

Gonadotrophin Suppression to Prevent Chemotherapy-Induced Ovarian Damage

A Randomized Controlled Trial

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OBJECTIVE: To estimate the effectiveness of gonadotrophin-releasing hormone (GnRH) analogues cotreatment in preventing chemotherapy-induced amenorrhea in young breast cancer patients undergoing cyclophosphamide-based chemotherapy.

METHODS: One hundred hormone-insensitive breast cancer participants (aged 18-40 years) were recruited from two university-affiliated oncology centers in Egypt. Opting for type of cotreatment was based on available timeframe until start of chemotherapy. Fifty women ready for early chemotherapy were randomized to receive either chemotherapy alone (arm I) or chemotherapy after downregulation (estradiol less than 50 pg/mL) by GnRH antagonist and agonist (arm II). Then, GnRH antagonist was discontinued and agonist was continued until the end of chemotherapy. When chemotherapy was to start later than 10 days after study

inclusion, 50 women were randomized to receive either chemotherapy alone (arm III) or chemotherapy after downregulation with GnRH agonist (arm IV). Resumption of menstruation at 12 months after end of chemotherapy was the primary outcome. Postchemotherapy hormonal and ultrasound changes were secondary outcomes.

RESULTS: Twelve months after termination of chemotherapy, there were no differences in menstruation resumption rates between GnRH-treated patients and control group individuals in either early (80% in arms I and II, risk ratio 1, 95% confidence interval 0.7-.32; $P=1.00$) or delayed chemotherapy groups (80% and 84% in arms III and IV, risk ratio 0.95, 95% confidence interval 0.73-1.235; $P=.71$). There were no differences in hormonal and ultrasound markers between GnRH analogue users and control group individuals. The use of GnRH analogue cotreatment did not predict independently the odds of menstruating at 12 months.

CONCLUSION: GnRH analogue cotreatment does not offer a significant protective effect on ovarian function in patients treated by cyclophosphamide-based chemotherapy.

CLINICAL TRIAL REGISTRATION: Australian New Zealand Clinical Trials Registry. www.anzctr.org.au, ACTRN12609001059257.

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LEVEL OF EVIDENCE: I

One in 233 women younger than age 40 years will have development of breast cancer, accounting for more than 40% of all cancers in women of reproductive age.¹ Breast cancer in young women is characterized by a higher incidence of undifferentiated and hormone-insensitive tumor cells, and the majority of these patients receive systemic treatment with chemotherapy.² Anthracycline-based and cyclophosphamide-based chemotherapy remains the standard of care.^{3,4} Cyclophosphamide has a four-fold increased risk of inducing amenorrhea

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when compared with other chemotherapeutic agents, mostly by causing apoptotic oocyte death in primordial follicles. Anthracyclines also have been reported to be gonadotoxic, in contrast to other chemotherapeutic agents like 5-fluorouracil that seem to have no reported adverse effects on ovarian reserve.⁵

One of the suggested strategies to preserve fertility is ovarian suppression by pituitary downregulation with gonadotropin-releasing hormone (GnRH) analogues before and during chemotherapy. This creates a pseudo-menopausal state with decreased ovarian function. The rationale behind this approach is the observation that prepubertal administration of chemotherapeutics causes less ovarian damage compared with in older women.^{6,7} Suppression of the pituitary ovarian axis, decreased ovarian perfusion, and direct gonad effect are proposed mechanisms of protection.⁸ Two recent systematic reviews^{8,9} suggest a possible protective role of GnRH agonists. Even so, they concluded that well-designed and implemented trials are still needed to determine the role of GnRH agonist in preserving fertility in this patient population.

Importantly, GnRH agonist must be administered 10–14 days before starting chemotherapy, so that the initial increase in gonad activity (“flare”) is followed by downregulation.⁸ This, however, appears to limit the applicability of this approach and cannot be adhered to in many oncologic illnesses because of time pressures; therefore, the question was raised regarding whether the flare-up can be avoided.¹⁰ One possibility appears to be the combination of GnRH agonist with GnRH antagonist to combine the quick onset of action of GnRH antagonist with the long-lasting effects of GnRH agonist.^{11–13} Until now, few studies have been published regarding the combination of both drugs and the results have been conflicting or incomplete. This could be attributed to the very small case numbers, the absence of a control group,¹⁰ and different timings of initiation of antagonist in relation to chemotherapy.^{11,13} In addition, there were reported concerns that the addition of antagonists could have a negative effect on the probable protective effect of GnRH agonist.¹⁴

Therefore, we initiated a two-center, randomized, controlled trial (RCT) to test the hypothesis that cotreatment with GnRH agonist or GnRH antagonist and agonist combination may prevent chemotherapy-induced amenorrhea in young patients with hormone-insensitive breast cancer treated with cyclophosphamide-based chemotherapy.

MATERIALS AND METHODS

During the period from December 2009 to August 2011, we conducted a two-center, four-armed, open-label RCT

of GnRH analogues cotreatment (GnRH antagonist and agonist combination or GnRH agonist), each compared with chemotherapy alone at two university-affiliated oncology centers in Egypt (Medical Oncology Departments, Zagazig University School of Medicine and National Cancer Institute, Cairo University). Institutional Review Board was obtained and all patients provided written informed consent. The trial protocol was a priori registered (ACTRN12609001059257).

Participants were randomized in a 1:1 ratio using a computer-generated block randomization scheme with variable block sizes (range from four to eight participants per block) and stratification for the timing of the first cycle of chemotherapy. The randomization list was produced by a statistician not involved with patient recruitment. Allocation concealment was achieved with sequentially numbered, dark, opaque, sealed envelopes.

Women (18–40 years old) with primary hormone-insensitive breast cancer (stage I–IIIa) scheduled for cyclophosphamide-based chemotherapy were recruited. Additional inclusion criteria were a history of regular menstrual periods, transvaginal ultrasound-confirmed presence of both ovaries, and absence of ovarian tumors or cysts larger than 40 mm. Exclusion criteria were advanced breast cancer (stage IIb–IV), primary ovarian cancer or pelvic metastases, history of chemotherapy or abdominal or pelvic radiation, and receiving or planning to receive hormone therapy. Pregnant and nursing women also were excluded. Participants were instructed to use adequate nonhormonal contraceptive measures during the study period.

A total of 100 women were included in the study. Opting for the type of cotreatment was based on available timeframe until start of chemotherapy. To prevent the flare-up produced by the GnRH agonist, 50 women ready for early chemotherapy within 1 week of enrollment were randomly allocated to receive either early chemotherapy alone (EC_{Control}, arm I, 25 patients) or chemotherapy after downregulation by a combined GnRH antagonist and agonist cotreatment (EC_{GnRH}, arm II, 25 patients). The GnRH antagonist (Cetrotide, cetrorelix 0.25 mg daily; Merck Serono) and GnRH agonist (Decapeptyl CR, triptorelin 3.75 mg; Ferring) were administered until downregulation (estradiol [E2] less than 50 pg/mL) was confirmed. Then, women were instructed to discontinue the GnRH antagonist and to continue using the GnRH agonist every 4 weeks until the end of chemotherapy.

When chemotherapy was to start at least 10 days after study inclusion (delayed chemotherapy), 50 women were randomized to receive either chemotherapy alone (DC_{Control}, arm III, 25 patients) or



chemotherapy after downregulation with GnRH agonist (DC_{GnRH}, arm IV, 25 patients), which was continued every 4 weeks until the end of chemotherapy.

The standard regimen of intravenous 5-fluorouracil (500/m²), adriamycin (50 mg/m²), and cyclophosphamide (500 mg/m²) every 21 days for six cycles, in absence of disease progression or toxicity, was used in all participants. Adverse events were assessed clinically and by means of hematologic and biochemical measurements. Surgical intervention and lymph node dissection were performed on a case-by-case basis. Regional adjuvant radiotherapy was allowed if indicated by the managing oncologist.

Resumption of menstruation at 12 months after the end of chemotherapy was the primary outcome. Resumption of “regular” menstrual cycles (defined as three consecutive periods within 21–35 days) at 12 months and biochemical (follicle-stimulating hormone, luteinizing hormone, E2) and ultrasound parameters of ovarian reserve (antral follicle count) at 6 and 12 months postchemotherapy were secondary outcomes. Antimüllerian hormone was measured basally and at 12 months postchemotherapy. Women were followed-up on a 6-month basis to measure biochemical and ultrasound parameters and to document menstrual cycle changes or tumor recurrence, pregnancies, or deaths.

Statistical analysis was performed according to the intention-to-treat principle. All analyses of significance were two-sided and tested at the 5% level; values of $P < .05$ were considered to indicate significant differences. Continuous variables were tested if they presented normal distribution using the t test. The results of the two groups were compared using the t test or Mann-Whitney U test for parametric and nonparametric data, respectively. Qualitative variables were compared with the use of the χ^2 test with Yates correction or Fisher exact test, when necessary, and the 95% confidence intervals (95% CIs). Risk ratios and 95% CIs were calculated to examine the risk of improving clinical outcomes. The confounding effects of women’s age, dose of chemotherapy, participating center, and treatment modality (GnRH agonist or GnRH antagonist and agonist) on resumption of menses at 12 months postchemotherapy were tested using logistic regression. Clinical and demographic data are presented as mean (\pm standard deviation), median, and interquartile ranges, or as frequency distribution for simplicity. Statistical analysis was performed using SPSS 11 for Windows.

Previous data indicated that the rate of resumption of menstruation among control group individuals is 35%.¹⁵ If resumption rate for experimental partici-

pants is assumed to be 80%, using a continuity-corrected χ^2 statistic to evaluate this null hypothesis we needed to recruit 22 women in each study arm to be able to reject the null hypothesis that the failure rates are equal with a probability (power) of 0.8 and a type I error probability of 0.05 using the χ^2 statistic. Because this trial was stratified according to planned time of starting chemotherapy, we intended on enrolling 25 women in each arm to allow for loss to follow-up and further comparisons within the strata.

RESULTS

One hundred women (aged 18–40 years) with hormone-insensitive breast cancer who received the allocated treatment and who were followed-up for at least 1 year were included in the study (Fig. 1). Five women died during the follow-up period: one woman in each group except the EC_{GnRH} group, in which two women died. Two women had tumor recurrences diagnosed: one in the EC_{GnRH} group and one in DC_{GnRH} group. Regardless of survival or tumor recurrence, all women remained included in the intention-to-treat analyses.

Baseline characteristics were comparable between each intervention group and its respective control individuals (Table 1). There was no difference in menstruation resumption rates between patients treated with GnRH analogues and their respective control individuals at 12 months (study endpoint).

Menses resumed in 72% and 80% of women at 6 and 12 months after termination of chemotherapy in the EC_{Control} group (arm I) compared with 64% and 80% in the EC_{GnRH} group (arm II) ($P = .54$ and $P = 1.00$, respectively). At 12 months, fewer women had resumption of regular menstruation in the DC_{Control} group than in the DC_{GnRH} group, but the difference was not statistically significant (48% compared with 60%; $P = .39$; Table 2).

Menses also was resumed in 76% and 80% of women at 6 and 12 months after termination of the chemotherapy in DC_{Control} (arm III) compared with 68% and 84% of cases in DC_{GnRH} (arm IV) ($P = .35$ and $P = .71$, respectively). At 12 months, 52% of DC_{Control} had regular menstruation compared with 72% in DC_{GnRH} ($P = .145$; Table 2).

During the study period, 68 women (17 in each arm) were followed-up for 18 months postchemotherapy. Cycle resumption and regularity were comparable in women receiving GnRH analogues and their respective control individuals (Table 2). Three spontaneous pregnancies were reported during the follow-up period, one in each group except the DC_{Control}. All



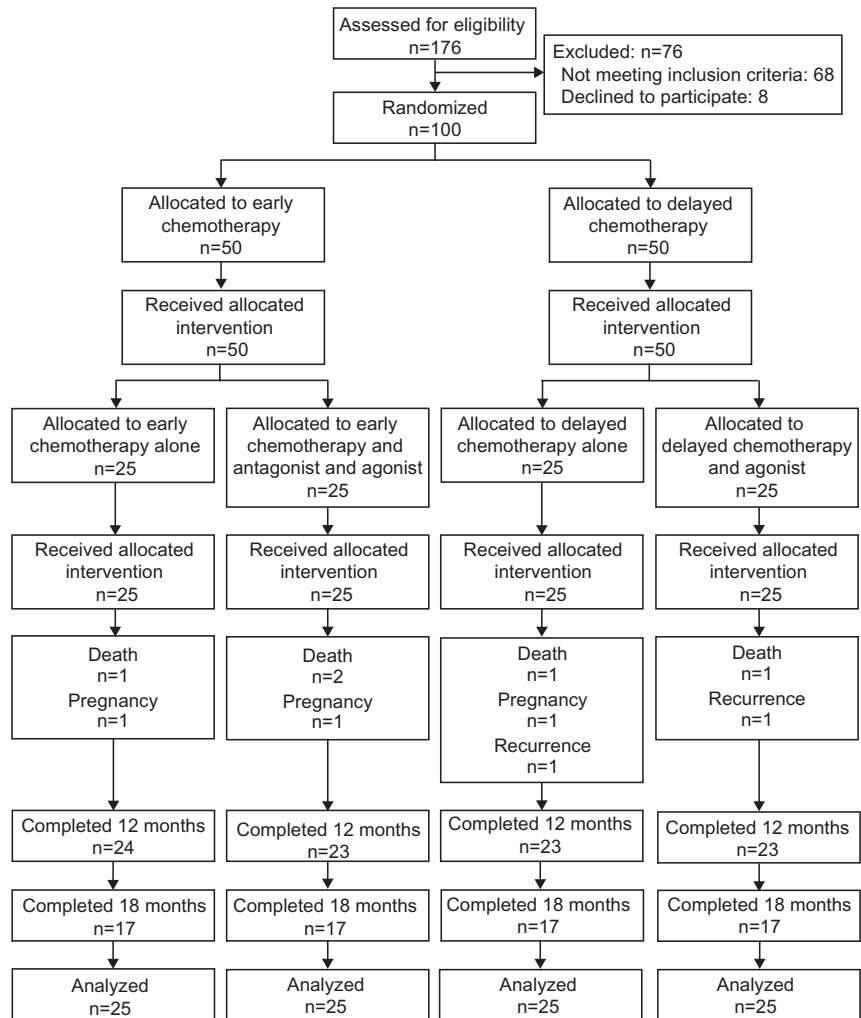


Fig. 1. Flow of participants through the study.

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pregnancies were reported to have resulted in normal term deliveries.

There were no significant differences between the GnRH-treated patients and their respective control individuals regarding any of the values of hormonal (follicle-stimulating hormone, luteinizing hormone, and E2) and ultrasound (antral follicle count) markers for ovarian reserve, whether at 6, 12, or 18 months. Antimüllerian hormone levels also were comparable at 12 months after end of chemotherapy (Figs. 2 and 3).

Multivariable logistic regression analysis did not identify any significant correlation between the tested variables and the resumption of menstruation at 12 months postchemotherapy (age: odds ratio [OR] 0.93, 95% CI 0.75–1.15; chemotherapy dose: OR 0.99, 95% CI, 0.98–1.01; treatment modality using GnRH antagonist and agonist: OR 1.02, 95% CI 0.32–3.54; treatment modality using GnRH agonist: OR 1.26,

95% CI 0.35–4.5; participating center: OR 1.45, 95% CI 0.23–4.7).

DISCUSSION

The results of this RCT demonstrate that GnRH analogue cotreatment does not offer any protective effect on ovarian function for young women with breast cancer receiving cyclophosphamide-based chemotherapy. At 1 year after the end of chemotherapy, menstruation resumption rates did not differ between women receiving chemotherapy alone and those receiving either a GnRH agonist or a combined GnRH antagonist and agonist cotreatment. The use of GnRH analogues cotreatment did not predict independently the odds of menstruating at 12 months and did not offer any beneficial effect on cycle resumption or regularity at 6, 12, and 18 months after the end of chemotherapy.



Table 1. Baseline Characteristics of the Participants According to Treatment Group

Characteristic	Early Chemotherapy		Delayed Chemotherapy	
	Control (n=25)	Agonist and Antagonist Cotreatment (n=25)	Control (n=25)	Agonist (n=25)
Age (y)	32.32±3.99	33.28±3.3	32.84±4.3	33±3.8
Body mass index (kg/m ²)	24.9±3	24.9±3.4	24.9±4.2	25.9±2.7
Doxorubicin (mg)	568±21	556±31	552.8±26	553.6±31
5-fluorouracil (mg)	5,680±214	5,560±308	5,528±264	5,536±309
Cyclophosphamide (mg)	5,680±214	5,564±308	5,528±264	5,536±309
Basal FSH (international units/L)*	7.6±1.19	6.7±1.5	7.1±1.27	6.97±0.93
Basal LH (international units/L)*	4.55±1.22	4.78±0.99	4.1±0.87	4.07±0.71
Estradiol (pg/mL)*	50.13±11	47.4±9.8	49.46 ±12	47.6±9.2
Antral follicle count†	12.24±2.5	10.75 ±2.5	10.95±2.5	12±1.75
Antimüllerian hormone (ng/mL)	2.34±0.56	2±0.63	2.29±0.74	2±0.63
Married	22 (88)	21 (84)	22 (88)	20 (80)
Parity				
Nulliparous	2 (8)	6 (24)	4 (16)	4 (16)
Primiparous	5 (20)	3 (12)	2 (8)	4 (16)
Multiparous	18 (72)	16 (64)	19 (76)	17 (68)
Radiography	21(84)	20 (80)	20 (80)	19 (76)
Stage				
1				
2	2 (8)	4 (16)	2 (8)	2 (8)
3	13 (52)	10 (40)	15(52)	15 (52)
Grade	10 (40)	11 (44)	8 (40)	8 (4)
1				
2	0	0	3 (12)	2 (8)
3	15 (60)	16 (64)	16 (64)	13 (52)

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Data are mean±standard deviation or n (%).

All baseline differences between groups were nonsignificant ($P>.05$).

* Values were not recorded for 12 participants in all groups except for the agonist and antagonist group, in which it was not recorded for 11 participants.

† Values were not recorded for eight participants in the early chemotherapy control group, nine in the agonist–antagonist group, six in the delayed chemotherapy control group, and seven in the agonist group.

Concerns that might arise regarding age and dose of chemotherapy were alleviated on performing regression analysis in which none of the tested confounders had any independent effect on cycle

resumption. The fact that all the women were aged from 18 to 40 years and 24% of them were in their later reproductive years (35–40 years old) might explain the nonsignificant effect of age. Actually, all

Table 2. Resumption of Menstruation in the Participants According to Treatment Group

	Early Chemotherapy		Delayed Chemotherapy	
	Control (n=25)	Agonist–Antagonist Cotreatment (n=25)	Control (n=25)	Agonist (n=25)
Menses at 6 mo	18 (72) 1.13 (0.77–1.65)	16 (64)	19 (76) 1.12 (0.79–1.58)	17 (68)
Menses at 12 mo	20 (80) 1.00 (0.76–1.32)	20 (80)	20 (80) 0.95 (0.73–1.24)	21 (84)
Regular at 12 mo	12 (48) 0.8 (0.48–1.34)	15 (60)	13 (52) 0.72 (0.46–1.13)	18 (72)
Menses at 18 mo*	14 (82) 1.1 (0.76–1.52)	13 (76.5)	13 (76.5) 0.93 (0.66–1.31)	14 (82)
Regular at 18 mo*	11 (64.7) 0.85 (0.55–1.31)	13 (76.5)	11 (64.7) 0.85 (0.55–1.31)	13 (76.5)

Data are n (%) or risk ratio (95% confidence interval).

All differences between groups are nonsignificant.

* Data were recorded for 17 cases.



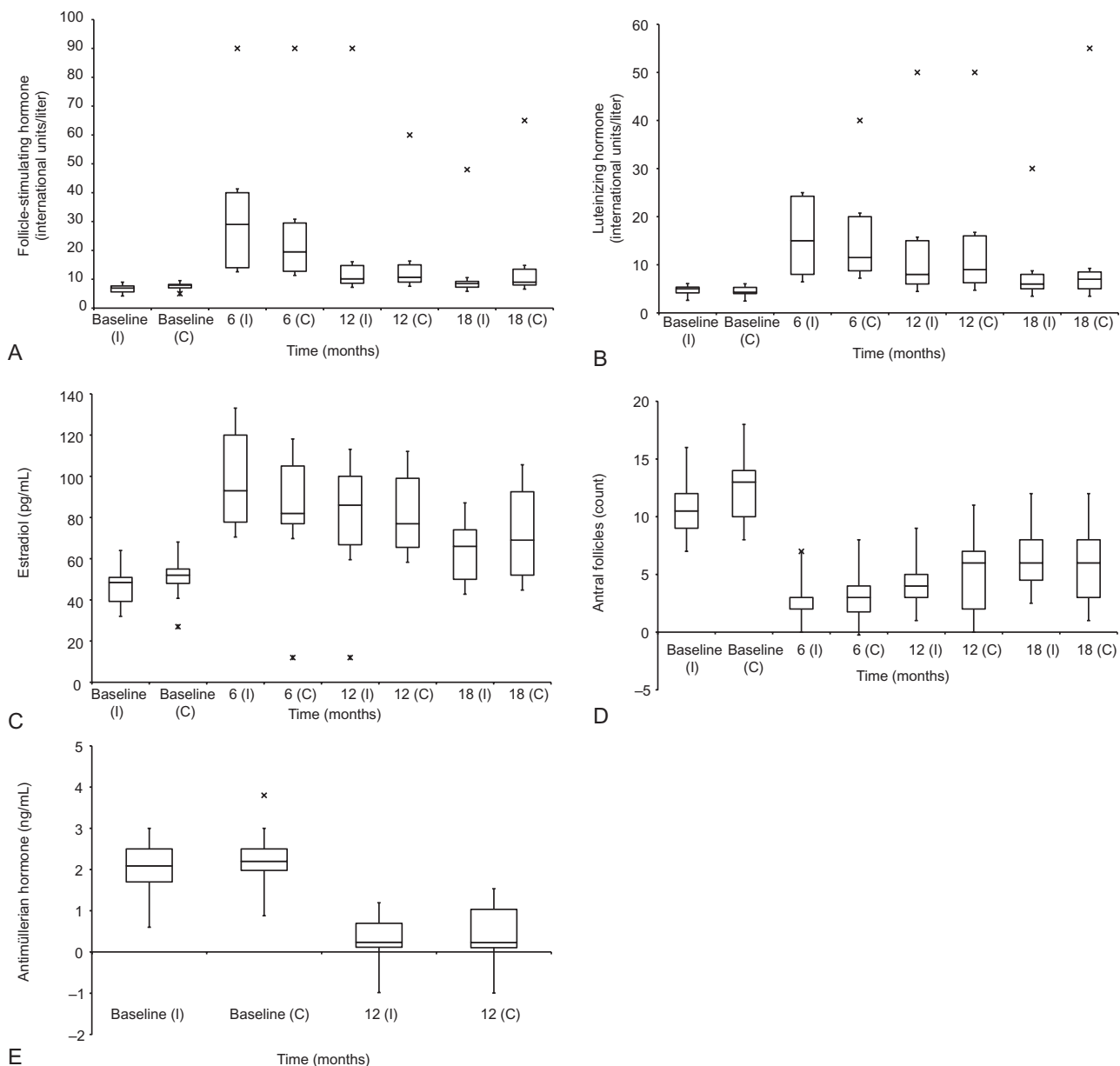


Fig. 2. Laboratory values and antral follicle count before start of chemotherapy (baseline) and at 6, 12, and 18 months after end of chemotherapy for early chemotherapy group with gonadotropin-releasing hormone (GnRH) antagonist and agonist cotreatment (I) and early chemotherapy group without GnRH analogues cotreatment (C). The line within the box is the median. Bottom and top of the box represent the 25th and 75th percentiles. The whiskers represent the lowest and highest datum within interquartile range of 1.5 of the lower and upper quartiles. **A.** Follicle-stimulating hormone. **B.** Luteinizing hormone. **C.** Estradiol. **D.** Antral follicle count. **E.** Antimüllerian hormone.

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fertility preservation methods are relevant to patients younger than 40 years, and ovarian reserve after this age is so low that the efficiency of fertility preservation is questionable.¹⁶ The reported incidence of amenorrhea varies widely, from 21% to 71% in women younger than 40 receiving chemotherapy.¹⁷ This might reflect the differing patient populations, chemother-

apy regimens, or length of the follow-up period in the respective trials. The World Health Organization defines menopause as no menstrual periods for 12 months.¹⁸ We used the last chemotherapy cycle as the start of the 12-month period and found amenorrhea ranging from 16% to 20% in the various groups. Fornier et al¹⁹ also reported that the rate of



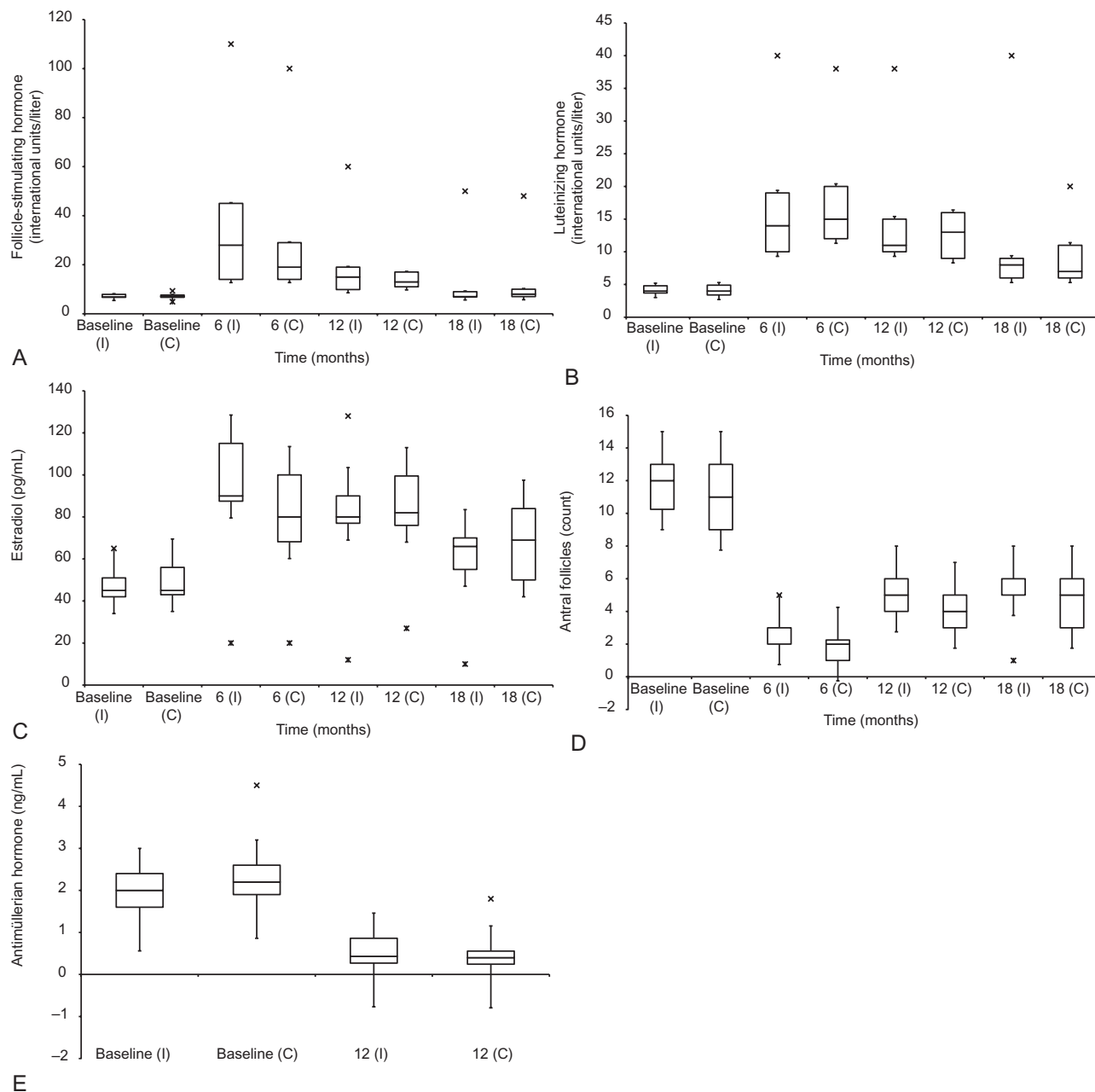


Fig. 3. Laboratory values and antral follicle count before start of chemotherapy (baseline) and at 6, 12, and 18 months after end of chemotherapy for delayed chemotherapy group with gonadotropin-releasing hormone (GnRH) agonist cotreatment (I) and early chemotherapy group without GnRH agonist cotreatment (C). The line within the box is the median. Bottom and top of the box represent the 25th and 75th percentiles. The whiskers represent the lowest and highest datum within interquartile range of 1.5 of the lower and upper quartiles. **A.** Follicle-stimulating hormone. **B.** Luteinizing hormone. **C.** Estradiol. **D.** Antral follicle count. **E.** Antimüllerian hormone.

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amenorrhea decreases to 15% in patients younger than 40 when a long duration of follow-up is considered (ie, 12 months or more after the end of chemotherapy).

The protective effect of GnRH agonist against chemotherapy-induced gonadal toxicity is quite debatable.^{8,9,15,20–25} Keeping with our results, two recent RCTs failed to prove the significant protective



effects of agonist cotreatment.^{20,21} In our trial, all women were younger than 40 years and received the same chemotherapy regimen (5-fluorouracil, adriamycin, and cyclophosphamide), and none received hormonal treatment, which are factors known to affect occurrence of amenorrhea. In a recent RCT, Del Mastro et al²² reported that triptorelin cotreatment reduced chemotherapy-induced early menopause; however, their patients were older (median age 39 years, range 25–45 years), received different regimens of chemotherapy (only 12 of 281 women received the most gonadotoxic cyclophosphamide-based chemotherapy) and less than 20% of them had hormone-negative breast cancer.

Earlier trial, limited by significant methodologic flaws, reported a potential benefit of agonist cotreatment.¹⁵ The positive effect of GnRH agonist also was underscored by a Cochrane review²³ as well as a systematic review and meta-analysis.⁸ The Cochrane review included four RCTs; the largest one involving 80 women was a previously criticized study¹⁵ and the total of women in the three remaining ones were 77. Meanwhile, the aforementioned systematic review and meta-analysis of Bedaiwy et al⁸ included not only the criticized trial¹⁵ but also the short 6-month follow-up of the ZORO trial,²⁰ which brings into question the validity of their conclusions.²⁴ Currently, the full 24-month follow-up is available and shows no difference in menses resumption rates between women receiving GnRH agonists and the control group, which is in keeping with our results. In an informative letter to the editor, Balkenende et al in 2011²⁵ performed a new meta-analysis for the included studies in the Bedaiwy meta-analysis,⁸ but with exclusion of the controversial study¹⁵ and inclusion of the follow-up of 24 months in the ZORO study.²⁰ According to this new meta-analysis,²⁵ the potential benefit of agonist was excluded (OR 2.25, 95% CI, 0.65–7.78) in contrast to the original meta-analysis in which a potential benefit of GnRH analogues was suggested (OR 3.46, 95% CI 1.13–10.57). Taking all these arguments into account, we believe that there is not enough high-quality evidence yet to consider cotreatment with GnRH agonist in women receiving chemotherapy as a standard practice to preserve ovarian function.

For women starting early chemotherapy, GnRH antagonists may help GnRH agonists to achieve faster downregulation.^{10–13} Some investigators have used antagonist or agonist combination simultaneously with the start of chemotherapy.¹³ The classic flare-up effect associated with GnRH agonists, however, cannot be suppressed with first dose of antagonist. Mardesic et al¹¹ reported a suppression of gonadotropin

secretion after 96 hours in women using agonist and antagonist. We opted to assure downregulation with less than 50 pg/mL E2 before stopping antagonist and starting chemotherapy.

In contrast to agonist, Danforth et al,¹⁴ in a murine model, reported that antagonists have a negative effect on the ovaries by depletion of primordial follicles. It has been speculated that the positive effect of additional antagonists on the reduction of the flare-up is reduced by the possible negative effect of antagonists on the ovaries.¹⁰

In the current trial, addition of antagonists to agonists neither improved nor averted the resumption of menses in comparison with the control group. Even so, we performed subgroup analysis between participants treated with antagonist and agonist compared with those using agonist only in addition to chemotherapy, and resumption of menses was comparable at 1 year after end of chemotherapy for both groups (80% compared with 84%, risk ratio 0.95, 95% CI 0.73–1.23; $P = .26$).

Regarding ovarian reserve markers, values and pattern of change of hormonal and ultrasound markers were comparable between GnRH analogue users and their respective control individuals. Hormones secreted by pituitary gland (follicle-stimulating hormone, luteinizing hormone) increased at 6 months, and then declined thereafter at 12 and 18 months. Estradiol increased at 6 months with no noticeable change of pattern thereafter. Antral follicle count decreased at 6 months and started to increase at 12 and 18 months. Antimüllerian hormone decreased significantly at 1 year after end of chemotherapy in all groups. Gracia et al²⁶ reported that even in young survivors with normal menstrual cycles, hormone and ultrasound measures of ovarian reserve suggest more decreased underlying ovarian reserve than in age-matched healthy women. The predictive value of measures for pregnancy and later menopause must be studied in longer follow-up trials. In the current study, spontaneous unplanned pregnancies occurred in three women. Definitely, longer follow-up for all participants will enable better studying of ovarian reserve, fertility potential, and timing of menopause. Admittedly, speculative mechanisms have been suggested regarding how GnRH analogues may protect ovarian reserve against chemotherapy.²⁷ These mechanisms, however, never have been proven to be functional in human oocytes, and development from primordial follicles to small preantral follicles are a gonadotropin-independent process²⁸; therefore, biologic plausibility for analogue preservation of ovarian reserve is lacking.



In conclusion, the results of this trial do not provide evidence that GnRH analogue cotreatment offers a significant protective effect on ovarian function in patients with hormone-insensitive breast cancer treated with cyclophosphamide-based chemotherapy.

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