity.

Assessment of Reporting Biases

We evaluated the possibility of within-study selective outcome reporting for each of the study included in the meta-analysis. First, by constructing a matrix of the outcomes for all studies, we identified studies and specific outcomes that were not reported. Then we searched for published protocols of such studies at ClinicalTrials.gov and other forms of publications of the same studies, in order to find the missing outcomes. Finally, the authors were contacted with a request to provide the missing data, but they did not send us the data. The possibility of between-study publication bias was examined by construction of funnel plots for continuous outcomes and by Egger regression[27] for discrete outcomes. The Klein number was also calculated for all outcomes.[28]

Data Synthesis

The random effects model (which includes both within-study and between-study variations in calculation of the weighted average) was used to combine the results from the studies. The Mantel-Haenszel method (fixed effect model) was also used to estimate how our conclusions could be influenced by assumptions about the model and by the study heterogeneity. Although significant heterogeneity of the studies was found, subgroup analysis was not performed owing to the small number of the included studies.

Table 1 Characteristics and Main Outcomes of the Included Studies

Study	Design	No. Patients	Mean Duration of Aromatase Inhibitor Therapy, mos	Local Hormonal Preparation	Type of Local Hormonal Preparation	Measured Hormones in Serum	Type of Bias With High Risk
Melisko et al 2017	Randomized, noncomparative trial	Estradiol, 35 Testosterone, 34	21	Estradiol or testosterone	Estradiol vaginal ring or testosterone cream	Estradiol, testosterone	Performance bias
Donders et al 2014	Open label, bicentric, phase I pharmacokinetic study	16	25	Estriol	Vaginal tablets	Estriol, FSH, LH, SHBG	Selection bias
Simmons et al 2012	Case series	14	NR	Estradiol	Vaginal tablets or vaginal rings	Estradiol	Reporting bias
Goldfarb et al 2012	Cohort study	18	NR	Estradiol	Vaginal tablets	Estradiol	Selection and performance bias
Witherby et al 2011	Phase I/II pilot clinical trial	20	NR	Testosterone	Vaginal cream	Estradiol, testosterone	Selection and performance bias
Pfeiler et al 2011	Prospective cohort study	10	26	Estriol	Vaginal tablets	Estradiol, FSH, LH, SHBG	Selection, performance and detection bias
Wills et al 2012	Prospective cohort study	48	>1	Estradiol	Vaginal tablets or vaginal rings	Estradiol, estriol, FSH, LH, SHBG	Selection and performance bias
Kendall et al 2006	Prospective cohort study	7	NR	Estradiol	Vaginal tablets	Estradiol, FSH, LH	Selection, performance and detection bias
Davis et al 2018	Double-blind, randomized, placebo-controlled trial	37	28	Testosterone vs. placebo	Vaginal cream	Estrone, estradiol, testosterone	Attrition bias
Blissafe study, current	Phase II, prospective, randomized, double-blind, placebo-controlled, international and multicenter study	61	>6	Estriol vs. placebo	Vaginal gel	Estriol, FSH, LH	None
Niravth et al 2017	Prospective randomized study	8	NR	Estrogen vs. non-estrogen	Vaginal tablets or vaginal lubricant	Estradiol	None

Abbreviations: FSH = follicle stimulating hormone; LH = luteinizing hormone; NR = not reported; SHBG = sex hormone binding globulin.

All calculations were done by calculators in Excel 2016 designed and constructed by the senior author (SJ).

Sensitivity Analysis

Sensitivity analysis was performed by excluding individual trials one at a time and recalculating the pooled mean serum levels of hormones or adverse effects rates for the remaining studies. In this way, we got insight into the impact of individual studies on conclusions.

Results

Results of the literature search are shown in the Figure 1. Eleven studies (2 randomized clinical trials and 9 observational studies) were identified that satisfied the set of inclusion and exclusion criteria. Characteristics of the included studies and their measured risk of bias are shown in detail in Table 1.

None of the included studies reported breast cancer relapse rates and mortality rates; hence, we were unable to evaluate these outcome measures. Assessment of other outcomes was possible only for serum levels of estradiol, estriol, FSH, LH, and SHBG, as well as for rates of certain adverse effects. However, estriol and SHBG levels were reported in only 2 of the included studies each, and details are shown in Table 2. Summaries of estradiol serum levels at the baseline, and after 2, 4, 8, and 12 weeks from the introduction of local hormonal therapy for vaginal atrophy are shown by the diagram in Figure 2. Serum levels of estradiol were available at baseline, and 4 and 8 weeks after the onset of local hormonal therapy in 7 studies with moderate heterogeneity. Estradiol level showed a clear trend for decline in the levels compared with the base line. (Table 2, Figure 2). The SHBG levels decreased, LH levels remained stable, estriol levels tripled, and FSH almost doubled 8 weeks after the onset of local hormonal therapy (Table 2). Only the following adverse effects rates could be summarized (although with high heterogeneity of the included studies): vaginal discharge, facial hair growth, urinary tract or yeast infection ("frequent" adverse effects as rates were between 1 and 10%), and vaginal or vulvar itching and/or irritation ("rare" adverse effect, with a rate less than 1%) (Table 3). Outcome details are summarized in the Tables 2 and 3. Sensitivity analysis did not show significant changes with the exclusion of single trials.

The reporting bias was assessed by funnel plot, using the "trim and fill" method for continuous outcomes: serum estradiol levels 4 weeks after the onset of local hormonal treatment. The central symmetry axis of funnel plots did not change place significantly after the "trim and fill" exercise (mean estradiol level for whole meta-analysis was 343 pg/mL before, and 326 pg/mL after the

Table 2 Overall (Combined) Effect of Local Hormonal Therapy on Serum Levels of Estradiol, Estriol, FSH, LH, and SHBG (Mean ± 95% Confidence Interval) Based on the Random Effects Model, With Heterogeneity Measures

Hormone	Baseline	1 Day	2 Weeks	4 Weeks	8 Weeks	12 Weeks
Estradiol, pg/mL	5.45 ± 1.19 Q = 1.250 I^2 = 87.60 *P* = .990 n = 6	20.7 ± 0.10 Q = 1.000 I^2 = 0.00 *P* = .000 n = 2	5.61 ± 1.53 Q = 0,132 I^2 = 46.47 *P* = .860 n = 4	3.43 ± 2.66 Q = 0.150 I^2 = 36.36 *P* = .840 n = 7	1.39 ± 1.22 Q = 0.277 I^2 = 21.66 *P* = .720 n = 5	4.75 ± 2.38 Q = 0.020 I^2 = 69.40 *P* = .970 n = 4
Estriol, pg/mL	0.62 ± 12.25 Q = 1.000 I^2 = 0.00 *P* = .000 n = 2	NA	NA	NA	1.88 ± 1.10 Q = 1.000 I^2 = 0.00 *P* = .000 n = 2	2.2 ± 0.89 Q = 1.000 I^2 = 0.00 *P* = .000 n = 2
FSH, mIU/mL	68.96 ± 0.288 Q = 0.050 I^2 = 59.76 *P* = .940 n = 4	NA	66.37 ± 0.60 Q = 0.580 I^2 = 0.00 *P* = .420 n = 3	93.67 ± 0.07 Q = 0.840 I^2 = 0.00 *P* = .160 n = 2	101.71 ± 0.08 Q = 0.860 I^2 = 0.00 *P* = .130 n = 2	NA
LH, mIU/mL	30.89 ± 1.12 Q = 0.180 I^2 = 37.66 *P* = .810 n = 4	NA	29.19 ± 0.92 Q = 0.850 I^2 = 0.00 *P* = .150 n = 3	35.32 ± 0.24 Q = 0.920 I^2 = 0.00 *P* = .070 n = 2	35.66 ± 41.48 Q = 0.360 I^2 = 0.41 *P* = .630 n = 3	30.88 ± 1.12 Q = 0.180 I^2 = 37.66 *P* = .810 n = 4
SHBG, nM/L	48.36 ± 0.55 Q = 0.400 I^2 = 0.00 *P* = .590 n = 2	NA	46.29 ± 0.54 Q = 0.420 I^2 = 0.00 *P* = .570 n = 2	NA	NA	NA

Abbreviations: FSH = follicle stimulating hormone; I^2 = I^2 analysis; LH = luteinizing hormone; n = number of studies; NA = not applicable; *P* = cumulative probability of calculated Q – test value according to the χ^2 distribution; Q = Cochran Q-test; SHBG = sex hormone binding globulin.

exercise). In Figure 3, the funnel plots are shown before and after the "trim and fill" exercise.

For discrete outcomes (frequencies of adverse effects), the reporting bias was assessed by the Klein number and Egger regression. The Klein number for the rate of vaginal discharge was 0.36, and the Egger regression showed a large correction of the summary effect estimate: from the rate = 0.0427 to rate = 0.744 (Figure 4). The Klein number for the rate of vaginal or vulvar itching and/or irritation was −7.27, whereas the Egger regression showed a relatively small correction of the summary effect estimate: from the rate = 0.0029 to the rate = 0.0006. The Klein number for the rate of vaginal hair growth and urinary tract or yeast infection was 0.64 and −1.04, respectively, whereas the Egger regressions for both outcomes produced unrealistic summary effect estimates larger than 1 (100%), owing to the small number of data points (only 3).

Discussion

Vaginal estrogen treatment is the most effective treatment of moderate to severe VVA that occurs during or after the use of AIs in postmenopausal women with ER^+ breast cancer. This meta-analysis is an attempt to establish an evidence-based estimate of breast cancer risk recurrence with the use of topical hormone therapy in patients with breast cancer. According to the best of our knowledge, there is only one retrospective study to date that estimates the risk of long-term breast cancer relapse in such a population.[19] In light of this, we had to adopt an indirect assessment of such a risk based on the quantification of systemic absorption of estrogens at different time points during the use of local hormone therapy. The most relevant finding of the study is a notable, but relatively small transient rise of the serum levels of estriol and FSH after inception of local hormone therapy, whereas the estradiol, LH, and SHBG levels showed no significant change compared with baseline. There was an increase in the rate of immediate dose-related and expected mild to moderate adverse effects of local estrogen therapy.

A significant short-term increase in serum estriol without concurrent rise of the estradiol or estrone blood levels following the onset of local hormone therapy deserves special attention with respect to the long-term safety profile of topical estrogens in women with breast cancer. Estriol data was based on only 2 studies included in the meta-analysis in which vaginal estriol was administered in conventional or low doses, but this finding is in concordance with many other studies, which were mostly conducted in postmenopausal women without breast cancer.[12,29-33] Estriol is far less potent than estradiol or estrone, and "in vivo" does not exhibit the conversion back to these more powerful natural estrogens. In addition, the estriol is considered to be a short-acting estrogen[32] owing to the relatively short biological half-life primarily caused by the short-lived interaction between estriol and its receptor (eg, after binding for receptor, the retention time in endometrial cell nucleus is twice as short as that shown for estradiol),[34] as well as by rapid plasma removal. Moreover, there is evidence that with the maturation of the vaginal epithelium, which occurs within a few weeks after the commencement of local therapy with estriol, its bioavailability starts to decline gradually.[35] Consequently, the short-lived increase in serum estriol levels following vaginal administration is probably unlikely to affect the endometrium and breast,[36] as its stimulatory effects requires the sustained presence of a high concentration of estriol in these tissues.[37]

The absence of a clinically relevant elevation of circulating estradiol during the use of low or ultra-low doses of local estrogen

Figure 2 Serum Levels of Estradiol (pg/mL) Before and After Introduction of Local Hormonal Therapy of Vaginal Atrophy in Women With Breast Cancer on Aromatase Inhibitors. Error Bars Indicate 95% Confidence Intervals

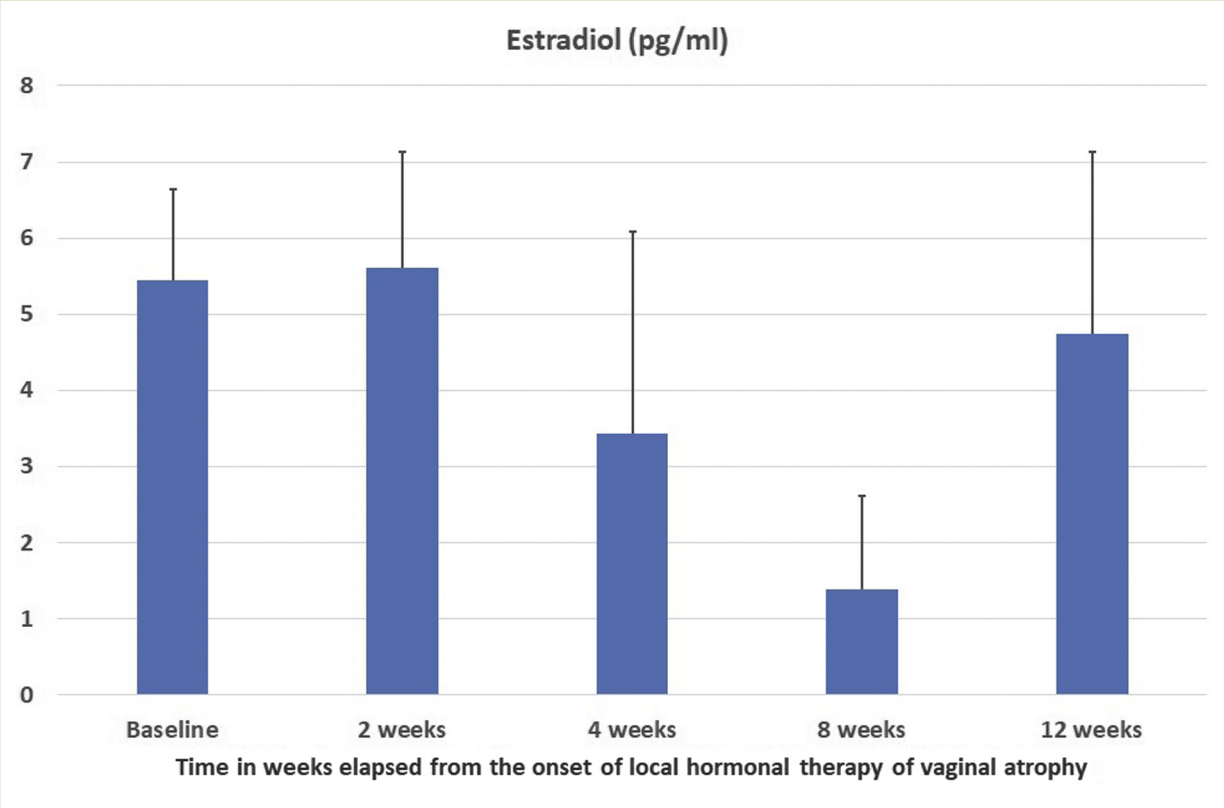


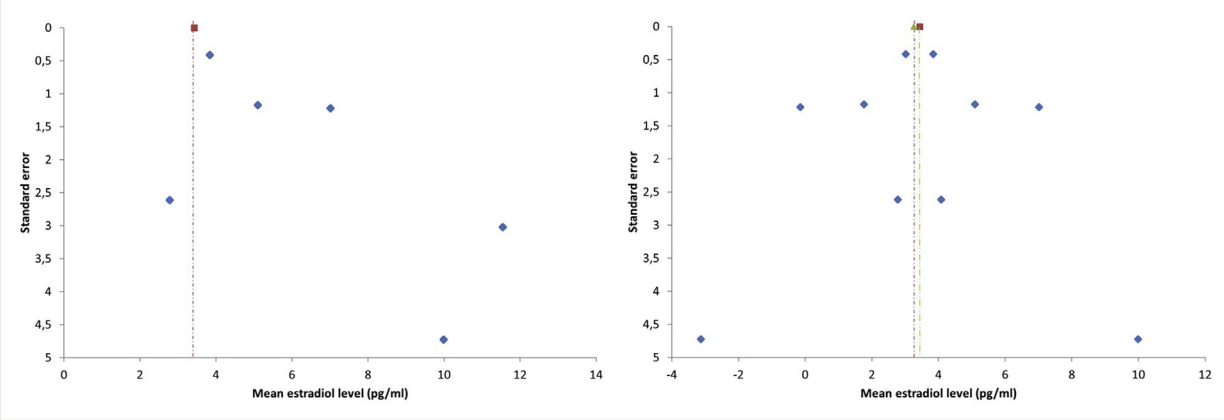
Table 3 Overall (Combined) Adverse Effects Rates of Local Hormonal Therapy (Mean ± 95% Confidence Interval) Based on the Random Effects Model, With Heterogeneity Measures

Adverse Effect	Rate and Heterogeneity
Vaginal discharge	4.27% ± 0.20% Q = 0.083 I^2 = 0.00 *P* = .006 n = 4
Facial hair growth	6.06% ± 0.15% Q = 0.112 I^2 = 0.00 *P* = .055 n = 3
Vaginal or vulvar itching and/or irritation	0.29% ± 0.64% Q = 0.141 I^2 = 0.00 *P* = .068 n = 3
Urinary tract or yeast infection	4.86% ± 0.16% Q = 0.035 I^2 = 0.00 *P* = .017 n = 3

Abbreviations: I^2 = I^2 analysis; n = number of studies; NA = not applicable; *P* = cumulative probability of calculated Q — test value according to the χ^2 distribution; Q = Cochran Q-test.

therapy (irrespective of active principle [ie, estradiol or estrone or estriol]) for AI-induced atrophic vaginitis was observed in numerous earlier prospective studies, particularly when formulation other than vaginal tablets or rings containing estradiol were used.[12,16,22,38,39] This was also shown when intra-vaginal testosterone had been administered for the same purpose.[22,25,26] Contrary to the above studies, Kendall et al demonstrated a significant short-term rise in estradiol concentration in patients with AI-related vaginal atrophy treated with estradiol vaginal tablets,[40] whereas Wills and associates revealed a notable persistent elevation of estradiol with the use of a slow-release vaginal ring containing estradiol that lasted until the end of the study period.[41] Drug manufacturers of one of the preparations investigated changed the quantitative composition of estradiol in vaginal tablets to reduce its systemic bioavailability, while preserving efficacy in the treatment of VVA.[42] Regardless, concerns remain regarding the effect of even the minimal systemic absorption of estradiol (the most potent natural estrogen) following local application of estrogens in counteracting the effects of AIs and consequently increase the risk of breast cancer relapse. However, the clinical significance of results of these studies remain uncertain and is debated owing to the following reasons. Several of the following confounding factors that could not be fully controlled by the study investigators include: (1) baseline serum levels of estradiol before intervention varied considerably between the studies, indicating a

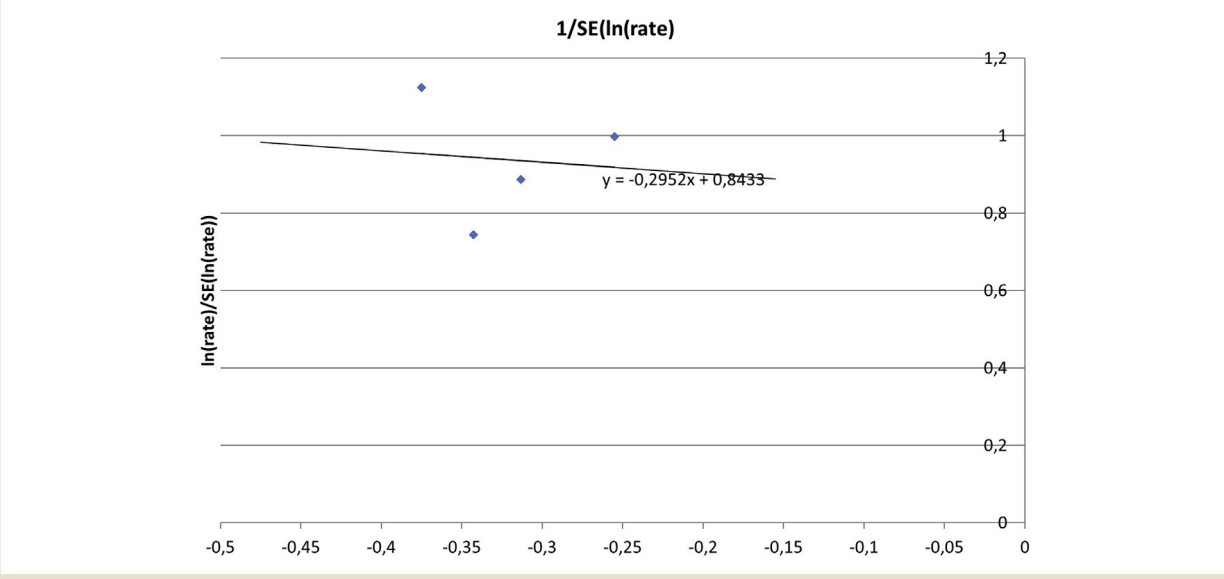
Figure 3 Funnel Plot and "Trim and Fill" Exercise for Mean Serum Level of Estradiol 4 Weeks After Introduction of Local Hormonal Treatment in Women With Breast Cancer on Aromatase Inhibitors



possible inconsistent efficacy of AIs in terms of suppression of estrogen synthesis,[43,44] which may be caused by differences in residual ovarian function and body mass index of the enrolled patients,[45] as well as by poor adherence/compliance to AIs, and self-administration with unlabeled nutritional supplements or plant-based products containing estrogens[46]; (2) analytical methods that were used to detect circulating estradiol in these studies were quite different, including the methods for obtaining and storage of the serum samples[47]; (3) there was no established minimum level of circulating estradiol that was linked to a meaningful increased risk of breast cancer; (4) studies have sample size and were non-representative with lack of controls. The results of several observational studies that favor the use of vaginal estrogens for VVA showed negligible recurrence risk, but additional randomized controlled trials are required to confirm this finding.[19,48,49]

Use of intravaginal testosterone, particularly in the lowest effective doses, is a promising therapeutic alternative to local estrogen preparations for the treatment of VVA in patients with breast cancer who receive AIs. Theoretically, if any amount of testosterone is being systematically absorbed, its conversion to estrogens is going to be suppressed by AIs. However, the risk of breast cancer can certainly be elevated in the presence of high levels of testosterone in blood.[50] On the other hand, an earlier observational study indicated that higher than usual postmenopausal levels of testosterone at baseline had not been associated with the increased risk of recurrent breast cancer after 7 years of follow-up.[51] Although a recent double-

Figure 4 The Egger Regression for Rate of Vaginal Discharge



Abbreviation: SE = standard error.

blind randomized placebo-controlled trial has found vaginal testosterone to be effective in symptomatic relief of AI-induced vaginal atrophy without any increase in serum sex hormone levels,[25] further prospective studies involving significantly more patients are warranted in order to clearly elucidate the long-term risk of relapse in patients with history of breast cancer.

Our study revealed an elevated FSH concentration with vaginal estrogen use, which offers indirect evidence of absence of the systemic absorption of sex hormones. This finding is contrary to earlier studies conducted on a sample of postmenopausal women without breast cancer in which an important reduction of FSH and LH plasma levels (compared with baseline) was observed following use of vaginal estriol along with increase in its serum estriol concentration.[52,53] However, in a study by Schiff et al, estriol elevation was not noticed after oral consumption of the drug,[52] indicating its systemic absorption in a significantly larger extent through altered vaginal mucosa owing to atrophic changes. Moreover, whether a transient elevation of sex hormones after vaginal use is sufficient to significantly decrease the concentration of gonadotropins, as well as to what extent the recurrent breast cancer is influenced by reduced gonadotropin levels in the absence of permanently elevated sex hormones, which was shown in a study by Pfeiler et al,[39,40] is still a matter of debate.

Our meta-analysis has several limitations. There was pronounced heterogeneity of the studies involved. The included studies differed in terms of design and the overall methodological approach, which means that the results should be used cautiously, based on the individual assessment of a patient's risk factors. The duration of prospective studies, size and representativeness of study samples, inclusion of control groups, values of baseline serum sex hormone levels, analytical methods that were used to measure blood concentration of hormones, time points used to measure hormone levels, and the lack of data on such measurement in different time points are just some of the numerous factors that have contributed to these differences. Also, the small number of included studies preclude a firm conclusion regarding surrogate markers of long-term safety following the use of vaginal estrogens or testosterone to treat VVA in patients on AI therapy.

In conclusion, this analysis demonstrated the absence of systemic absorption of sex hormones after intravaginal administration in postmenopausal women with a history of breast cancer who underwent treatment with AIs, an indirect clue for a low risk of recurrence of breast cancer. Large prospective randomized clinical control trials are needed in order to establish the long-term safety of use of vaginal hormone therapy in these patients.

Acknowledgments

This study was partially supported by Grant No 175007 given by the Serbian Ministry of Education.

Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental material accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2019.07.007.

References

1. Kang H, Xiao X, Huang C, et al. Potent aromatase inhibitors and molecular mechanism of inhibitory action. *Eur J Med Chem* 2018; 143:426-37.
2. Aydiner A. Meta-analysis of breast cancer outcome and toxicity in adjuvant trials of aromatase inhibitors in postmenopausal women. *Breast* 2013; 22:121-9.
3. Goss PE, Ingle JN, Pritchard KI, et al. Extending aromatase-inhibitor adjuvant therapy to 10 years. *N Engl J Med* 2016; 375:209-19.
4. Haque R, Shi J, Schottinger JE, et al. Cardiovascular disease after aromatase inhibitor use. *JAMA Oncol* 2016; 2:1590-7.
5. Blaes A, Beckwith H, Florea N, et al. Vascular function in breast cancer survivors on aromatase inhibitors: a pilot study. *Breast Cancer Res Treat* 2017; 166:541-7.
6. Rydén L, Heibert Arnlind M, Vitols S, Höistad M, Ahlgren J. Aromatase inhibitors alone or sequentially combined with tamoxifen in postmenopausal early breast cancer compared with tamoxifen or placebo - meta-analyses on efficacy and adverse events based on randomized clinical trials. *Breast* 2016; 26:106-14.
7. Goldvaser H, Barnes TA, Šeruga B, et al. Toxicity of extended adjuvant therapy with aromatase inhibitors in early breast cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2018:110.
8. Sini V, Botticelli A, Lunardi G, Gori S, Marchetti P. Pharmacogenetics and aromatase inhibitor induced side effects in breast cancer patients. *Pharmacogenomics* 2017; 18:821-30.
9. Lintermans A, Laenen A, Van Calster B, et al. Prospective study to assess fluid accumulation and tenosynovial changes in the aromatase inhibitor-induced musculoskeletal syndrome: 2-year follow-up data. *Ann Oncol* 2013; 24:350-5.
10. Weitzman SP. The use of vaginal estrogen in women with a history of estrogen-dependent breast cancer. *Breast Dis Year B Q* 2016; 27:228-9.
11. Archer DF, Kimble TD, Lin FDY, Battucci S, Sniukiene V, Liu JH. A randomized, multicenter, double-blind, study to evaluate the safety and efficacy of estradiol vaginal cream 0.003% in postmenopausal women with vaginal dryness as the most bothersome symptom. *J Womens Health (Larchmont)* 2018; 27:231-7.
12. Biglia N, Peano E, Sgandurra P, et al. Low-dose vaginal estrogens or vaginal moisturizer in breast cancer survivors with urogenital atrophy: a preliminary study. *Gynecol Endocrinol* 2010; 26:404-12.
13. Sassarini J, Perera M, Spowart K, et al. Managing vulvovaginal atrophy after breast cancer. *Post Reprod Health* 2018, 2053369118805344.
14. Chin SN, Trinkaus M, Simmons C, et al. Prevalence and severity of urogenital symptoms in postmenopausal women receiving endocrine therapy for breast cancer. *Clin Breast Cancer* 2009; 9:108-17.
15. Dahir M, Travers-Gustafson D. Breast cancer, aromatase inhibitor therapy, and sexual functioning: a pilot study of the effects of vaginal testosterone therapy. *Sex Med* 2014; 2:8-15.
16. Donders G, Neven P, Moegele M, et al. Ultra-low-dose estriol and Lactobacillus acidophilus vaginal tablets (Gynoflor(®)) for vaginal atrophy in postmenopausal breast cancer patients on aromatase inhibitors: pharmacokinetic, safety, and efficacy phase I clinical study. *Breast Cancer Res Treat* 2014; 145:371-9.
17. Fernandes T, Costa-Paiva LH, Pedro AO, Baccaro LFC, Pinto-Neto AM. Efficacy of vaginally applied estrogen, testosterone, or polyacrylic acid on vaginal atrophy: a randomized controlled trial. *Menopause* 2016; 23:792-8.
18. Zuo SW, Wu H, Shen W. Vaginal estrogen and mammogram results: case series and review of literature on treatment of genitourinary syndrome of menopause (GSM) in breast cancer survivors. *Menopause* 2018; 25:828-36.
19. Le Ray I, Dell'Aniello S, Bonnetain F, Azoulay L, Suissa S. Local estrogen therapy and risk of breast cancer recurrence among hormone-treated patients: a nested case-control study. *Breast Cancer Res Treat* 2012; 135:603-9.
20. Lester J, Bernhard L, Ryan-Wenger N. A self-report instrument that describes urogenital atrophy symptoms in breast cancer survivors. *West J Nurs Res* 2012; 34:72-96.
21. Lethaby A, Ayeleke RO, Roberts H. Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev* 2016; 8:CD001500.
22. Melisko ME, Goldman ME, Hwang J, et al. Vaginal testosterone cream vs estradiol vaginal ring for vaginal dryness or decreased libido in women receiving aromatase inhibitors for early-stage breast cancer: a randomized clinical trial. *JAMA Oncol* 2017; 3:313-9.
23. Nappi RE, Kokot-Kierepa M. Women's voices in the menopause: results from an international survey on vaginal atrophy. *Maturitas* 2010; 67:233-8.
24. Management of symptomatic vulvovaginal atrophy: 2013 position statement of The North American Menopause Society. *Menopause* 2013; 20:888-902, quiz: 903-4.
25. Davis SR, Robinson PJ, Jane F, White S, White M, Bell RJ. Intravaginal testosterone improves sexual satisfaction and vaginal symptoms associated with aromatase inhibitors. *J Clin Endocrinol Metab* 2018; 103:4146-54.
26. Witherby S, Johnson J, Demers L, et al. Topical testosterone for breast cancer patients with vaginal atrophy related to aromatase inhibitors: a phase I/II study. *Oncologist* 2011; 16:424-31.
27. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315:629-34.
28. Gioacchino L. *Meta-analysis in medical research: the handbook for the understanding and practice of meta-analysis*. 1st ed. Malden, MA: Blackwell Publishing Ltd; 2005.
29. Moegele M, Buchholz S, Seitz S, Ortmann O. Vaginal estrogen therapy in postmenopausal breast cancer patients treated with aromatase inhibitors. *Arch Gynecol Obstet* 2012; 285:1397-402.
30. Mariani L, Gadducci A, Vizza E, Tomao S, Vici P. Vaginal atrophy in breast cancer survivors: role of vaginal estrogen therapy. *Gynecol Endocrinol* 2013; 29:25-9.

31. Labrie F, Cusan L, Gomez J-L, et al. Effect of one-week treatment with vaginal estrogen preparations on serum estrogen levels in postmenopausal women. *Menopause* 2009; 16:30-6.
32. Al-Baghdadi O, Ewies AA. Topical estrogen therapy in the management of postmenopausal vaginal atrophy: an up-to-date overview. *Climacteric* 2009; 12: 91-105.
33. Rueda C, Osorio AM, Avellaneda AC, Pinzón CE, Restrepo OI. The efficacy and safety of estriol to treat vulvovaginal atrophy in postmenopausal women: a systematic literature review. *Climacteric* 2017; 20:321-30.
34. Anderson JN, Peck EJ, Clark JH. Estrogen-induced uterine responses and growth: relationship to receptor estrogen binding by uterine nuclei. *Endocrinology* 1975; 96:160-7.
35. Buhling KJ, Eydeler U, Borregaard S, Schlegelmilch R, Suesskind M. Systemic bioavailability of estriol following single and repeated vaginal administration of 0.03 mg estriol containing pessaries. *Arzneimittelforschung* 2012; 62:378-83.
36. Head KA. Estriol: safety and efficacy. *Altern Med Rev* 1998; 3:101-13.
37. Lattrich C, Stegerer A, Häring J, Schüler S, Ortmann O, Treeck O. Estrogen receptor β agonists affect growth and gene expression of human breast cancer cell lines. *Steroids* 2013; 78:195-202.
38. Goldfarb SB, Dickler M, Dnistrian A, et al. Abstract P2-12-05: Limited absorption of low dose 10-μg intravaginal 17-β estradiol (Vagifem®) in postmenopausal women with breast cancer on aromatase inhibitors. *Cancer Res* 2012; 72(24 Suppl), P2-12-05.
39. Pfeiler G, Glatz C, Königsberg R, et al. Vaginal estriol to overcome side-effects of aromatase inhibitors in breast cancer patients. *Climacteric* 2011; 14:339-44.
40. Kendall A, Dowsett M, Folkerd E, Smith I. Caution: vaginal estradiol appears to be contraindicated in postmenopausal women on adjuvant aromatase inhibitors. *Ann Oncol* 2006; 17:584-7.
41. Wills S, Ravipati A, Venuturumilli P, et al. Effects of vaginal estrogens on serum estradiol levels in postmenopausal breast cancer survivors and women at risk of breast cancer taking an aromatase inhibitor or a selective estrogen receptor modulator. *J Oncol Pract* 2012; 8:144-8.
42. Lindahl SH. Reviewing the options for local estrogen treatment of vaginal atrophy. *Int J Womens Health* 2014; 6:307-12.
43. Kunovac Kallak T, Baumgart J, Stavreus Evers A, et al. Higher than expected estradiol levels in aromatase inhibitor-treated, postmenopausal breast cancer patients. *Climacteric* 2012; 15:473-80.
44. Bellet M, Gray KP, Francis PA, et al. Twelve-month estrogen levels in premenopausal women with hormone receptor-positive breast cancer receiving adjuvant triptorelin plus exemestane or tamoxifen in the Suppression of Ovarian Function Trial (SOFT): The SOFT-EST substudy. *J Clin Oncol* 2016; 34:1584-93.
45. Ingle JN, Buzdar AU, Schaid DJ, et al. Variation in anastrozole metabolism and pharmacodynamics in women with early breast cancer. *Cancer Res* 2010; 70:3278-86.
46. Guns ES, Goldenberg SL, Brown PN. Mass spectral analysis of PC-SPES confirms the presence of diethylstilbestrol. *Can J Urol* 2002; 9:1684-8, discussion: 1689.
47. Jones ME, Folkerd EJ, Doody DA, et al. Effect of delays in processing blood samples on measured endogenous plasma sex hormone levels in women. *Cancer Epidemiol Biomark Prev* 2007; 16:1136-9.
48. Dew JE, Wren BG, Eden JA. A cohort study of topical vaginal estrogen therapy in women previously treated for breast cancer. *Climacteric* 2003; 6:45-52.
49. Vassilopoulou-Sellin R, Theriault R, Klein MJ. Estrogen replacement therapy in women with prior diagnosis and treatment for breast cancer. *Gynecol Oncol* 1997; 65:89-93.
50. Tamimi RM, Byrne C, Colditz GA, Hankinson SE. Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 2007; 99:1178-87.
51. Rock CL, Flatt SW, Laughlin GA, et al. Women's Healthy Eating and Living Study Group. Reproductive steroid hormones and recurrence-free survival in women with a history of breast cancer. *Cancer Epidemiol Biomark Prev* 2008; 17: 614-20.
52. Schiff I, Tulchinsky D, Ryan KJ, Kadner S, Levitz M. Plasma estriol and its conjugates following oral and vaginal administration of estriol to postmenopausal women: correlations with gonadotropin levels. *Am J Obstet Gynecol* 1980; 138:1137-41.
53. Mattsson LA, Cullberg G. Vaginal absorption of two estriol preparations. A comparative study in postmenopausal women. *Acta Obstet Gynecol Scand* 1983; 62:393-6.