



Ovarian reserve in premenopausal women with breast cancer

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ARTICLE INFO

Keywords:

Breast cancer
Ovarian function
Anti-Müllerian hormone (AMH)
Chemotherapy
Gonadotropin-releasing hormone analogs (GnRHa)

ABSTRACT

Background: As a special reproductive hormone and ovarian reserve indicator, the role of anti-Müllerian hormone (AMH) in premenopausal women with breast cancer deserves further study.

Methods: We conducted an in-depth analysis of the data from the EGOFACT study (NCT02518191), a phase III, randomized, controlled trial involving premenopausal female breast cancer patients in two parallel groups: chemotherapy with or without gonadotropin-releasing hormone analogs (GnRHa). Three hundred thirty premenopausal women aged 25–49 years with operable stage I to III breast cancer were included in this study. The characteristics of ovarian reserve changes marked by AMH in the EGOFACT study and the factors affecting ovarian function in premenopausal women with breast cancer were analyzed.

Results: The AMH level of the chemotherapy alone group decreased gradually within one year, while the AMH level of the GnRHa group was significantly higher as early as 6 months after chemotherapy and recovered to close to the baseline level 12 months after chemotherapy ($F = 34.991$, $P < 0.001$). Correlation analysis showed that the factors affecting AMH levels mainly included age, menarche age, body mass index (BMI), reproductive history, baseline follicle stimulating hormone (FSH) level, pathological stage and GnRHa application, but they had different effects on the incidence of premature ovarian insufficiency (POI) at different periods. Multivariate logistic regression analysis showed that menarche age younger than 14 years (OR 0.470 [0.259, 0.852], $P = 0.013$), baseline AMH level higher than 0.5 ng/mL (OR 9.590 [3.366, 27.320], $P < 0.001$), pathological stage I (OR 0.315 [0.124, 0.798], $P = 0.015$) and GnRHa application (OR 0.090 [0.045, 0.183], $P < 0.001$) were independent factors conducive to protection of ovarian reserve, as well as to recovery of ovarian reserve.

Conclusions: Age, menarche age, baseline AMH level, and GnRHa application are the most important influencing factors for ovarian reserve in premenopausal women with breast cancer.

Trial registration: ClinicalTrials.gov, NCT02518191, registered on Aug 5, 2015.

1. Introduction

The treatment-related reproductive toxicity and ovarian function protection of women with cancer will be an important issue in the present and for a long time in the future [1–3]. Oncologists and gynaecologists have paid attention to this issue and have formed some consensus and guidelines for clinical diagnosis and treatment [4–7]. In the field of breast cancer treatment, this issue is particularly important because chemotherapy and endocrine therapy may be associated with

reproductive toxicity in several young breast cancer patients and impair their ovarian function [8–10]. In this context, anti-Müllerian hormone (AMH) and gonadotropin-releasing hormone analogs (GnRHa) have become the hot spots in research on reproductive function protection in breast cancer patients.

Since the 1980s, GnRHa has been used to protect ovarian function in women with cancer. Two major clinical studies in the 2010s, PROMISE-GIM6 and POEMS-SWOG S0230, both obtained positive results, confirming that GnRHa can reduce the reproductive toxicity of

Abbreviations: AMH, anti-Müllerian hormone; GnRHa, gonadotropin-releasing hormone analogs; POI, premature ovarian insufficiency; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; OFS, ovarian function suppression; DFS, disease-free survival; AFC, antral follicle count; CTX, cyclophosphamide.

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<https://doi.org/10.1016/j.breast.2022.05.009>

Received 12 April 2022; Received in revised form 29 May 2022; Accepted 30 May 2022

Available online 2 June 2022

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chemotherapy in premenopausal women with breast cancer and reduce the risk of premature ovarian insufficiency (POI) after chemotherapy [11,12]. This established the clinical status of GnRHa for ovarian function protection in premenopausal women with breast cancer [3,9]. However, for many years, the understanding of the mechanism of GnRHa has been limited to the effect of inhibiting follicle stimulating hormone (FSH) and LH (luteinizing hormone) levels, and few studies on the direct mechanism have been reported [5]. In addition to ovarian function protection, GnRHa is also used as an adjuvant endocrine therapy for breast cancer as a mode of ovarian function suppression (OFS). The TEXT/SOFT study confirmed that OFS combined with tamoxifen or aromatase inhibitors can improve disease-free survival (DFS) in premenopausal breast cancer patients [13].

AMH is widely used in obstetrics and gynecology as an indicator of ovarian reserve. Because it is less directly affected by the hypothalamic-pituitary-gonad axis and fluctuates less in the physiological cycle, it is more stable and sensitive than E2/FSH and other indicators [14,15]. AMH has been clinically used to assess ovarian function damage. ESMO Clinical Practice Guidelines and ESHRE Guidelines recommend antral follicle count (AFC) or AMH as a standard test for ovarian reserve [4,5]. Additional research evidence suggests that AMH may be a potential target for ovarian function protection: recombinant AMH prevented cyclophosphamide (CTX)-induced loss of primordial follicles, and protected ovarian reserve and reproductive function during chemotherapy [16–18].

Over the past ten years, our team has focused on the clinical and basic research of reproductive function in breast cancer [19]. Our previous research indicated that AMH is an efficient marker for predicting postchemotherapy ovarian function exclusively in premenopausal female patients with breast cancer older than 35 years [20]. We discovered that GnRHa protects granulosa cells from chemotherapeutic toxicity in vivo and in vitro, CTX-induced endoplasmic reticulum (ER) stress inhibits the secretion of AMH, and treatment with GnRHa relieves

ER stress and the subsequent unfolded-protein response by modulating mTOR signaling to induce autophagy [21]. The EGOFACT study confirmed from a clinical perspective that administration of GnRHa with chemotherapy in premenopausal breast cancer patients reduces the risk of POI and promotes the recovery of ovarian function [22]. In this study we conducted an in-depth analysis of the data from the EGOFACT study to explore the changing characteristics of AMH levels in premenopausal women with breast cancer and various clinical factors affecting the ovarian reserve at different stages.

2. Methods

2.1. Participants

The EGOFACT study (NCT02518191) was a phase III, randomized, controlled trial involving premenopausal female patients aged from 18 to 49 years with operable stage I to III breast cancer who needed neoadjuvant or adjuvant chemotherapy in two parallel groups: chemotherapy with or without GnRHa treatment. The trial was conducted at the Shanghai Jiao Tong University Affiliated Shanghai Sixth People's Hospital in Shanghai and Zhejiang Cancer Hospital in Hangzhou, China. The trial protocol and all amendments that approved by an independent ethics committee or the institutional review board at each site were published [22]. Written informed consent was provided by all the patients. In total, 330 patients (165 in the GnRHa group and 165 in the Control group) were included in this statistical analysis (Fig. 1).

Following the established criteria of the EGOFACT study, POI was still defined as AMH < 0.5 ng/mL in this study [22,23]. Patients were divided into a high E2 group and a low E2 group, a high FSH group and a low FSH group with median baseline E2 (105.5 pg/mL) and FSH (5.67 mIU/mL) as cut-off values, respectively (eTable 3).

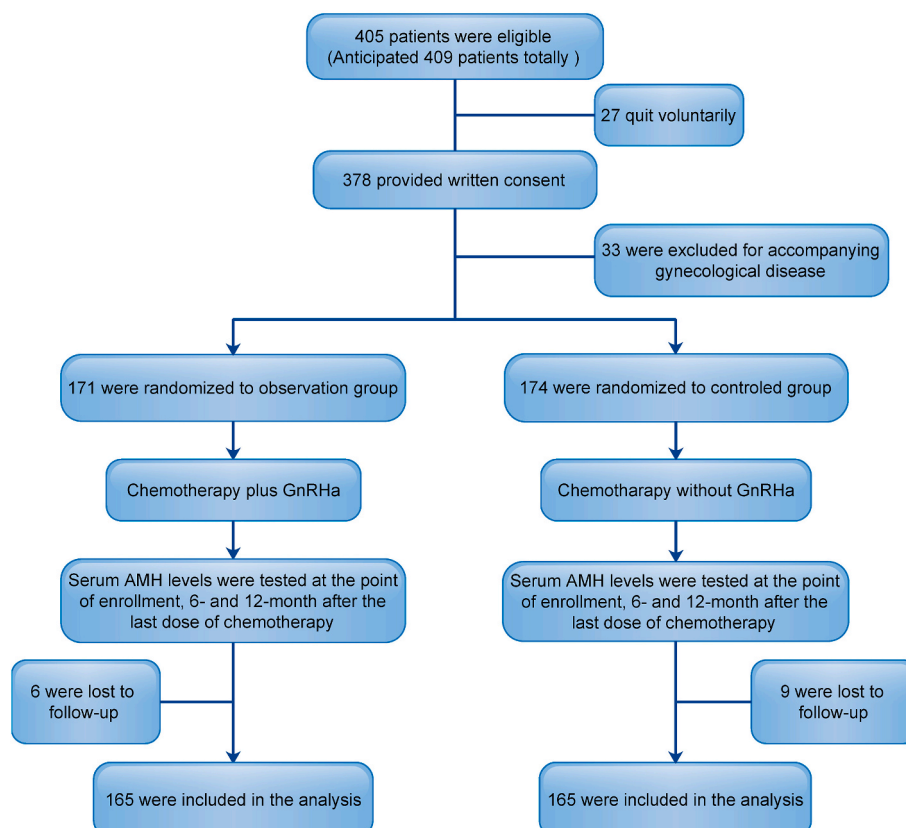


Fig. 1. CONSORT diagram.

2.2. Statistical analysis

Categorical data were recorded as frequencies and percentages. Continuous data were recorded as the mean ± standard deviation. The mean difference was analyzed by *t*-test. A general linear model was used to analyze the variance in AMH continuous measurement data. Spearman correlation analysis or Pearson correlation analysis was used to explore the correlation between various clinical/pathological factors and AMH levels. The risk ratios of various clinical/pathological factors for POI were calculated by crosstabs analysis. Multivariate logistics regression analysis was used to analyze factors promoting POI occurrence and AMH recovery. Effect sizes were reported as odds ratios with 95% confidence intervals. All tests were two-sided, and a *P* value of less than 0.05 was considered to indicate statistical significance. Data were analyzed with the use of SPSS software, version 19.0 and R software.

3. Results

The characteristics of patients in the EGOFACT study are shown in eTable 1 and eTable 2, and there was no significant difference in baseline characteristics between the two groups. There was no significant difference in AMH levels between the two groups before chemotherapy.

3.1. Changes in AMH levels in the EGOFACT study

Repeated measurements of serum AMH levels (0, 6 and 12 months after the last cycle of chemotherapy) were examined by a general linear model. As shown in Fig. 2A, in both the GnRHa group and the control group, there were significant differences in AMH measurements before, 6 months after and 12 months after chemotherapy, and AMH levels showed a significant trend of change over time ($F = 113.187, P < 0.001$). The mean AMH level of the GnRHa group was significantly different from that of the control group according to analysis of variance (ANOVA, $F = 8.025, P = 0.005$). As time changed, the difference in AMH levels between the GnRHa group and the control group also changed, indicating that time and treatment had an interaction ($F = 34.991, P < 0.001$).

Different results were obtained when the enrolled patients were grouped according to age: In patients younger than 35 years, AMH measurements in the GnRHa group and the control group still showed a significant trend over time ($F = 35.731, P < 0.001$), but there was no statistically significant difference in AMH levels between the GnRHa group and the control group ($F = 0.623, P = 0.525$), and there was no interaction between time and group ($F = 1.180, P = 0.311, Fig. 2B$). Among patients older than 35 years, AMH measurements before chemotherapy, 6 months after chemotherapy, and 12 months after chemotherapy showed significant differences between the two groups. AMH levels showed a significant trend of change over time ($F = 96.942, P < 0.001$), and AMH levels between the two groups showed significant differences ($F = 10.289, P = 0.001$). Time and grouping had an interaction ($F = 35.048, P < 0.001, Fig. 2C$), which was consistent with pooled analyses of all patients.

3.2. Factors influencing AMH levels in newly diagnosed premenopausal women with breast cancer by correlation analysis

As Table 1 shows, among breast cancer patients initially diagnosed without any treatment, the factors influencing the AMH level in premenopausal women included age ($r = -0.671, P < 0.001$), menarche age ($r = -0.176, P = 0.001$), BMI ($r = -0.162, P = 0.003$), reproductive history ($r = -0.225, P < 0.001$) and baseline FSH level ($r = -0.200, P < 0.001$). However, only age ($r = -0.322, P < 0.001$), menarche age ($r = -0.114, P = 0.038$) and baseline FSH level ($r = -0.326, P < 0.001$) were associated with primary POI. Compared with female patients older than 35 years, women younger than 35 years had higher AMH levels ($r = -0.597, P < 0.001$) and were less likely to develop primary POI ($r =$

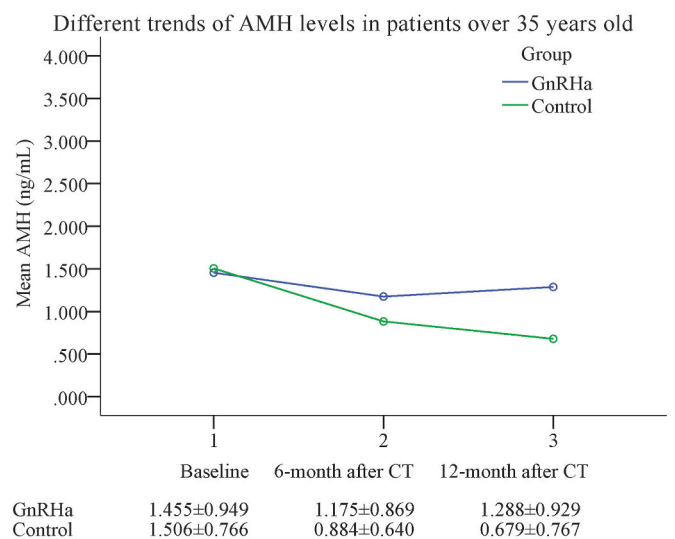
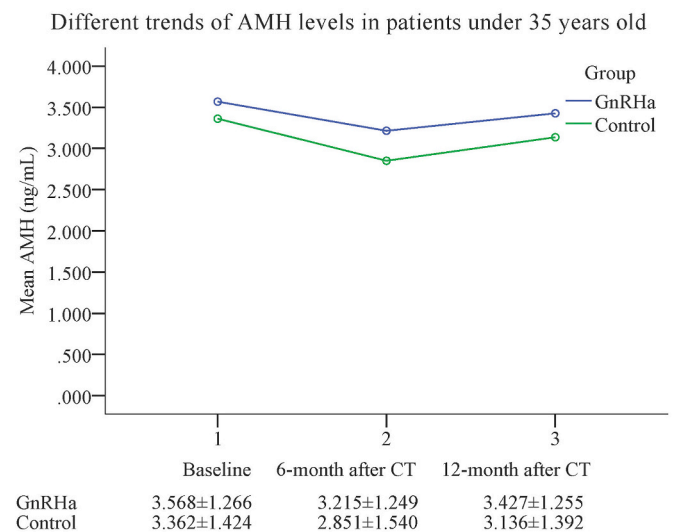
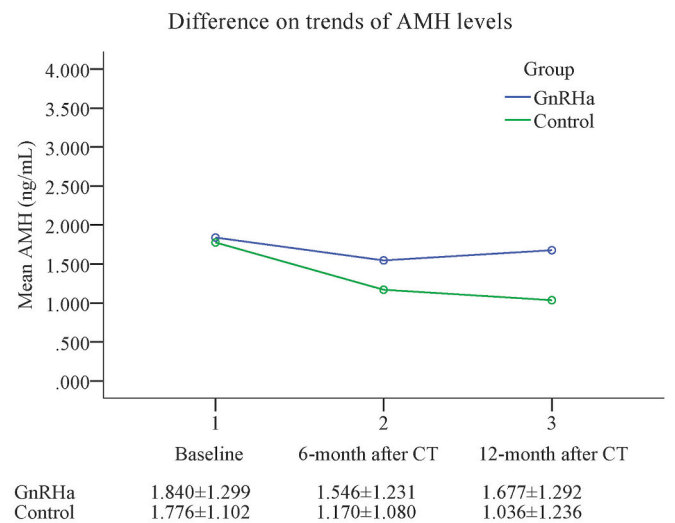


Fig. 2. Different trends in AMH levels between the GnRHa and the control groups.

Table 1
Correlations among AMH levels and patients' baseline characteristics.

Partial correlation		Before chemo		6 months after chemo		12 months after chemo	
		AMH [#]	POI [#]	AMH	POI	AMH	POI
Age	r	-0.671	-0.322	-0.681	-0.370	-0.741	-0.490
	P value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
<35yrs vs. ≥35yrs	r	-0.597	-0.149	-0.628	-0.270	-0.657	-0.317
	P value	<0.001*	0.007*	<0.001*	<0.001*	<0.001*	<0.001*
Menarche age	r	-0.176	-0.114	-0.159	-0.044	-0.145	-0.077
	P value	0.001*	0.038*	0.004*	0.431	0.009*	0.163
<14yrs vs. ≥14yrs	r	-0.207	-0.091	-0.197	-0.090	-0.178	-0.138
	P value	<0.001*	0.100	<0.001*	0.105	0.001*	0.013*
BMI	r	-0.162	-0.030	-0.171	-0.042	-0.161	-0.038
	P value	0.003*	0.588	0.002*	0.450	0.003*	0.498
<25 vs. ≥25	r	-0.044	0.012	-0.080	0.003	-0.052	0.021
	P value	0.430	0.828	0.146	0.954	0.345	0.702
Reproductive history	r	-0.225	-0.086	-0.234	-0.152	-0.275	-0.146
	P value	<0.001*	0.122	<0.001*	0.006*	<0.001*	0.008*
Baseline E2	r	0.076	0.098	0.086	0.120	0.047	0.035
	P value	0.171	0.077	0.118	0.030*	0.391	0.532
Baseline FSH	r	-0.200	-0.326	-0.167	-0.110	-0.106	-0.108
	P value	<0.001*	<0.001*	0.002*	0.047*	0.056	0.051
Pathological stage (I,II,III)	r			0.188	-0.018	0.175	-0.044
	P value			0.001*	0.751	0.001*	0.425
Grouping (GnRHa vs. Control)	r			-0.161	-0.131	-0.246	-0.339
	P value			0.003*	0.018*	<0.001*	<0.001*

Note: [#]Partial correlation adjusted by pathological stage and grouping. * Correlation was significant by two-sided test.

-0.149, $P = 0.007$). Those who had menarche earlier than 14 years had higher AMH levels than those who had menarche later ($r = -0.207$, $P < 0.001$), but menarche age was not associated with primary POI risk ($r = -0.091$, $P = 0.100$).

At 6 months after chemotherapy, AMH levels were significantly correlated with age ($r = -0.681$, $P < 0.001$), menarche age ($r = -0.159$, $P = 0.004$), reproductive history ($r = -0.234$, $P < 0.001$), BMI ($r = -0.171$, $P = 0.002$), baseline FSH level ($r = -0.167$, $P = 0.002$), pathological stage ($r = 0.188$, $P = 0.001$) and treatment grouping ($r = -0.161$, $P = 0.003$). However, menarche age, BMI and pathological stage did not affect the risk of POI.

Twelve months after chemotherapy, AMH levels were significantly correlated with age ($r = -0.741$, $P < 0.001$), menarche age ($r = -0.145$, $P = 0.009$), reproductive history ($r = -0.275$, $P < 0.001$), BMI ($r = -0.161$, $P = 0.003$), pathological stage ($r = 0.175$, $P = 0.001$) and treatment grouping ($r = -0.246$, $P < 0.001$). However, menarche age, BMI and pathological stage did not affect the risk of POI.

3.3. Factors influencing POI incidence in premenopausal breast cancer patients exposed to chemotherapy toxicity with or without GnRHa by correlation analysis

As shown in Table 2, in the control group that received chemotherapy without GnRHa cotreatment, age and reproductive history had significant effects on the risk of POI at both 6 and 12 months after chemotherapy. Patients younger than 35 years had lower risk of POI than those older than 35 years. The risk of POI was higher in women with reproductive history than in those without childbearing. The age of menarche did not affect the risk of POI. Patients with BMI less than 25 were more likely to have POI 6 months after chemotherapy ($r = 0.171$, $P = 0.029$) but not 12 months after chemotherapy ($r = 0.136$, $P = 0.083$).

In the treatment group receiving chemotherapy combined with GnRHa, age, reproductive history and BMI had significant effects on the risk of POI at both 6 and 12 months after chemotherapy. Patients younger than 35 years had lower risk of POI than those older than 35 years. In contrast to the control group, patients with a BMI greater than 25 were more likely to have POI 6 months after chemotherapy. The age of menarche did not affect the risk of POI. Patients with high baseline E2 levels before treatment were not prone to POI at either 6 months ($r = 0.156$, $P = 0.046$) or 12 months ($r = 0.162$, $P = 0.038$) after

Table 2
Factors affecting POI incidence in premenopausal breast cancer patients exposed to chemotherapy toxicity with or without GnRHa.

Partial correlation		6-month POI		12-month POI	
		GnRHa group	Control group	GnRHa group	Control group
Age	r	-0.421	-0.320	-0.422	-0.557
	P value	<0.001*	<0.001*	<0.001*	<0.001*
<35yrs vs. ≥35yrs	r	-0.250	-0.285	-0.233	-0.381
	P value	0.001*	<0.001*	0.003*	<0.001*
Menarche age	r	-0.066	-0.035	-0.112	-0.075
	P value	0.403	0.654	0.154	0.340
<14yrs vs. ≥14yrs	r	-0.118	-0.066	-0.136	-0.145
	P value	0.132	0.403	0.082	0.064
BMI	r	-0.218	0.093	-0.196	0.052
	P value	0.005*	0.236	0.012*	0.507
<25 vs. ≥25	r	-0.187	0.171	-0.119	0.136
	P value	0.016*	0.029*	0.130	0.083
Reproductive history	r	-0.150	-0.161	-0.164	-0.152
	P value	0.055	0.040*	0.036*	0.051
Baseline E2	r	0.156	0.125	0.162	0.015
	P value	0.046*	0.111	0.038*	0.853
Baseline FSH	r	-0.125	-0.113	-0.169	-0.100
	P value	0.111	0.149	0.031*	0.205
Pathological stage (I,II,III)	r	-0.083	0.044	-0.230	0.097
	P value	0.287	0.575	0.003*	0.214

Note: Partial correlation adjusted by pathological stage. * Correlation was significant by two-sided test.

chemotherapy, and patients with low FSH had a lower risk of POI at 12 months after chemotherapy ($r = -0.169$, $P = 0.031$).

3.4. Factors promoting POI and influencing the recovery of impaired ovarian function in premenopausal breast cancer patients

As shown in eTable 3, multivariate logistics regression analysis found that menarche age, pathological stage, baseline AMH level and GnRHa application were important factors affecting POI occurrence and AMH recovery. The respective risk ratios for POI were calculated by crosstabs analysis and are displayed in Figs. 3 and 4.

At 12 months after chemotherapy, baseline AMH level, pathological stage, menarche age and GnRHa treatment were important factors affecting the incidence of POI: patients with baseline AMH levels lower than 0.5 ng/mL were more likely to have sustained POI ($OR = 9.590$, $[3.366-27.320]$, $P < 0.001$), and patients who received chemotherapy with GnRHa had a lower risk of POI ($OR = 0.090$, $[0.045-0.183]$, $P < 0.001$). Patients with pathological stage I cancer ($OR = 0.315$, $[0.124-0.798]$, $P = 0.015$) or with menarche younger than 14 years ($OR = 0.470$, $[0.259-0.852]$, $P = 0.013$) had a lower risk of POI (eTable 3, Fig. 3).

Among 87 patients who developed POI 6 months after chemotherapy, factors affecting the recovery of ovarian reserve (AMH level returning to normal range) included menarche age, baseline AMH level, pathological stage, and GnRHa application. Patients with primary POI, i.

e., baseline AMH < 0.5 ng/mL ($OR = 0.087$, $[0.008-0.906]$, $P = 0.041$), had difficulty restoring normal ovarian reserve. Patients who received chemotherapy with GnRHa ($OR = 18.487$, $[3.630-94.161]$, $P < 0.001$), with menarche younger than 14 years ($OR = 5.436$, $[1.188-24.873]$, $P = 0.029$), or with pathological stage I cancer ($OR = 65.883$, $[4.220-1028.474]$, $P = 0.003$) were more likely to have restored normal ovarian reserve (eTable 3, Fig. 4).

4. Discussion

ANOVA of repeated measurement data was performed on the mean serum AMH level of patients in the GnRHa group and the control group before chemotherapy, 6 months after chemotherapy and 12 months after chemotherapy by a general linear model, and the effect of GnRHa treatment on AMH level could be clearly seen. There was no significant difference in AMH levels between the two groups before treatment, while the level of AMH in the GnRHa group was significantly higher than that in the control group after chemotherapy, indicating that the combination of GnRHa with chemotherapy can protect ovarian reserve compared with chemotherapy alone. The AMH level of the chemotherapy alone group decreased gradually within one year, while the AMH level of the GnRHa group was significantly higher than that of the control group as early as 6 months after chemotherapy and recovered to close to the initial level 12 months after chemotherapy, suggesting that GnRHa can reverse the trend of ovarian reserve changes after

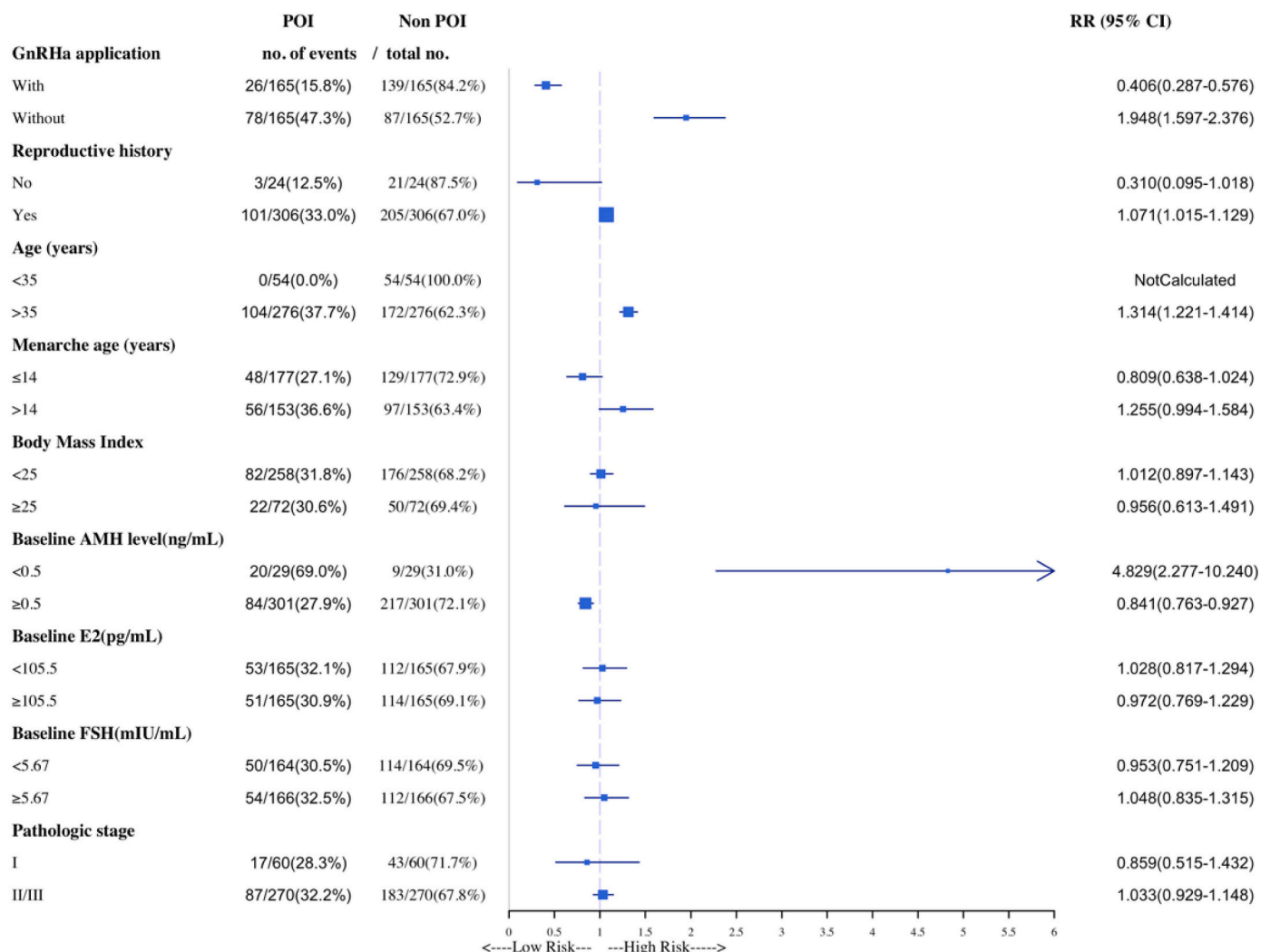


Fig. 3. Risk ratios for POI in premenopausal breast cancer patients.

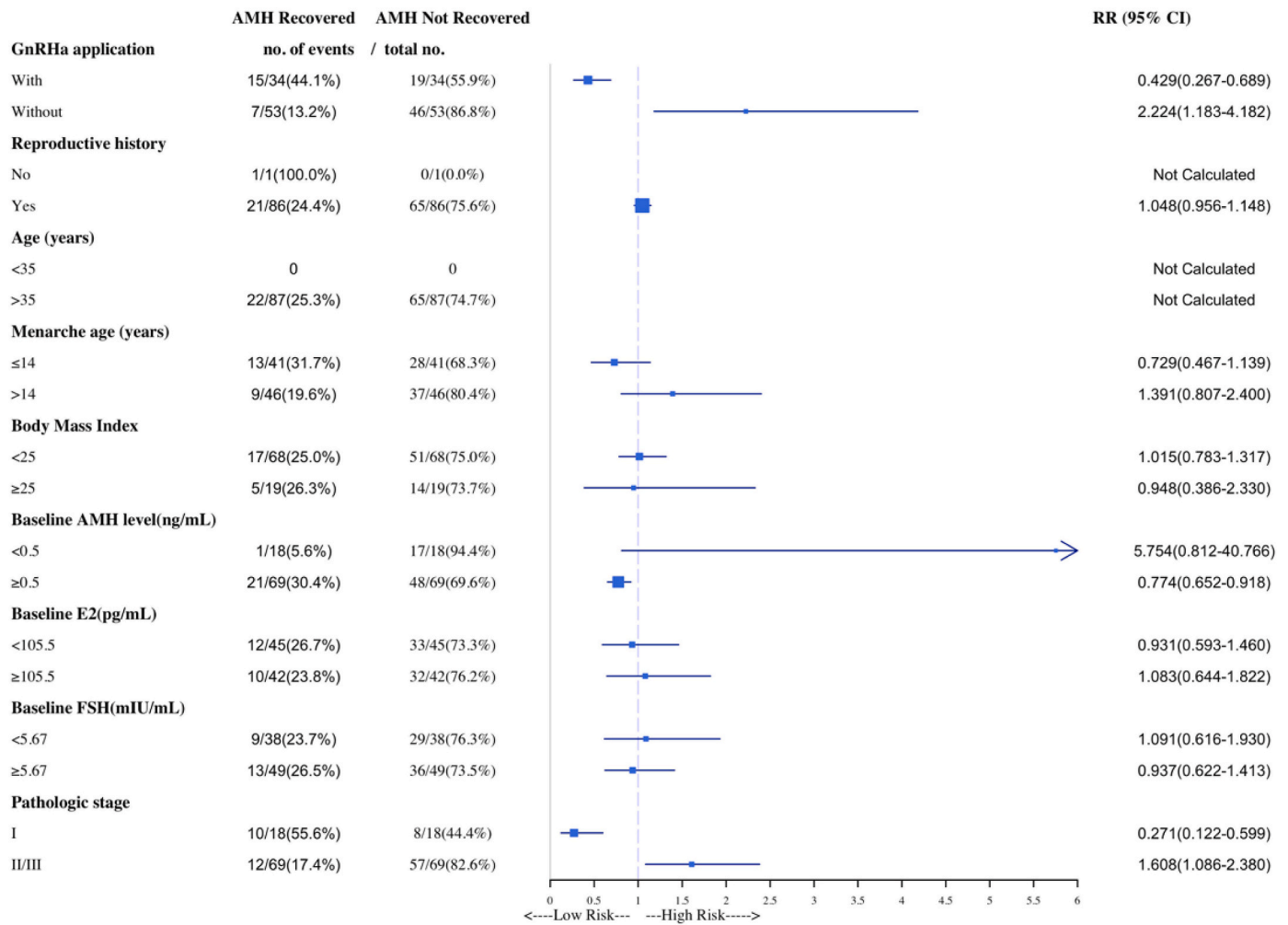


Fig. 4. Risk ratios for AMH not recovered in 87 patients suffering POI at 6 months after chemotherapy.

chemotherapy.

Age group analysis was also conducted in this study, and the results were consistent with the conclusions of our previous study [20]. Among patients older than 35 years, the variation trend of AMH levels in the two groups over time and the differences between the two groups were basically consistent with the results of all samples. However, in patients younger than 35 years, regardless of whether GnRHa was used, AMH levels in the two groups showed no significant difference and showed the same trend over time. AMH levels in both groups dropped to a low point 6 months after chemotherapy and then rose to close to the normal value 12 months after chemotherapy. There are two possibilities for this result: first, patients younger than 35 years have rapid replacement of growing follicles, and ovarian function damage caused by chemotherapy cannot be reflected through changes in AMH levels [24]; Second, the ovarian reserve of young women is sufficient, enough to withstand the ovarian function damage caused by the toxicity of chemotherapy and can recover by themselves [25].

Regardless of the stage of treatment, female patients younger than 35 years old had higher AMH levels and were less likely to develop primary or secondary POI than those older than 35 years old, which was consistent with the results of most studies [24–26]. The average AMH level of female patients with menarche earlier than 14 years old was higher than that of those with menarche later than 14 years old, and the risk of POI was reduced by more than half at 12 months after chemotherapy. The odds of recovery from temporary ovarian impairment in patients with menarche earlier than 14 years old increased by more than 5 times, while the specific reasons are not yet clear. To test whether

primary POI in young breast cancer patients is reversible, especially when treated with GnRHa, 29 patients with baseline AMH levels lower than 0.5 ng/mL were also included in the analysis. The proportion of primary POI cases was low enough (<10%) not to affect the overall analysis. It is clear that subsequent treatment with or without GnRHa has difficulty restoring normal ovarian reserve in patients with baseline AMH levels lower than 0.5 ng/mL, which has been confirmed by several studies [27–29]. This study showed that GnRHa treatment was an important factor affecting the incidence of POI: The risk of POI in patients with combined GnRHa was only 0.09 times that in those without combined GnRHa. Meanwhile, the probability of ovarian function recovery in patients with short-term ovarian function impairment after chemotherapy was 18.49 times higher in patients with GnRHa combination than in patients without GnRHa combination, which was consistent with the results of our own laboratory and clinical studies and with the results of most studies [3,9,10,20–22,30].

Baseline E2 levels and FSH levels were correlated with AMH levels to some extent, which has only been mentioned in a few references. [29, 31] Patients with higher E2 and lower FSH had a lower risk of POI in the early stage after chemotherapy, especially in the GnRHa group. High baseline FSH levels may be the cause of primary POI, as well as the result and clinical presentation of POI. For reasons that are not clear, patients with reproductive history were more likely to develop POI after chemotherapy. There have been previous studies on the types and molecular characteristics of breast cancer and ovarian function after chemotherapy, but the effect of breast cancer stage on ovarian function is rarely mentioned [32–35]. We found that compared with patients

with pathological stage II or III disease, patients with stage I disease had a lower risk of POI and were more likely to recover from temporary impairment of ovarian function, which may be related to systemic changes in the natural course of breast cancer, suggesting that patients with earlier stages of disease have better body function, including ovarian function. The effect of BMI on AMH and POI presented interesting results, which is different from literature reports [35]: The analysis of all patients found that BMI was negatively correlated with AMH level at all stages but not with POI risk. Analysis of the treatment group and the control group showed that 6 months after chemotherapy, patients with BMI < 25 in the control group were more likely to develop POI, while those with BMI ≥ 25 in the GnRHα group were more likely to develop POI. At 12 months after chemotherapy, patients with a lower BMI in the GnRHα group had a lower incidence of POI. This may be because the ovarian function of patients with low BMI is more likely to be damaged by CTX, while in the treatment group, the same dose of GnRHα has a slower effect on patients with high BMI, and the effect is weaker in patients with high BMI than in patients with low BMI.

In the design and execution of the EGOFACt study, we tried our best to reduce the bias in chemotherapy regimen and CTX dose. There was basically no difference in regimen or dose between the two groups, so the analysis of these two factors was not repeated in this study. We also conducted correlation analysis of HR status with baseline AMH levels and AMH levels after chemotherapy, and the results showed no correlation between HR status and AMH level change ($P > 0.05$, the results were not shown).

5. Conclusions

There are few reports on the dynamic changes in ovarian reserve in premenopausal women before and after chemotherapy using AMH as the indicator. In addition, we analyzed the dynamic changes in AMH and its influencing factors from multiple dimensions, which is helpful to deepen the understanding of ovarian function damage and protection.

Taking AMH < 0.5 ng/mL as the quantitative index of POI can accurately reflect the damage and recovery of ovarian function during chemotherapy. Age, menarche age, BMI, reproductive history, pathological stage, baseline AMH level, and the use of GnRHα all have significant effects on the AMH level of premenopausal women with breast cancer [36]. Age, menarche age, baseline AMH level, and GnRHα application are the most important influencing factors for ovarian function injury and recovery after chemotherapy.

Credit author statement

Xiangyun Zong, Yang Yu: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – original draft; Wenhu Chen, Hongjian Yang, Xuan Chen: Resources, Data curation; Xiangyun Zong, Weiwei Zong: Formal analysis; xiangyun Zong, Yang Yu, Wenhu Chen, Hongjian Yang, Xuan Chen: Writing- Reviewing and Editing

Ethics, consent and permissions

The EGOFACt trial protocol and all amendments were approved by the ethics committee of Shanghai Sixth People's Hospital and the ethics committee of Zhejiang Cancer Hospital. Written informed consent was provided by all the patients.

Contributors

The study was designed by X Zong and Y Yu. Statistical analysis was performed by X Zong and W Zong. X Zong, Y Yu, and W Chen contributed equally to this study. The manuscript was reviewed by all the authors. X Zong and Y Yu had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding/support

This study was funded by the Science and Technology Commission of Shanghai Municipality Medical Program 15411966500 and Zhejiang Medical Association Cancer Research Program 2015ZYC-A07.

Role of the funder/sponsor

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data sharing

After publication, trial data will be made available on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.breast.2022.05.009>.

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