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CLINICAL TRIAL

Pretreatment anti-Mullerian hormone-based nomogram predicts menstruation status after chemotherapy for premenopausal women with hormone receptor-positive early breast cancer

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Abstract

Purpose Ovarian function is important for optimizing endocrine treatment in patients with hormone receptor-positive (HR+) early breast cancer (eBC). The aim of the study was to determine whether patients' pretreatment levels of anti-Mullerian hormone (AMH) were associated with menses status after chemotherapy and to build a predictive nomogram model for amenorrhea in women with HR+ eBC.

Methods Between August 2013 and December 2014, 120 premenopausal patients with HR+ eBC were included retrospectively. The associations among age, prechemotherapy levels of AMH, follicle-stimulating hormone (FSH),and estradiol (E2) and the 2-year postchemotherapy menses status were analyzed. We determined the cutoff values of hormone levels by using the biostatistical tool (Cutoff Finder). A novel nomogram was established to predict the 2-year amenorrhea status based on the logistic analysis. Concordance index (C-index) was used to validate the capacity.

Results One hundred nine women (90.8%) experienced amenorrhea after chemotherapy. AMH < 0.965 ng/ml predicted amenorrhea at 2 years (AUC 0.84, sensitivity 74% and specificity 81.8%), independent of age. The predictive nomogram based on age and pretreatment AMH and FSH levels was developed to predict the probability of 2-year postchemotherapy amenorrhea with a C-index of 0.88 (95% CI 0.84–0.91).

Conclusions In premenopausal patients with HR+ eBC, prechemotherapy AMH concentration was associated with the patient's 2-year amenorrhea status, independent of age. The nomogram model based on age and pretreatment AMH and FSH levels accurately predicted the 2-year amenorrhea status.

Keywords Amenorrhea · Anti-Mullerian hormone · Breast cancer · Chemotherapy · Nomogram

Introduction

Breast cancer is the most common cancer in women. Unlike in western countries, more than half of the patients with breast cancer in China are premenopausal [1]. Endocrine therapy is an essential part of the treatment plan for hormone receptor-positive (HR+) early breast cancer (eBC). Recently, the choice of adjuvant endocrine regimens in premenopausal women has developed rapidly, including tamoxifen, aromatase inhibitor (AI), and ovarian function suppression (OFS). The SOFT and TEXT trials have shown that adding OFS [including gonadotropin-releasing hormone agonist (GnRHa), bilateral oophorectomy, or bilateral ovarian irradiation] to endocrine therapy reduced the recurrence rate in premenopausal women at high risk [2, 3]. Patients at

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high risk of recurrence are advised to undergo chemotherapy and OFS in their treatment [3].

Chemotherapy results in irreversible destruction on ovarian function,presented as amenorrhea and high levels of follicle-stimulating hormone (FSH) [4]. Menstruation is often used to measure postchemotherapy ovarian function. Compared to normal menses or temporary amenorrhea (< 6–12 months) after chemotherapy, prolonged or permanent amenorrhea is related to a better prognosis for premenopausal HR+ eBC [5]. A previous study showed that regardless of whether patients were over 40 years old, the prognosis of patients with normal menses after chemotherapy was poorer than that of patients who became amenorrhoeic [6]. However, menstruation does not return predictively. There were cases of regained menses or even pregnancy with AI in patients in their late 40 s or 50 s after long-term amenorrhea [7]. FSH levels play a conflicting role in predicting menstruation return in former studies [4, 8, 9]. A more accurate predictor is needed to evaluate ovarian function after chemotherapy.

Anti-Mullerian hormone (AMH), which is synthesized by preantral ovarian follicles, is now the standard measurement of ovarian reserve [10]. Previous studies have reported that AMH could predict menses return in western patients [8, 9, 11–13]. However, data are lacking for Chinese women. Furthermore, no easily performed predictive model has been developed for amenorrhea after chemotherapy in patients with HR+ eBC [8]. Therefore, we carried out this study primarily to evaluate the association among pretreatment AMH, FSH, and estradiol (E2) levels with amenorrhea status two years postchemotherapy in Chinese women with HR+ eBC and to generate a predictive model for amenorrhea after chemotherapy. A nomogram is an easy-to-understand tool that provide a statistical predictive model in a visual form. Nomograms have been used in many malignancies for the prediction of survivals [14]. We assumed that the nomogram would be an easily applicable, predictive tool for the estimation of menses status after chemotherapy.

Patients and methods

Patients

We set up this study in February 2016. The data of 1531 consecutive patients with mastectomy or breast-conserving surgery treated in Sun Yat-sen University Cancer Center between August 2013 and December 2014 were retrospectively analyzed. The exclusion criteria included (i) postmenopausal women ($N = 631$); (ii) patients undergoing OFS ($N = 345$); (iii) patients who were unwilling to be enrolled or had an incomplete medical record ($N = 253$); (iv) women with negative estrogen receptor (ER) and progesterone receptor (PR) status ($N = 86$); (v) women with metastatic disease ($N = 42$); (vi) patients without chemotherapy ($N = 36$); and (vii) patients with special pathological types of breast cancer such as sarcoma and lymphoma, etc. ($N = 18$). In total, 120 premenopausal patients with HR (+) early stage breast cancer were included in this study. We divided patients above 50 years old or had irregular menses over last 12 months to the postmenopausal group. To test whether AMH or other endocrine measures could predict the regain of menses after chemotherapy, the enrollment included patients who received adjuvant chemotherapy and for whom GnRHa was excluded from endocrine therapy. The study was approved by the Institutional Review Boards at the Sun Yat-sen University Cancer Center. All enrolled women gave informed consent in writing.

Amenorrhea was defined as no menses for more than three consecutive months, irrespective of the resumption of menstruation afterwards [5, 15, 16]. Return of menses was defined as the resumption of menses (lasting at least three consecutive months) after amenorrhea.

Study procedures

Hormone analyses were analyzed prior to chemotherapy (pretreatment). FSH and E2 were also analyzed 1 and 2 years after the last cycle of chemotherapy. Because of the urgency in starting therapy, baseline blood specimens were drawn across the menstrual cycle. Follow-up blood specimens were also not scheduled with menstrual cycles due to the irregularity of menstrual cycles after chemotherapy.

All samples were assayed in duplicate using commercial kits. AMH was measured via chemiluminescent immunoassay (CLIA), which has a limit of detectability of 0.08 ng/ml (YHLO Biotech, Shenzhen, China). Intra-assay and interassay variations in AMH assays were < 6% and < 11%, respectively. FSH and E2 levels were measured by electrochemiluminescence immunoassay (ECLIA) using a Cobas 8000 Module 602 (Roche Diagnostics, Basel, Switzerland). For FSH and E2, the intra- and interassay variation was < 6%. The E2 level might not be exact around the marginal value of 18.35 pmol/l via immunoassays [17]. Because it was not a study concerning the sensitivity of the testing method, we assumed that immunoassays were sufficient. Values below the detection thresholds were given the threshold value in analyses.

The menstruation status of 120 patients included were followed every 3 months. Patients in the clinic needed to take a card home and bring it back on their next visit. The card was used to record the side effects of endocrine therapy, as well as the patients' menses cycles. If patients did not return in time, they received telephone follow-up until the menses returned, they received OFS, cancer recurrence, death, or until study withdrawal.

Statistical analysis

Analyses were performed using the software packages SPSS (version 23.0; SPSS Inc., Chicago, United States). The Wilcoxon rank sum test was used to assess univariate associations between the 2-year menses status and each of the following: age, pretreatment AMH, FSH and E2 levels, chemotherapy regimen, and tamoxifen treatment. The multivariate analysis included only those factors that were significant in the univariate analysis.

We performed the time-to-event analysis to assess the relationship of the above characterists and time to menses resumption. Time to menses resumption was measured from the end of chemotherapy to the first episode of returned menses.A case was censored at the last follow-up, if a patient began OFS, upon cancer relapse, upon death, or upon study withdrawl. Menses resumption was compared according to baseline characteristics.

A web-based system, R software engineered and designed by Budczies et al. (http://molpath.charite.de/cutoff/), was used to define the optimal cutoff value of pretreatment AMH, E2 and FSH levels [18]. The ROC curve, sensitivity, and specificity were also provided by the system. The steps of data processing were as follows: (1) the data is uploaded; (2) the biomarker and outcome were selected; (3) the method for cutoff determination was selected; (4) the inquiry was sent to the statistical engine R; and the optimal cutoff point was determined.

On the basis of the results of the logistic multivariable analysis, a nomogram was formulated to provide visualized probability prediction using R 3.5.0 (http://www.r-project.org) with the survival and rms package. The use of nomogram was convenient. A line was drawn vertically to determine the number of points received for every variable value. The sum of these points was located on the total points axis, and a line was then drawn downwards to the probabilities axis to determine the 2-year amenorrhea likelihood. An internal validation study was conducted using 1000 bootstrap samples of size 100 to determine final variables for the 2-year amenorrhea status. A calibration plot was constructed to determine whether the predicted and observed probabilities were in concordance.

Two-sided p value of less than 0.05 was considered statistically significant.

Results

A total of 120 premenopausal women with HR + eBC were recruited to this study between August 2013 and December 2014 in Sun Yat-sen University Cancer Center. The baseline clinical characteristics are listed in Table 1. The median age was 43 years old (range 24–49 years). One hundred two women (85%) had doxorubicin/epirubicin (A) and cyclophosphamide (C) in their chemotherapy regimens. Eighty-eight women (73.3%) were treated with taxanes (T). The most common chemotherapy regimens included AC-T ($N=36$, 30%), AC ($N=25$, 20.8%) and TAC ($N=21$, 17.5%). Ninety-one women (75.8%) had stage I-II disease. Seventy-four women (61.7%) received tamoxifen in their adjuvant endocrine therapy, while forty-six women (38.3%) received toremifene.

Table 1 Baseline characteristics

Characteristics	No. of patients (%)
Age (years, median)	43 (range 24–49)
<40 years	32 (26.7)
≥40 years	88 (73.3)
Histology	
Invasive ductal carcinoma	111 (92.5)
Others	9 (7.5)
Molecular features	
ER positive	118 (98.3)
PR positive	113 (94.2)
HER2 positive	41 (34.2)
Stage	
I/II	91 (75.8)
III	26 (21.7)
Unknown	3 (2.5)
Therapy	
AC-T[a]	36 (30)
AC	25 (20.8)
TAC	21 (17.5)
TC	13 (10.8)
TA	13 (10.8)
Others	12 (10)
Trastuzumab-containing therapy in HER2 positive ($N=41$)	18 (43.9)
Tamoxifen	74 (61.7)
Toremifene	46 (38.3)

The results are presented as the number of patients and proportions

T taxanes, *A* doxorubicin/epirubicin, *C* cyclophophamide

[a]Containing dose dense and regular schedule

The median follow-up was 1074 days (range 182–1502 days). One hundred nine patients (90.8%) experienced amenorrhea after chemotherapy. Amenorrhea occured after the first chemotherapy cycle in fifty-seven women (52.2%). All patients reported menses status at 1 year post chemotherapy, 82 women were amenorrhoeic, and 38 patients had ongoing menses. Two years after chemotherapy, except for 15 women (nine lost follow-ups, three had GnRHa, two relapsed and one was diagnosed with lung cancer), 55 women were still amenorrhoeic while the remaing 50 women had ongoing menses. The median time

of menses return was 334.5 days (range 61–1095 days) from the last cycle of chemotherapy. Baseline characteristics are presented in Table 2 for patietns grouped by their 2-year menses status (amenorrhea vs. ongoing menses).

We found that age (grouped by age < 40 years or age ≥ 40 years) was significantly associated with the 2-year menses status. Patients < 40 years old were more likely to have ongoing menses than patients over 40 years (age < 40 years and age ≥ 40 years: 70.0% vs. 29.1%, $p < 0.001$). However, the chemotherapy regimens (with or without C, with or without A, with or without T) were not associated with ongoing menses ($p = 0.112$, $p = 0.960$ and $p = 0.241$, respectively).Moreover, the usage of tamoxifen did not have significant impact on the menses status ($p = 0.626$).

The pretreatment AMH level of those who had menses at 1 and 2 year ($N = 50$) postchemotherapy was significantly higher than the pretreatment AMH level of those who were amenorrhoeic ($N = 55$, Fig. 1, 3.11 ± 0.38 ng/ml vs.

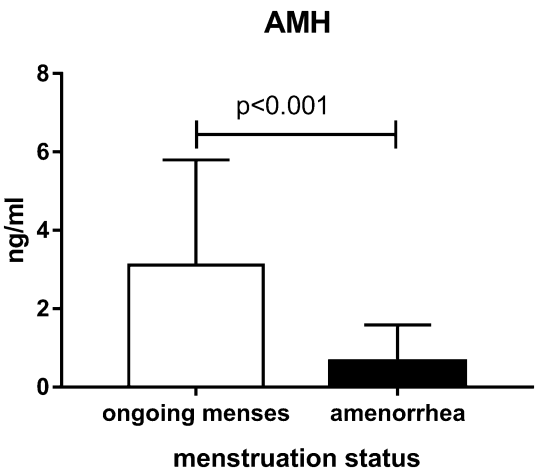

Fig. 1 AMH concentration at pretreatment in women with ongoing menses(white bars, $N = 50$) and those with amenorrhea at 2 years postchemotherapy (black bars, $N = 55$). Data are the mean ± SEM, $p < 0.05$ indicates a significant difference between these two group

Table 2 Baseline characteristics of patients stratified by 2-year menses status (ongoing menses vs. amenorrhea)

Characteristics	Ongoing menses ($N = 50$) No. of patients (%)	Amenorrhea ($N = 55$) No. of patients (%)	*p*
Age (years, median)	39 (range 24–49)	46 (range 28–49)	0.000
< 40 years	25 (50)	4 (7)	
≥ 40 years	25 (50)	51 (93)	
Histology			0.030
Invasive ductal carcinoma	49 (98)	48 (87)	
Others	1 (2)	7 (13)	
Molecular features			
ER positive	49 (98)	54 (98)	0.779
PR positive	48 (96)	52 (95)	0.530
HER2 positive	17 (34)	19 (35)	0.799
Stage			0.596
I/II	41 (82)	44 (78)	
III	7 (14)	11 (20)	
Unknown	2 (4)	1 (2)	
Therapy			0.071
AC-T[a]	15 (30)	18 (33)	
AC	13 (26)	10 (18)	
TAC	5 (10)	10 (18)	
TC	3 (6)	9 (18)	
TA	7 (14)	5 (9)	
Others	7 (14)	3 (5)	
Trastuzumab-containing therapy in HER2 positive	6 (35)	9 (47)	
Endocrine therapy			0.554
Tamoxifen	29 (58)	35 (64)	
Toremifene	21 (42)	20 (36)	

The results are presented as the number of patients and proportions

T taxanes, *A* Doxorubicin/Epirubicin, *C* cyclophophamide

[a]Containing dose dense and regular schedule

0.65 ± 0.13 ng/ml, $p < 0.001$). Younger women (< 40 years) showed significant higher AMH levels than those who were over 40 years old ($p < 0.001$). When the association among age and AMH level with menstruation status was further analyzed, we found that, for women who were over 40 years old, pretreatment AMH concentration was lower in women with subsequent amenorrhea at 2 years than in women with ongoing menses ($p < 0.001$). As there were only 4 women with amenorrhea at 2 years postchemotherapy, AMH levels did not demonstrate statistical significance between those with menses or amenorrhea in the subgroup below 40 years old ($p = 0.109$).

Compared to women with ongoing menses ($N = 47$), pretreatment FSH concentration was higher in women who experienced amenorrhea ($N = 53$) at 2 year (Fig. 2a. 6.11 ± 0.63 mIU/ml vs. 12.16 ± 11.68 mIU/ml, $p = 0.001$). However, when we analyzed the 1-year data, we did not find such a correlation (data not shown, $p = 0.593$). Some women had a series of FSH examination at 1 and 2 year postchemotherapy, and we found that the dynamic FSH concentration was significantly correlated with the patients' menstruation status. At 1 year, the FSH level of women with menses was higher than that of women were amenorrhoeic ($p < 0.001$). The same difference was found at the 2-year follow-up (menses vs. amenorrhea = 21 vs. 41, Fig. 2b. 16.78 ± 2.50 mIU/ml vs. 31.40 ± 2.52 mIU/ml, $p < 0.001$). Pretreatment E2 levels did not show any correlation with menses at 1 year ($p = 0.405$) or 2 year (menses vs. amenorrhea = 47 vs. 53, Fig. 2c, 533.18 ± 72.55 ng/ml vs. 459.44 ± 65.03 ng/ml, $p = 0.449$). Similar to the series of FSH levels, the E2 levels were in line with the menstruation status at 1 year ($p = 0.032$) and 2 years (menses vs. amenorrhea = 21 versus. 41, Fig. 2d, 919.13 ± 263.00 ng/ml vs. 23.15 ± 1.84 ng/ml, $p < 0.001$).

The optimal cutoff value of 0.965 ng/ml for pretreatment AMH concentration was determined for assessing 2 years menses status, with an AUC of 0.84, sensitivity of 74% and specificity of 81.8%. The optimal cutoff value of 6.995 mIU/ml of pretreatment FSH concentration was determined for assessing 2 years menses status, with an AUC of 0.69, sensitivity of 60.4% and specificity of 74.5%. The optimal cutoff value of 431.3 pmol/l for pretreatment E2 concentration was determined for assessing 2 years menses status, with AUC of 0.55, sensitivity of 51.4% and specificity of 35.2%. Age 40 years was chosen as the most widely used cutoff when discussing amenorrhea. These cutoff values were used in the following analysis.

In time-to-event analysis, younger age, higher AMH levels, and lower FSH levels were associated with shorter time until menses resumption (Table 3). When included in the multivariable model, AMH and FSH remained significantly associated with time of menses resumption (Table 4).

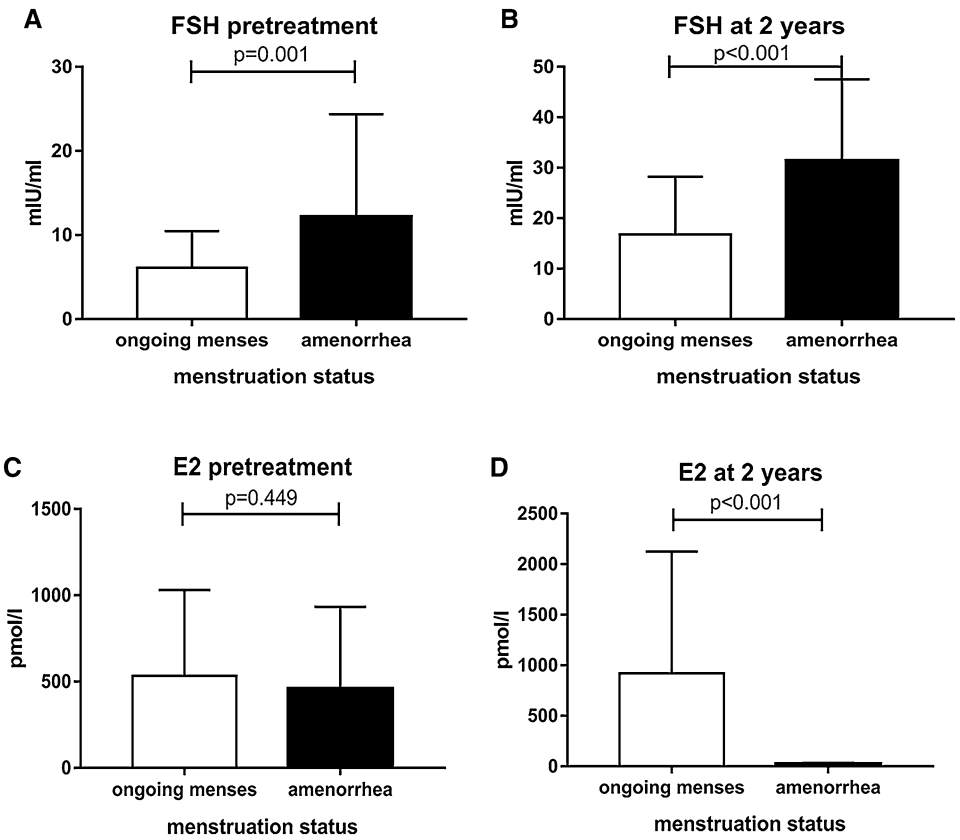

Fig. 2 Hormone concentrations (FSH and E2) at pretreatment and 2 years postchemotherapy in women with ongoing menses (white bars, pretreatment $N = 47$, 2-year $N = 21$) and those with amenorrhea at 2 years postchemotherapy (black bars, pretreatment $N = 53$, 2-year $N = 41$). Data are presented as the mean ± SEM, $p < 0.05$ indicates a significant difference between these two group. **a** E2 concentration measured at pretreatment; **b** E2 concentration measured at 2 years postchemotherapy; **c** FSH concentration measured at pretreatment; **d** FSH concentration measured at 2 years postchemotherapy

Table 3 Univariate analysis of prognostic factors related to menses rsumption by 2 years postchemotherapy, $N=95$

Variable	HR (95% CI)[a]	*p*
Age		
< 40 years	1.00 (Reference)	
≥ 40 years	0.283 (0.147–0.546)	0.000
AMH (ng/ml)		
< 0.965	1.00 (Reference)	
≥ 0.965	4.773 (2.435–9.357)	0.000
E2 (pmol/l)[b]		
< 431.3	1.00 (Reference)	
≥ 431.3	1.443 (0.757–2.750)	0.265
FSH (mIU/ml)[c]		
< 6.995	1.00 (Reference)	
≥ 6.995	0.260 (0.119–0.570)	0.001
C		
No	1.00 (Reference)	
Yes	1.240 (0.520–2.961)	0.628
A		
No	1.00 (Reference)	
Yes	0.288 (0.069–1.196)	0.087
T		
No	1.00 (Reference)	
Yes	1.248 (0.621–2.508)	0.533
Endocrine therapy		
Toremifene	1.00 (Reference)	
Tamoxifen	0.689 (0.367–1.293)	0.246

AMH anti-Mullerian hormone, *FSH* follicle-stimulating hormone, *HR* hazard ratio, *CI* confidence interval, *C* cyclophosphamide, *T* taxanes, *A* Doxorubicin/Epirubicin

[a]COX regression analysis was used to calculate HR and 95% CI values

[b]$N=91$ participants

[c]$N=91$ participants

Logistic regression was used to investigate which variables have independent predictors of 2-year menses status. Age and AMH remained significant predictors of amenorrhea at 2 years ($p=0.048$ and $p=0.012$ respectively, Table 5). These factors were selected into the multivariate model. FSH was also included due to significance near the decision boundary ($p=0.057$).

Nomograms that incorporated the predictive factors (age and pretreatment AMH and FSH levels) were established (Fig. 3). The nomograms demonstrated that FSH and AMH shared the largest contribution to 2-year amenorrhea, followed by age. Each of the variables was assigned a score on the point scale. By adding up the score of each variable and referring to the total point scale, we constructed a straight line to determine the estimated probability of 2-year amenorrhea.

Table 4 Multivariable analysis of prognostic factors related to menses rsumption by 2 years postchemotherapy, $N=95$

Variable	HR (95% CI)[a]	*p*
AMH (ng/ml)		
< 0.965	1.00 (Reference)	
≥ 0.965	4.029 (2.034–7.979)	0.000
FSH (mIU/ml)[b]		
< 6.995	1.00 (Reference)	
≥ 6.995	0.343 (0.161–0.731)	0.006
Age		
< 40 years	1.00 (Reference)	
≥ 40 years	0.577 (0.277–1.204)	0.143

AMH anti-Mullerian hormone, *FSH* follicle-stimulating hormone, *HR* hazard ratio, *CI* confidence interval

[a]COX regression analysis was used to calculate HR and 95% CI values

[b]$N=91$ participants

The calibration plots showed good statistical performance upon internal validation between the nomogram prediction and actual observation for the probability of 2-year amenorrhea (Fig. 4). Harrell's concordance index (C-index) for the established nomogram to predict 2-year amenorrhea was 0.88 (95% CI 0.84–0.91).

Discussion

Our study showed that prechemotherapy AMH had a significant correlation with 2-year menstruation status postchemotherapy. The ROC curve showed a cutoff value of AMH < 0.965 ng/ml for assessing 2-year amenorrhea, with an AUC of 0.84, sensitivity of 74% and specificity of 81.8%. Moreover, we built a predictive nomogram model with a C-index 0.88. To our knowledge, this is the first nomogram to predict postchemotherapy amenorrhea in HR + eBC. These data support the use of a prechemotherapy AMH-based model to prospectively discuss future ovarian function with premenopausal women with HR + eBC.

In our study, the majority of women (90.8%) experienced secondary amenorrhea postchemotherapy. When grouped by age (< 40 years and ≥ 40 years), the proportion of menses that had returned was significantly different in these subgroup (< 40 years and ≥ 40 years: 70.0% vs .29.1%, $p<0.001$). These percentages were in line with our previous study and others [5, 6]. However, the absolute age above which doctors can be certain that a woman will not have residual ovarian function has not been established [19–21]. There was concern that a proportion of women who were believed to have ovarian failure following chemotherapy might recover ovarian function [7]. Therefore, age should

Table 5 Univariate and multivariate analyses for 2-year amenorrhea status

Parameters	Univariate analysis	Multivariate analysis	
		OR (95% CI)	*p*
Age, years (< 40 vs. ≥ 40)	< 0.0001	1.15 (1.00, 1.31)	0.048
AMH, ng/ml (< 0.965 vs. ≥ 0.965)	< 0.0001	0.45 (0.30, 0.67)	0.012
FSH, mIU/ml (≥ 6.995 vs. < 6.995)	0.001		0.057
E2, pmol/l (≥ 431.3 vs. < 431.3)	0.450		
C	0.112		
A	0.960		
T	0.241		
Tamoxifen	0.626		

AMH anti-Mullerian hormone, *FSH* follicle-stimulating hormone, *E2* estradiol, *C* cyclophosphamide, *A* doxorubicin/epirubicin, *T* taxanes, *OR* odds ratio, *CI* confidence interval

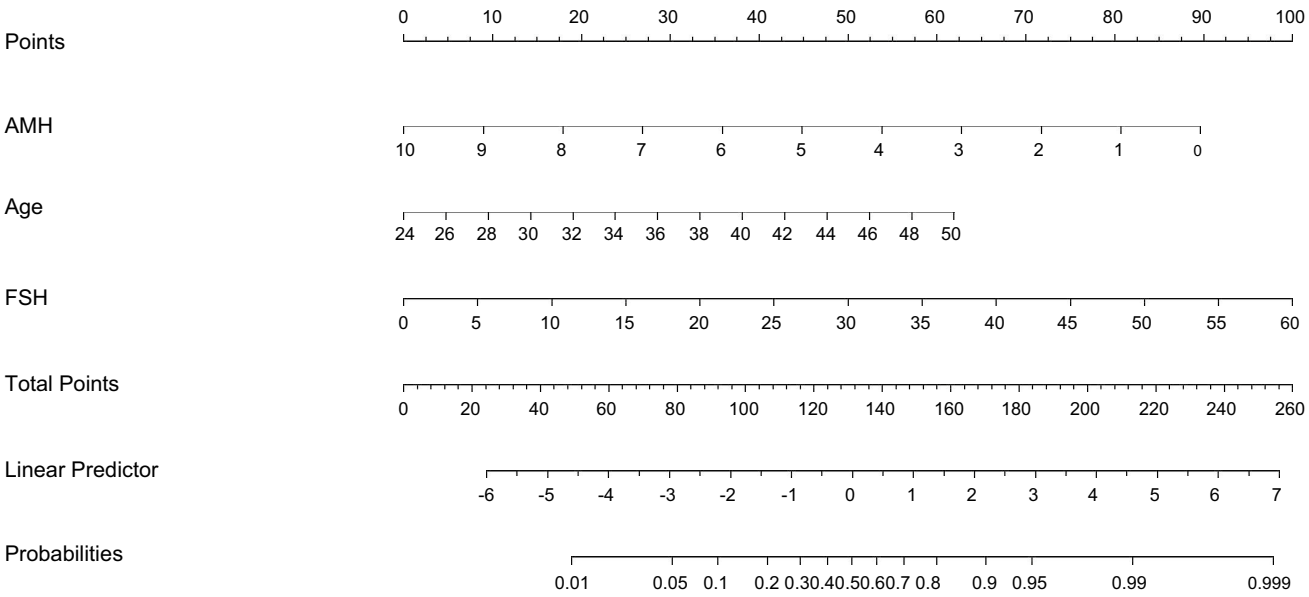

Fig. 3 Nomogram for 2-year amenorrhea prediction. A score for each predictor can be visualized at the top scale (score). All summed scores can be converted directly to the probability of 2-year amenorrhoeic status. *FSH* prechemotherapy level of serum FSH, *AMH* prechemotherapy level of serum AMH

be considered a relative but not absolute predictor of menses recovery.

Several studies have shown that pretreatment AMH and other markers of the ovarian reserve can predict postchemotherapy ovarian failure. The majority of these studies analyzed the correlation between pretreatment AMH levels and 1- or 2-year menses status. Yu et al. first reported that AMH decreased rapidly after chemotherapy and remained suppressed for 52 weeks [13]. Anderson et al. showed that pretreatment AMH could predict long-term ovarian function after chemotherapy. Higher AMH also predicted the greater bone mineral density loss at 5-year [11]. Anderson et al. examined the relationship among the pretreatment hormone levels (AMH, FSH, and E2) of 59 premenopausal women with eBC and their menstruation status. Those who were amenorrhea at 2 years had lower pretreatment AMH levels than those regained menses (4.0 pmol/l vs. 17.2 pmol/l, $p < 0.001$), whereas FSH and inhibin B levels were similar in these subpopulations [9]. Su et al. reported that higher AMH and lower FSH levels were associated with a shorter time to ovarian recovery [8]. Our findings were consistent with previously published data. Our cutoff value of AMH < 0.965 ng/ml was different from the cutoff values used in previous studies [8, 9, 11]. Due to the diversity of enrolled population, the cutoff value of predictive pretreatment AMH levels had not reach a consensus. We considered that a predictive model comprising several factors may be more correct and more reasonable than a single predictor.

As AMH was rapidly under detection postchemotherapy but was slightly elevated at 52 weeks, postchemotherapy

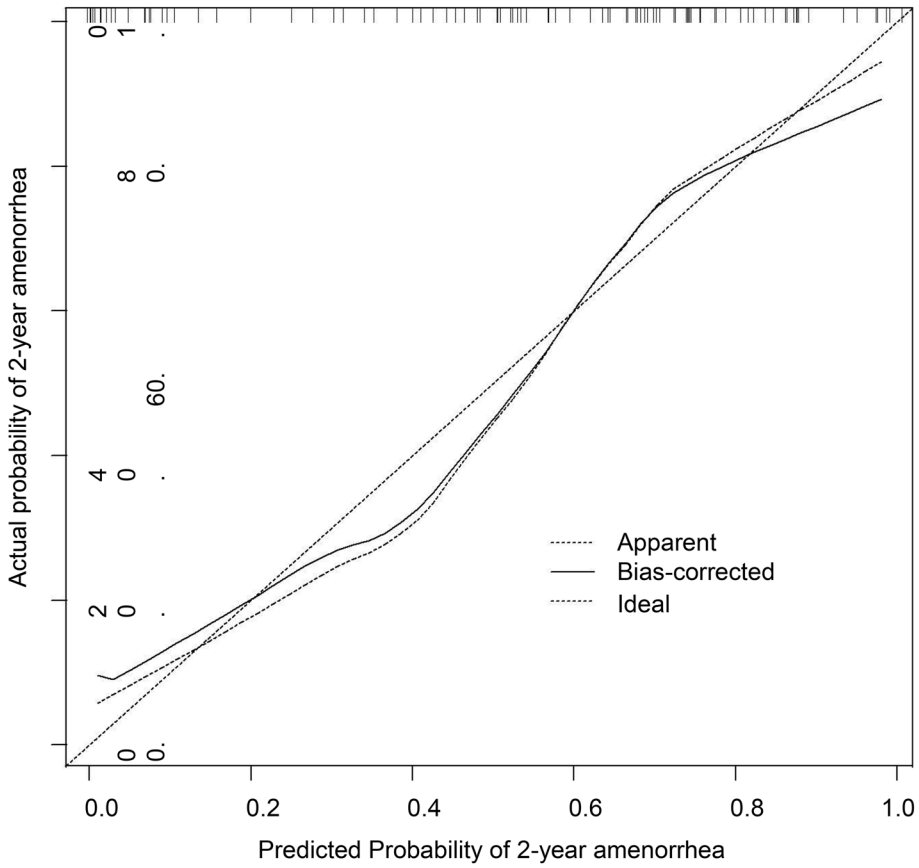

Fig. 4 Calibration plot of the predicted and observed probabilities of 2-year amenorrhea. The prediction calculated using the nomograms are plotted on the *X*-axis, and the observed rate of amenorrhea is plotted on the *Y*-axis

AMH assessment does not appear to be useful for endocrine therapy decision making [13, 22]. Recently, Anderson et al. reported a more sensitive method to detect postchemotherapy AMH concentration. Using this sensitive AMH assay, an undetectable AMH level in women aged > 40 years at the end of chemotherapy predicted that ovarian function would not return [4]. We did not examine AMH levels after chemotherapy in our study. The classic AMH assays were good enough for prediction, especially in a nomogram model with other factors.

The predictive power of FSH concentreation is also uncertain. Previous studies showed that FSH could not predict postchemotherapy ovarian function [4, 9], while Su et al. reported that FSH < 10 IU/l was associated with a shorter time to ovarian recovery [8]. The result that pretreatment FSH is a statistically significant predictor of only the 2-year menses status (not at 1 year) is interesting. It is possible that FSH is more informative about the later menstruation status. Alternatively, it is possible that AMH is more accurate than FSH to predict short-term and long-term ovarian function. With replication, this is potential advantage of AMH over FSH. However, FSH shared the largest contribution in our predictive nomogram model. This result suggested that when combined with other factors, FSH could better predict ovarian recovery than it could alone.

There might be questions as to few patients who are young or who have stage III in our study received OFS in their adjuvant endocrine therapy. First, patients who received OFS upfront were excluded from our study (Patients and Methods). In fact, because of the results of the SOFT and TEXT studies, most patients < 40 years or with stage III in our daily clinical management would have OFS in their adjuvant treatment plan. Next, we found that in patients with stage III disease ($N = 26$), 23 of them received dose dense AC-T or TAC during their adjuvant chemotherapy, and 19 of them were over 40 years old. Maybe their doctors considered that they would become amenorrhea after treatment due to their age. For women < 40 years old with stage I/II eBC ($N = 27$), except for two who were lost to follow-up, only two of them were amenorrheoic at the 2-year postchemotherapy period. This finding suggests that OFS might be important in this kind of young patients.

There were some limitations in our study, including a small sample size, lack of series AMH detection, and hormonal levels that were not measured in the early follicular phase of menstrual cycles. Moreover, tamoxifen or toremifene might influence patients' menses status. However, in the univariate analysis, tamoxifen did not have significant impact. Imaging methods such as transvaginal ultrasound were not performed. External validation was not performed due to the

lack of an external cohort. Finally, this study was limited by its relatively short follow-up period. A long-term follow-up, for example 4–5 years, might result in more details of ovarian function after chemotherapy.

Conclusions

In summary, prechemotherapy AMH concentration was associated with the 2-year amenorrea status, independent of age. The novel nomogram based on age and pretreatment AMH and FSH levels for predicting amenorrhea postchemotherapy in premenopausal patients with HR +eBC might promote personalized treatment approaches in the future. Prospective validation of this hypothesis is warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent It was obtained from all individual participants included in the study.

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