European Review for Medical and Pharmacological Sciences 2022; 26: 3261-3268

Analysis and prediction of risk factors of ovarian hyperstimulation caused by Long-acting GnRH agonist protocol in follicular phase

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Abstract. – OBJECTIVE: The aim of the study was to explore the risk factors of ovarian hyperstimulation in patients undergoing long-acting gonadotropin-releasing hormone (GnRH) agonist protocol in follicular phase of ovulation induction therapy and to establish a predictive model.

PATIENTS AND METHODS: A total of 1289 patients who received Long-acting GnRH agonist protocol in follicular phase for ovulation induction in the Fujian Provincial Maternity and Child Health Hospital from July 1, 2018, to July 31, 2019, were selected. Among them, 33 patients developed moderate/severe ovarian hyperstimulation syndrome. The relevant indicators of the two groups were followed up for comparison, and Lasso regression was used to screen independent risk factors and construct a nomogram prediction model. A receiver operating characteristic (ROC) curve and calibration curve were used to evaluate the discrimination and calibration of the prediction model.

RESULTS: Univariate analysis suggested that the woman's age, basal antral follicle number (AFC), total gonadotropin (Gn) dose, Gn starting dose, basal estradiol (E2) level, basal anti-Müllerian hormone (AMH) value, number of follicles obtained, Gn start day E2, the difference in follicle-stimulating hormone (FSH) value and Gn starting day were statistically significant. Significant indicators of univariate analysis and clinical significance were included in the Lasso regression model, and AFC, woman's age, polycystic ovary syndrome, Gn starting dose and number of follicles obtained were finally screened as final predictors. The ROC curve indicated that the area under the curve (AUC) was 0.812.

CONCLUSIONS: Ovarian hyperstimulation caused by long-acting GnRH agonist protocol in follicular phase for ovulation stimulation has a certain predictability. Paying attention to the patient's age, AFC, Gn starting dose, number of follicles obtained, and whether PCOS is evident may lead to early detection of ovarian hyperstimulation syndrome, which has clinical guiding significance.

Key Words:
Long-acting GnRH agonist protocol in follicular phase, Ovarian hyperstimulation, Predictive model.

Introduction

The popularization and promotion of assisted reproductive technology allowed increasing numbers of infertile couples to have children, but a series of complications, associated with the assisted reproduction process require clinical attention. Among them, the occurrence of ovarian hyperstimulation syndrome (OHSS) has a greater impact on mothers and children[1]. Ovarian hyperstimulation syndrome has been fully studied with the development of assisted reproductive technology, and its incidence varies greatly in different literature reports. The incidence of moderate ovarian hyperstimulation syndrome fluctuates between 8%-23%, and the incidence of severe ovarian hyperstimulation is between 0.8%-10%[2,3]. In recent years, replacing the human chorionic gonadotropin trigger with a gonadotropin-releasing hormone (GnRH) agonist trigger has significantly reduced the incidence of OHSS, but it has not eliminated it completely[4]. Long-acting GnRH agonist protocol in follicular phase for ovulation stimulation has the advantages of improving hormone levels, as well as pelvic and endometrial environment. It is widely used in patients with endometriosis and adenomyosis in the early stage and has become one of the main clinical superovulation protocols. However,

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the overstimulation of the ovaries should not be underestimated[5]. This article uses a retrospective cohort to study the outcome of ovulation induction in patients, treated with the long-acting GnRH agonist protocol in follicular phase, explores the risk factors of OHSS, and constructs a predictive model in order to provide a certain reference for clinical work.

Patients and Methods

Female patients who received a reproductive assistance treatment (long-acting GnRH agonist in follicular phase) at the Reproductive Center of Fujian Maternity and Child Health Hospital from July 1, 2018, to July 30, 2019, were included. The Ethics Committee of our hospital approved this study (No: 2022KYLLR03020, Date: 2022-03-10). Follow up records of the outcome of ovulation induction were retrospectively analyzed. Inclusion criteria were as follows: 1) age 20-40 years old; 2) causes of infertility are fallopian tube factors, male factors, ovulation disorders, or unexplained infertility, etc. Exclusion criteria were as follows: infertility accompanied by systemic diseases that are not suitable for assisted reproduction, such as poorly controlled hypertension, Cushing syndrome and other diseases.

The following protocol of the GnRH-a long-acting long-term program during the follicular phase was used: on days 2-5 of the menstrual cycle, a long-acting gonadotropin-releasing hormone agonist (GnRH-a, Dafeilin, France Beaufort-Ipsen Pharmaceutical and Biological Company) was administered by intramuscular injection. After reaching the pituitary hyporegulation standard, the recombinant human Follicle Stimulating Hormone Alpha (Gnnal-F, Gonafen, Merck Serono, Germany) was started at the baseline dose of 100-300 IU. The size of the follicle was monitored by B-ultrasound, and the serum hormone levels were measured at the same time. The dosage of Gn was adjusted according to the growth of the follicle until ovulation induction. The follicles were harvested 36 hours after subcutaneous injection of hCG6000-10000IU on the induction day. The number of retrieved follicles ≤ 5 was defined as low response, the number of retrieved follicles greater than 5 and ≤ 18 was defined as a normal response, and the number of retrieved follicles greater than 18 was defined as a high response[6].

We used 2016 American Society of Reproductive Medicine (RCOG) guidelines as the diagnostic criteria. Moderate OHSS was diagnosed in cases of moderate abdominal pain, nausea with or without vomiting, ascites on ultrasound, and ovarian size usually in the range of 8-12 cm. Severe OHSS was diagnosed in cases of clinical ascites (with or without pleural effusion), oliguria (<300 ml/day or <30 ml/hour), HCT>0.45, hyponatremia (blood sodium <135 mmol/L), low osmolality (osmotic pressure <282 mOsm/kg), hyperkalemia (serum potassium >5 mmol/L), hypoalbuminemia (serum albumin<35 g/L), ovarian size often>12 cm (one of the above).

R3.6.0 software was used for data analysis and graphing. Continuous variables were expressed as mean ± standard deviation, and normal distribution test was performed. *t*-test was used for continuous variables with normal distribution and homogeneous variance. For continuous variables with non-normal distribution or uneven variance, the rank sum test was used. The enumeration data was expressed by the number of cases (percentage), and the chi-square test or Fisher's exact test was used. $p<0.05$ indicated that the difference was statistically significant. The meaningful data and clinically significant data of univariate analysis were included in the lasso regression to screen variables, establish a nomogram prediction model, and use ROC curve and calibration curve to evaluate the model discrimination and calibration, respectively.

Results

Baseline Data

According to the inclusion and exclusion criteria, a total of 1,299 patients with infertility were selected for the study. Among them, 10 patients gave up assisted reproduction due to personal reasons, and a total of 1,289 cases with complete follow-up data were included. Of them, 33 cases (2.56%) had moderate (15/33) or severe (18/33) ovarian hyperstimulation. The overall patient age was 31.31±3.94 years old, and the number of follicles obtained was 12.78±6.18. There were 60 cases (5%) of polycystic ovary syndrome, 278 cases (22%) of endometriosis, and 89 cases (7%) of adenomyosis in all patients.

The Comparison Between the Two Groups

As shown in Table I, univariate analysis suggests that there is no statistical significance between the occurrence of moderate to severe

Table I. Comparison of general data, hormone levels and number of follicles between the two groups if moderate to severe OHSS occurs.

	Without OHSS/Mild OHSS (n = 1256)	Moderate to severe OHSS (n = 33)	T/Z	*p*-value
Female age	31.35 ± 3.96	29.85 ± 2.54	3.792	< 0.001
Male age	33.24 ± 4.65	31 ± 2.76	5.106	< 0.001
BMI (kg/m^2)	21.34 ± 2.8	20.73 ± 2.5	1.758	0.088
Years of infertility (years)	3.68 ± 2.54	3.61 ± 2.4	0.273	0.787
Gn Starting dose (IU)	168.09 ± 48.66	141.67 ± 40.46	28109	< 0.001#
Gn total dose (IU)	2795.04 ± 939.59	2250.38 ± 860.09	28769.5	< 0.001#
Basal FSH (IU/L)	5.96 ± 1.6	5.45 ± 1.14	2.757	0.009
Basal LH (IU/L)	3.68 ± 1.76	4.37 ± 2.95	-1.419	0.166
Basal FSH/LH	2.04 ± 2.08	3.49 ± 11.19	24791.5	0.05
Basal E2	46.26 ± 115.5	40.58 ± 45.09	26108.5	0.011#
AMH (ng/ml)	3.96 ± 2.47	6.29 ± 4.25	11872.5	< 0.001#
Gn first day FSH (IU/L)	3.29 ± 16.84	2.76 ± 2.03	20164.5	0.501#
Gn first day LH (IU/L)	0.49 ± 0.49	0.45 ± 0.36	21182.5	0.731#
Gn first day FSH/LH	9.55 ± 56.4	7.11 ± 5.36	1.291	0.198
Gn figure day E2	14.89 ± 18.22	18.42 ± 21.51	19208.5	0.526#
HCG before use FSH (IU/L)	13.08 ± 4.53	10.44 ± 3.83	27918.5	< 0.001#
HCG before use LH (IU/L)	1.14 ± 1.03	1 ± 0.69	21570	0.658#
HCG before use FSH/LH	20.01 ± 21.52	16.94 ± 15.22	23209	0.212#
HCG before use E2	3219.33 ± 1968.12	4923.52 ± 2323.92	10965.5	< 0.001#
Number of basal antral follicles (pcs)	14.72 ± 5.89	21.16 ± 9.78	-3.724	< 0.001
Number of eggs captured (a)	12.62 ± 6.07	18.76 ± 7.31	-4.858	< 0.001

#Mann-Whitney U test.

OHSS and the number of years of infertility, body mass index, basic LH, basic FSH/LH, Gn first day FSH, Gn first day LH, Gn first day FSH/LH, Gn first day E2, LH before HCG use, and FSH/LH difference before HCG use (p>0.05). Additionally, woman's age, initial Gn dose, total Gn dose, basic FSH, basic FSH/LH, basic E2, AMH, FSH before HCG use, E2 before HCG use, the number of antral follicles, polycystic ovary syndrome diagnosis, and the number of follicles obtained were all significantly associated with moderate or severe OHSS (p<0.05; Table I and Table II). There were no significant differences in FSH/LH on the first day of Gn, E2 on the first day of Gn, LH before HCG and FSH/LH before HCG (p>0.05). Similarly, as shown in Table II, the type of infertility (primary/secondary infertility), tubal infertility, infertility combined with adenomyosis or endometriosis and diagnosed uterine adhesions were not significantly associated with OHSS (p>0.05).

Screening of Predictor Variables

The identified 13 variables with 2 indicators that may have clinical significance (BMI, infertility years) were further analyzed using LASSO regression, where the total Gn is converted into a binary variable based on whether it is greater than 2400 units. Taking the logλ of the maximum distance of 1 standard error as the screening index (as shown in Figure 1 and Figure 2), a total of 5 predictors (number of basal antral follicles, age of the woman, PCOS diagnosis, total Gn dose and the number of follicles obtained) were screened.

Predictive Model Construction and Visualization

The coefficients and OR values of the final prediction model are shown in Table III. Multivariate analyses revealed independent risk factors for OHSS to be the polycystic ovary syndrome, high level of basal antral follicles and high level of follicles during oocyte retrieval follicles (p<0.05). The nomogram is constructed by R 3.6.1 software, and the total point is calculated to predict the occurrence of moderate to severe OHSS risk.

The Internal Verification of the Model

To test whether the occurrence of moderate to severe OHSS can be separately predicted by the number of basic antral follicles, the number of follicles obtained and the composite index (prediction model), we used ROC curve and calibration curve to evaluate the model discrimination and

Table II. Comparison of comorbidities, Gn dosage and ovarian reactivity between the two groups with moderate to severe OHSS.

	Without OHSS/Mild OHSS (n = 1256)	Moderate to severe OHSS (n = 33)	T/X2/Z	*p*-value
Type of infertility			0.261	0.609
Primary infertility	609 (48)	18 (55)		
Secondary infertility	647 (52)	15 (45)		
Tubal infertility			1.312	0.252
No	653 (52)	21 (64)		
Yes	603 (48)	12 (36)		
Adenomyosis			Fisher	0.493
No	1170 (93)	30 (91)		
Yes	86 (7)	3 (9)		
Endometriosis			1.259	0.262
No	982 (78)	29 (88)		
Yes	274 (22)	4 (12)		
Intrauterine adhesions			Fisher	0.398
No	1197 (95)	33 (100)		
Yes	59 (5)	0 (0)		
Polycystic Ovary Syndrome			Fisher	< 0.001
No	1205 (96)	24 (73)		
Yes	51 (4)	9 (27)		
Gn total dose			6.398	0.011
> 2400	864 (69)	30 (91)		
≥ 2400	392 (31)	3 (9)		
Ovarian reactivity			Fisher	< 0.001
Low response	164 (13)	0 (0)		
Normal reaction	874 (70)	17 (52)		
High response	218 (17)	16 (48)		

calibration, respectively. As shown in Figure 3, the prediction model had the best discrimination with an AUC of 0.81 that is significantly higher than the other two independent risk factors. The calibration curve indicates that the model has a moderate degree of calibration (Figure 4).

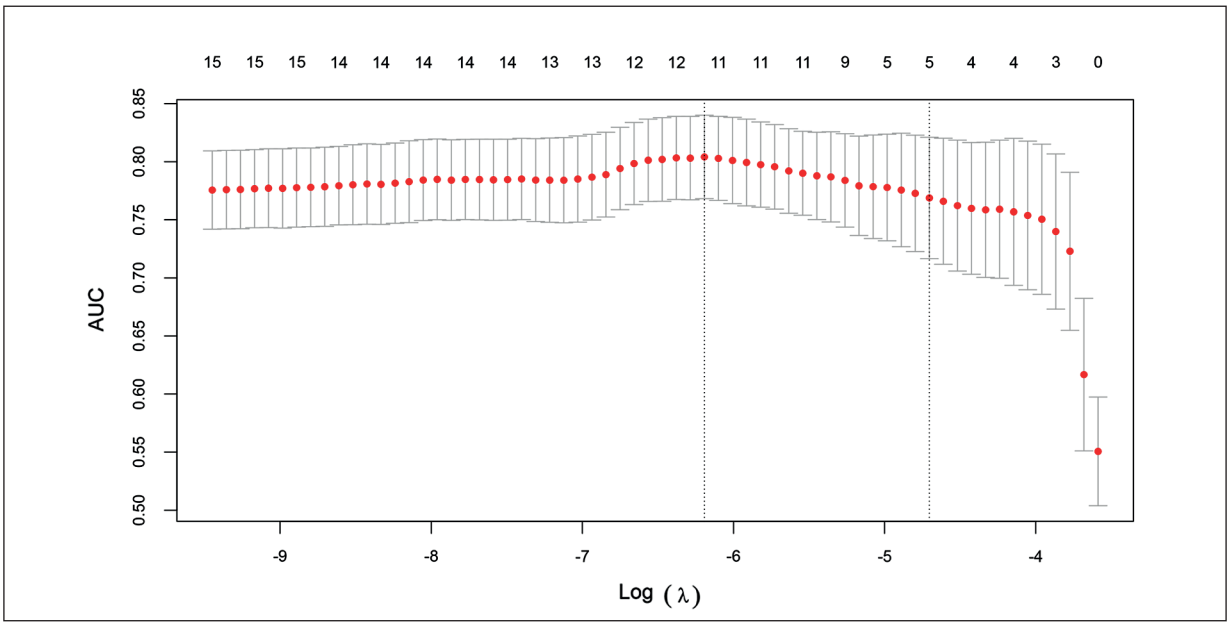

Figure 1. LASSO regression screening variables.

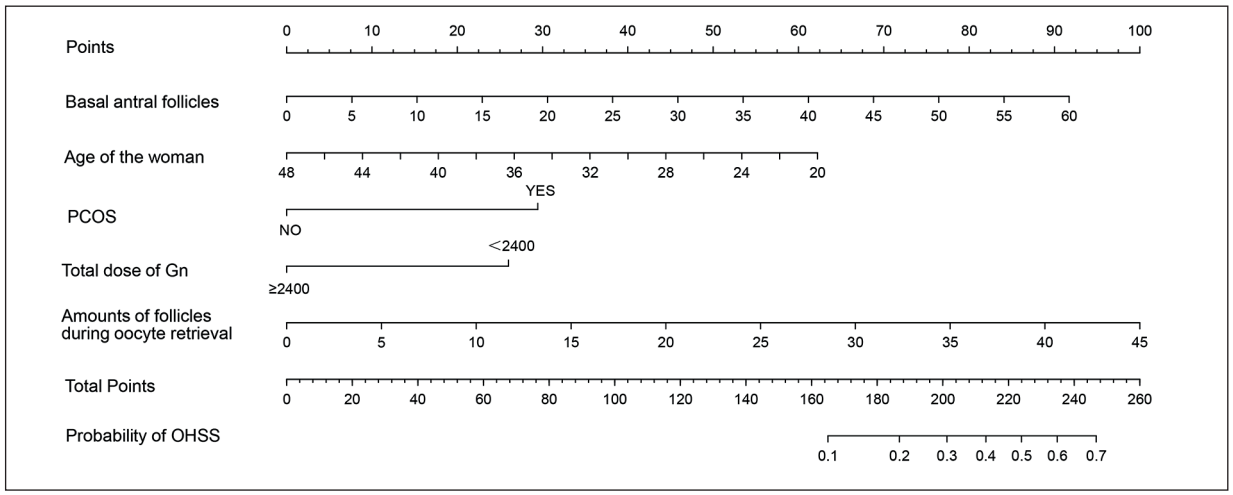

Figure 2. Moderate to severe OHSS prediction nomogram.

Discussion

OHSS is a syndrome of a series of pathophysiological changes that arise from administration of exogenous Gn in the process of assisted reproduction. The mechanism of OHSS is complicated, and timely diagnosis, as well as effective and specific prevention and treatment measures, have important clinical significance. Although the OHSS prediction model has been established in previous studies[7,8], recent years saw an increase in the implementation of the Long-acting GnRH agonist protocol in follicular phase for stimulated ovulation. Therefore, timely prediction of OHSS in patients, treated with this protocol has become a new problem. In this study of a retrospective cohort, we show that the occurrence of OHSS in patients with Long-acting GnRH agonist protocol in follicular phase for ovulation induction can also be predicted using routine testing items. We show that the number of basal antral follicles, the number of follicles obtained, and accompanied PCOS diagnosis are independent risk factors of OHSS.

The endocrine state of PCOS patients is very complicated. Small- and medium-sized follicles in the ovaries may respond poorly to exogenous Gn and may overreact[9]. Some studies[10] have suggested that PCOS is a high-risk factor for OHSS, and even polycystic ovarian changes (PCOM) that are not enough to diagnose PCOS may increase the risk of OHSS. The mechanism of PCOS causing OHSS may be related to insulin resistance, and the use of metformin can reduce the occurrence of OHSS[11]. Our results, therefore, are consistent with previous reports that PCOS is a predictor of OHSS.

The number of basic antral follicles (the number of bilateral ovarian antral follicles, AFC) is an important basis for ultrasound evaluation of ovarian reserve[12]. It has a predictive effect on ovarian responsiveness and on OHSS after ovulation induction[8]. Some studies[13,14] suggest that the number of unilateral follicles > 9 significantly increases the incidence of OHSS, but the results should be interpreted with caution because the number of basal antral follicles fluctuates greatly in different menstrual cycles. Similarly to previ-

Table III. Relevant parameters of the final model.

	β value	OR [95% confidence interval]	Statistics	*p*-value
(Intercept)	-2.432	0.09 [0.00, 3.29]	-1.316	0.188
Number of basal antral follicles (pcs)	0.057	1.06 [1.00, 1.12]	2.014	0.044
Female age	-0.083	0.92 [0.83, 1.02]	-1.549	0.121
PCOS	1.096	2.99 [1.00, 8.27]	2.047	0.041
Gn total dose (≥ 2400)	-0.969	0.38 [0.09, 1.11]	-1.557	0.12
Number of eggs captured	0.083	1.09 [1.03, 1.14]	3.338	0.001

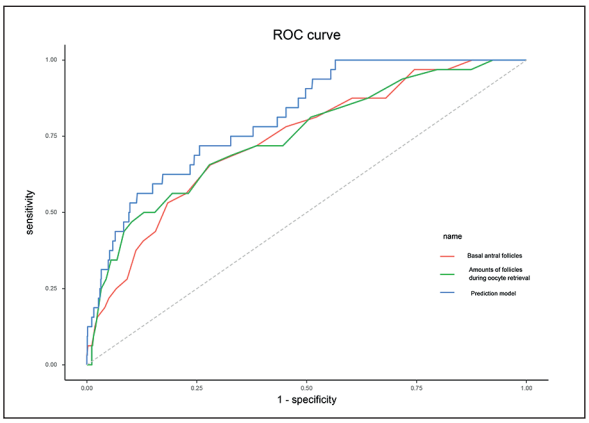

Figure 3. ROC curve of basic antral follicle number, number of follicles harvested and prediction model.

ous scholars[7], this study found that the number of basal antral follicles is positively correlated with ovarian responsiveness. Therefore, the number of basal antral follicles was included as a continuous variable in the prediction model. The ROC curve also suggested that simply using the number of basal antral follicles has certain predictive power, but it is weaker than the compound forecasting model.

Exogenous Gn stimulates proliferation, growth, and maturation of multiple follicles at the same time. The total amount and time of exogenous Gn used may be related to OHSS. While the dosage of exogenous Gn varies greatly among individuals, study by Su et al[15] classified a dose of 2400 IU as a threshold and showed that the Gn amount below 2400 IU increases the incidence of OHSS. Predisposition to OHSS may be related to the specific characteristics of ovaries of some patients, such as high sensitivity, high responsiveness to lower doses of exogenous Gn, and easier growth and maturation of follicles.

Some scholars[8] showed that the number of follicles obtained positively correlates with ovarian reactivity. Our results also report that there was no low ovarian response in the OHSS group, further confirming higher ovarian sensitivity of OHSS patients. Some studies[8,16], including a study by Steward et al[16] have shown that the number of puncture-retrieved follicles greater than 15 (as a cut-off value) has a good sensitivity and specificity for predicting OHSS. However, there is still no consensus on this threshold[17]. In view of the positive correlation between the number of follicles collected by puncture and ovarian reactivity and the occurrence of OHSS, this study included the number of follicles collected by puncture as a continuous variable into the prediction model. We show that irrespective of whether other confounding factors were adjusted or not, they were all related to the occurrence of OHSS. Therefore, the number of follicles, retrieved by puncture, is an independent risk factor for OHSS.

Our study has several limitations. Patients' BMI, basic sex hormone levels, and estrogen levels on the day of HCG injection were not included after screening variables based on LASSO. However, some studies suggest that these indicators

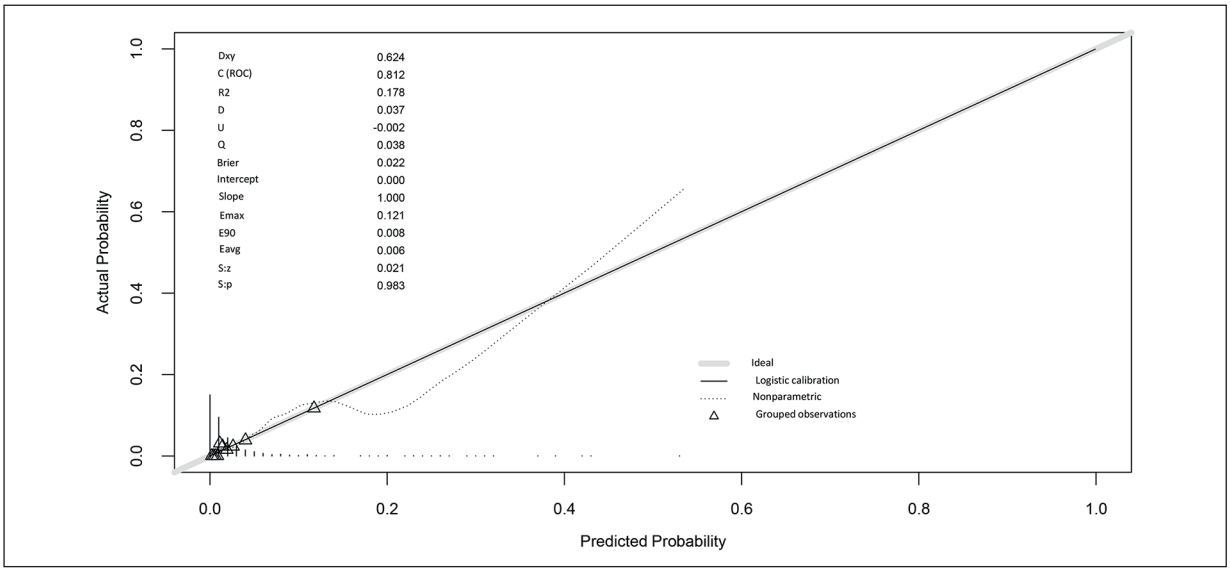

Figure 4. Calibration curve of predictive model.

may be related to OHSS occurrence[8,18,19]. Moreover, the results of the study may be affected by factors, such as race and the amount of Gn agonist used, and the predictive ability in this study is, therefore, limited. In addition, although some cytokines, such as vascular endothelial growth factor have a predictive effect on OHSS[20], they cannot be used as routine inspection items. There is a large amount of data that is not conducive to the loss of model promotion and was not included in the study.

Conclusions

In summary, we screened clinically available conventional indicators in patients undergoing follicular phase GnRH-a long-acting regimen for hyperstimulation. We show that the patient's age, AFC, Gn starting dose, number of follicles obtained, and the diagnosis of PCOS are associated with the occurrence of OHSS. Our results may help in predicting whether moderate to severe OHSS will occur and to build a model to predict the outcome of OHSS. This will provide decision-making basis for clinical individualized diagnosis and treatment and improve the safety of clinical treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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