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Original Article

Risk factors for methotrexate resistance in low-risk gestational trophoblastic neoplasia patients (FIGO score 0-4)

Tianzhe Jin[1*], Zijun Zhou[2*], Mengmeng Lin[3], Shuo Yuan[1], Yite Xue[1], Taotao Yin[1], Ruiyi Xu[1], Bingxin Chen[1], Jianwei Zhang[1], Jiehao Sun[2], Xiao Li[4], Yan Hu[3], Lili Chen[4,5], Hui Wang[1,4]

[1]Zhejiang Provincial Key Laboratory of Precision Diagnosis and Therapy for Major Gynecological Diseases, Women's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, Zhejiang, China; [2]Department of Anesthesiology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; [3]Department of Gynecology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; [4]Department of Gynecologic Oncology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, Zhejiang, China; [5]Zhejiang Provincial Clinical Research Center for Obstetrics and Gynecology, Hangzhou 310006, Zhejiang, China. [*]Equal contributors.

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Abstract: The challenge of methotrexate (MTX) resistance among low-risk gestational trophoblastic neoplasia (GTN) patients has always been prominent. Despite the International Federation of Gynaecology and Obstetrics (FIGO) score of 0-4 patients comprising the majority of low-risk GTN patients, a comprehensive exploration of the prevalence and risk factors associated with MTX resistance has been limited. Therefore, we aimed to identify associated risk factors in GTN patients with a FIGO score of 0-4. Between January 2005 and December 2020, 310 low-risk GTN patients received primary MTX chemotherapy in two hospitals, with 265 having a FIGO score of 0-4. In the FIGO 0-4 subgroup, 94 (35.5%) were resistant to MTX chemotherapy, and 34 (12.8%) needed multi-agent chemotherapy. Clinicopathologic diagnosis of postmolar choriocarcinoma (OR = 17.18, 95% CI: 4.64-63.70, $P < 0.001$) and higher pretreatment human chorionic gonadotropin concentration on a logarithmic scale (log-hCG concentration) (OR = 18.11, 95% CI: 3.72-88.15, $P < 0.001$) were identified as independent risk factors associated with MTX resistance according to multivariable logistic regression. The decision tree model and regression model were developed to predict the risk of MTX resistance in GTN patients with a FIGO score of 0-4. Evaluation of model discrimination, calibration and net benefit revealed the superiority of the decision tree model, which comprised clinicopathologic diagnosis and pretreatment hCG concentration. The patients in the high- and medium-risk groups of the decision tree model had a higher probability of MTX resistance. This study represents the investigation into MTX resistance in GTN patients with a FIGO score of 0-4 and disclosed a remission rate of approximately 65% with MTX chemotherapy. Higher pretreatment hCG concentration and clinicopathologic diagnosis of postmolar choriocarcinoma were independent risk factors associated with resistance to MTX chemotherapy. The decision tree model demonstrated enhanced predictive capabilities regarding the risk of MTX resistance and can serve as a valuable tool to guide the clinical treatment decisions for GTN patients with a FIGO score of 0-4.

Keywords: Gestational trophoblastic neoplasia, methotrexate resistance, decision tree model, choriocarcinoma, human chorionic gonadotropin

Introduction

Gestational trophoblastic neoplasia (GTN) comprises various pregnancy-related disorders, such as invasive mole, choriocarcinoma, placental-site trophoblastic tumors (PSTT) and epithelioid trophoblastic tumors (ETT). Non-PSTT/ETT forms of GTN are often effectively managed with chemotherapy. GTN is prognostically classified into two groups based on the International Federation of Gynecology and Obstetrics (FIGO) 2000 scoring system. The scoring system comprises eight factors to calculate the risk of developing resistance to first-line single-agent chemotherapy. Patients with a FIGO score ≤ 6 are categorized as low risk and typically undergo primarily first-line single-agent methotrexate (MTX) chemotherapy [1].

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Despite the widespread use of the FIGO 2000 scoring system, the initial remission rates of GTN patients receiving MTX treatment range from 53% to 87% [2-4]. The primary remission rate for patients with FIGO scores of 0-4 is between 50%-85%, whereas for those with scores of 5-6, it falls within the 12%-52% [4, 5]. These differences in responsiveness to MTX treatment between the two subgroups underscore the need to categorize and discuss these patients separately. However, although patients with a FIGO score of 0-4 constitute 80%-86% of low-risk GTN patients, investigation on MTX resistance in this subgroup remains restricted [6, 7]. Additionally, some risk factors within the FIGO 2000 scoring system may be relevant to tumor burden, which could be interrelated with MTX resistance [8, 9].

This study aimed to identify risk factors associated with resistance to MTX chemotherapy and introduce a decision tree model as a novel risk stratification model. A decision tree model based on retrospective analysis of clinical data from two hospitals was conducted by employing machine learning algorithms. This model offered an enhanced tool for guiding treatment decisions in GTN patents with a FIGO score of 0-4.

Materials and methods

Patients

We did a retrospective study of low-risk GTN patients treated primarily with MTX chemotherapy between January 2005 and December 2020. Patients were excluded if they did not receive MTX chemotherapy as primary treatment, had a histopathological diagnosis of ETT or PSTT, failed to complete treatment, were lost to follow-up or developed toxicity to MTX chemotherapy. The Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (Approval number: IRB-20230076-R) and the First Afflicted Hospital of Wenzhou Medical University (Approval number: KY2023-R069) approved the study. No informed consent was required because the data were anonymized.

Postmolar GTN was diagnosed after a plateau in hCG concentration for four consecutive tests over 3 weeks, any elevation in hCG concentration by ≥ 10% for three consecutive tests over 2 weeks or hCG concentration persistence ≥ 6 months after molar evacuation. The clinicopathologic diagnosis of choriocarcinoma was made by examination of tissue specimens showing hyperplastic and dysplastic trophoblast in the absence of villi or if GTN developed in association with a non-molar pregnancy, thus excluding the possibility of the invasive mole [10]. The number of MTX-resistant patients should be more than 50 based on the "one in ten rule" in model building. According to the efficacy of the MTX treatment reported in previous literature, we estimated that primary remission rates of MTX therapy are 60%. Therefore, at least 125 GTN patients of FIGO 0-4 are needed [11, 12]. A total of 310 low-risk GTN patients who met the criteria were recruited, of which 265 GTN patients with a FIGO score of 0-4 were recruited from the Women's Hospital, Zhejiang University School of Medicine (n = 206), and the First Afflicted Hospital of Wenzhou Medical University (n = 59).

Pathologists and gynecologists confirmed all diagnoses. Patients included in this study underwent a histopathological review when pathology samples were available.

Treatment protocols

All low-risk GTN patients were primarily treated with MTX chemotherapy (0.4 mg/kg per day, maximum 25 mg) injected intramuscularly or intravenously for 5 days every other week. The patients who developed resistance or toxicity to MTX chemotherapy switched to a 5-day administrated actinomycin D (Act-D) salvage regimen (10 μg/kg per day, intravenous injection, for 5 days every 2 weeks). Patients who failed sequential single-agent chemotherapy were managed with multi-agent chemotherapy.

The drug resistance criteria were: after 2 successive chemotherapy cycles, serum hCG concentration did not fall logarithmically, remained at a plateau level, or increased during 2 successive chemotherapy cycles or imaging exams, which indicated that the tumor size increased or new lesions appeared. Relapse was diagnosed as increasing serum hCG concentrations with or without the appearance of metastases and in the absence of pregnancy after complete remission. Drug toxicity was assessed in each cycle according to World Health Organization criteria. Complete remis-

sion was diagnosed as three consecutive weekly normal serum hCG concentrations (< 5.3 IU/L) [13]. The primary outcome was the incidence of resistance to MTX chemotherapy. Two additional courses of chemotherapy were given after the first normal hCG concentration. We performed a follow-up of at least 1 year to determine recurrence and survival.

Data collection

The following clinical, biochemical and pathological variables were recorded: age, antecedent pregnancy, FIGO risk score, maximum lesion diameters, number of metastases, the chemotherapy regimen and pretreatment hCG concentration.

Statistical analysis

The normally distributed variables were summarized using the mean, standard deviation (SD), counts and percentages. Median and interquartile range (IQR) were used for non-normal distributed variables. We used restricted cubic splines to investigate the nonlinear association between continuous variables and the risk of MTX resistance.

Risk factors associated with MTX resistance in GTN patients with a FIGO score of 0-4 were initially identified with univariable logistic regression analysis. Variables with a *P* Value < 0.05 were included in the multivariable logistic regression analysis. Multicollinearity was assessed using the variance inflation factors (VIF). A VIF > 4 indicated a collinearity problem [14]. We observed large odds ratios (ORs) for some risk factors associated with MTX resistance. To assess the robustness of these findings, we conducted a post hoc power analysis [15].

Then, novel models were built, including a parametric regression model and a nonparametric decision tree model, to predict the risk of MTX resistance. The decision tree model was utilized to select the most significant variables and was trained to optimize the treatment effect difference between leaves (subgroups). This approach aimed to identify specific patient subgroups with distinct responses to MTX chemotherapy and calculate the incidence of MTX resistance within these subgroups [16]. Moreover, for continuous variables (pretreatment hCG concentration), the decision tree model identified the cut-point that maximized the difference between subgroups [17]. To address the risk of overfitting and consider clinical applicability, we tuned the hyper-parameter with the 10-fold cross-validation and fine-tuned the cutoff values while ensuring accuracy.

We assessed the performance of the novel models utilizing traditional metrics. Model discrimination was quantified through the receiver operating characteristic (ROC) analysis. The test of AUROC (area under the receiver operating characteristic curve) was calculated using a bootstrapping approach involving two steps: 1) Bootstrapping samples were generated by randomly resampling with replacement from the original dataset 5000 times. 2) For each bootstrapped sample, AUROC was calculated using the R package "nsROC" [18]. Calibration curves were generated to evaluate the agreement between the predicted and observed risk of MTX resistance. To assess the clinical utility of the model, we calculated their net benefit through decision curve analysis. The statistical analysis was performed using the R statistical software version 4.3.1.

Results

Between January 2005 and December 2020, 369 patients diagnosed with low-risk GTN received primary first-line single-agent MTX chemotherapy at two hospitals, of whom 265 had a FIGO score of 0-4. 59 patients (16.0%) were excluded (**Figure 1**). The clinical characteristics of GTN patients with a FIGO score of 0-4 were summarized in **Table 1**. Baseline characteristics between two hospitals were similar (**Table 2**).

Among low-risk patients primarily treated with MTX chemotherapy, 118/310 (38.1%) developed resistance. Act-D chemotherapy was administered to MTX-resistant patients. 45/310 (14.5%) patients required multi-agent chemotherapy after developing resistance to single-agent chemotherapy. For GTN patients with a FIGO score of 0-4, 94/265 (35.5%) patients were resistant to MTX chemotherapy, and among them, 34/265 (12.8%) necessitating multi-agent chemotherapy for remission. The incidence of MTX resistance in GTN patients with a FIGO score of 0-4 and 5-6 was 35.5% (94/265) and 53.3% (24/45), respectively.

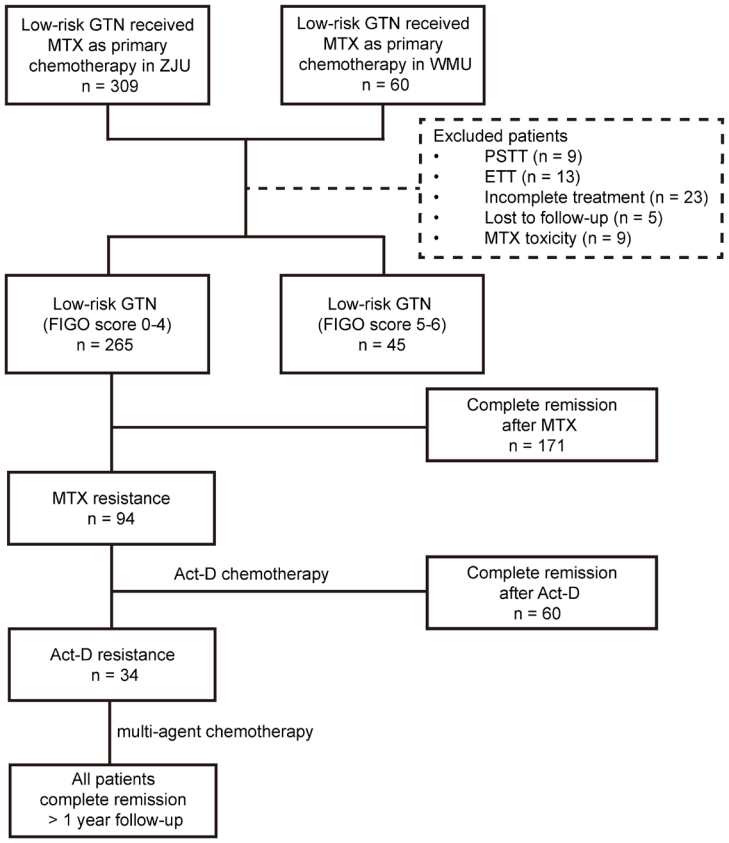

Figure 1. The flowchart of the included GTN patients. GTN = gestational trophoblastic neoplasia. MTX = methotrexate. ZJU = the Women's Hospital, Zhejiang University School of Medicine. WMU = the First Affiliated Hospital of Wenzhou Medical University. FIGO = International Federation of Gynecology and Obstetrics. PSTT = placental-site trophoblastic tumors. ETT = epithelioid trophoblastic tumors. Act-D = actinomycin D.

The relationship between pretreatment hCG concentration on a logarithmic scale (log-hCG concentration) and the risk of MTX resistance was linear. Consequently, log-hCG concentration was employed in subsequent logistic regression analysis (**Figure 2**). **Table 3** demonstrates the results of the univariable and multivariable logistic regression analysis for GTN patients with a FIGO score of 0-4 treated with MTX chemotherapy. In univariable logistic regression analysis age, clinicopathologic diagnosis of postmolar choriocarcinoma, pretreatment log-hCG concentration and the largest tumor mass diameter were significant risk factors associated with MTX resistance. Multivariable logistic regression identified clinicopathologic diagnosis of postmolar choriocarcinoma (OR = 17.18, 95% CI: 4.64-63.70, $P < 0.001$) and higher pretreatment log-hCG concentration (OR = 18.11, 95% CI: 3.72-88.15, $P < 0.001$) as independent risk factors for MTX resistance (**Figure 3A**). Multicollinearity was not observed between these variables (VIF < 4). Considering 2-sided $\alpha = 0.05$, sample size = 265, MTX-resistant rate = 35.5% and adjusted OR (postmolar CCA) = 17.18, power calculations yielded 0.85 for MTX-resistant and 0.91 for MTX-sensitive outcomes.

The decision tree model identified clinicopathologic diagnosis and pretreatment hCG concentration to give a new risk stratification model, where the thresholds of hCG were the logarithmic-scale split point determined by minimizing the impurity of the decision tree nodes. This model could divide the GTN patients with a FIGO score of 0-4 into three subgroups: high-risk, medium-risk and low-risk (**Figure 3B**). 29/265 (11.0%) patients with a pretreatment hCG concentration of 25,000 IU/L or more, regardless of the clinicopathologic diagnosis, were defined as high-risk patients. They had a 72.4% chance of developing MTX resistance. 100/265 (37.7%) patients with a clinicopathologic diagnosis of choriocarcinoma and hCG concentration < 2000 IU/L or with a pretreatment hCG concentration between 2,000 IU/L to 25,000 IU/L were classified into the medium-risk group, with a 41.9% probability of developing MTX resistance. Additionally, 136/265 (51.3%) patients diagnosed with postmolar GTN and hCG concentration < 2,000 IU/L were classified as the low-risk group, being the most sensitive to MTX chemotherapy with a 16.0% probability of MTX resistance (**Table 4**).

We further compared the performance of the decision tree model and the regression model to the FIGO 2000 scoring system. The discriminating power of these novel models was compared with the FIGO 2000 scoring system using ROC analysis (**Table 5**). The AUROC was 0.71 (95% CI: 0.65-0.77) for the decision tree model,

Table 1. Clinical characteristics of GTN patients with a FIGO risk score of 0-4 at two hospitals

Variables	Population n = 265
FIGO risk score (%)	
0	30 (11.3)
1	67 (25.3)
2	69 (26.1)
3	61 (23.0)
4	38 (14.3)
Clinicopathologic diagnosis (%)	
Postmolar GTN	226 (85.3)
Postmolar CCA	14 (5.3)
Postab/term CCA	25 (9.4)
Age (median [IQR])	30.00 [26.00, 40.00]
Metastatic disease status (%)	174 (65.7)
Antecedent pregnancy (%)	
Mole	240 (90.6)
Abortion	22 (8.3)
Term	3 (1.1)
Largest tumor mass diameter (cm) (%)	
< 3	202 (76.2)
3-5	54 (20.4)
> 5	9 (3.4)
hCG concentration (%)	
$< 10^3$	94 (35.5)
10^3 -< 10^4	104 (39.2)
10^4 - 10^5	66 (24.9)
$> 10^5$	1 (0.4)

Postab/term = postabortion/term. CCA = choriocarcinoma. IQR = interquartile range. hCG = human chorionic gonadotropin. cm = centimeter.

0.68 (95% CI: 0.61-0.75) for the regression model and 0.65 (95% CI: 0.58-0.72) for the FIGO 2000 scoring system. It indicated the decision tree model showed better discrimination than other models (decision tree model vs regression model, $P < 0.001$; decision tree model vs FIGO 2000 scoring system, $P < 0.001$) (**Figure 3C**). The calibration curves of the decision tree model and the FIGO 2000 scoring system illustrated satisfactory agreement between the actual observations and predictions for MTX resistance probabilities, while the regression model exhibited worse calibration performance (**Figure 3D**). According to decision curve analysis, the regression model and decision tree model had higher net benefits than the FIGO 2000 scoring system (**Figure 3E**).

Discussion

The FIGO 2000 scoring system is widely employed to guide the treatment of GTN patients, recommending single-agent chemotherapy for low-risk GTN patients and multi-agent chemotherapy for high-risk GTN patients [1]. Despite an almost 100% complete remission rate in low-risk GTN patients, 15%-50% still experience MTX resistance [19, 20]. Patients with a FIGO score of 0-4 accounted for 80%-86% of all low-risk GTN patients. Previous studies have proved that only around 60% of these patients have complete remission [7, 21]. In our study, the incidence of MTX resistance in GTN patients with a FIGO score of 0-4 and 5-6 was 35.5% (94/265) and 53.3% (24/45), respectively. This MTX-resistant rate in GTN patients with a FIGO score of 0-4 (35.5%) showed a slight decrease compared to all low-risk GTN patients (38.1%). However, previous studies have mainly focused on identifying risk factors associated with developing resistance to primary single-agent chemotherapy in all low-risk GTN patients or those with a FIGO risk score of 5-6 [3, 4, 22, 23]. Underscoring the critical need to address drug resistance in GTN patients with a FIGO score of 0-4, we aim to investigate risk factors associated with MTX resistance in this subgroup of patients.

We analyzed 265 low-risk GTN patients with a FIGO score of 0-4 in two hospitals. Using univariable and multivariable logistic regression analysis, we found higher pretreatment hCG concentrations and clinicopathologic diagnosis of postmolar choriocarcinoma were the independent risk factors associated with resistance to MTX chemotherapy. Our finding was consistent with previous studies that a higher risk of MTX resistance was associated with higher pretreatment hCG concentration. Taylor F et al. noted that resistance to MTX chemotherapy was more common with pretreatment hCG concentration greater than 100,000 IU/L [24]. The

Table 2. Clinical characteristics of GTN patients with a FIGO score of 0-4 at two hospitals

Variables	MTX-sensitive n = 171	MTX-resistance n = 94
FIGO score (%)		
0	25 (14.6)	5 (5.3)
1	52 (30.4)	15 (16.0)
2	45 (26.3)	24 (25.5)
3	35 (20.5)	26 (27.7)
4	14 (8.2)	24 (25.5)
Clinicopathologic diagnosis (%)		
Postmolar GTN	153 (89.5)	73 (77.7)
Postmolar CCA	3 (1.7)	11 (11.7)
Postab/term CCA	15 (8.8)	10 (10.6)
Age (median [IQR])	32.00 [26.00, 42.00]	28.00 [26.00, 35.00]
Metastatic disease status (%)	79 (63.7)	60 (73.2)
Antecedent pregnancy (%)		
Mole	156 (91.2)	84 (89.4)
Abortion	13 (7.6)	9 (9.6)
Term	2 (1.2)	1 (1.0)
Largest tumor mass diameter (cm) (%)		
< 3	139 (83.1)	63 (67.0)
3-5	27 (13.7)	27 (29.3)
> 5	5 (3.2)	4 (3.7)
hCG concentration (%)		
$< 10^3$	72 (42.1)	22 (23.4)
10^3 -< 10^4	67 (39.2)	37 (39.4)
10^4 - 10^5	32 (18.7)	34 (36.2)
> 10^5	0 (0.0)	1 (1.0)

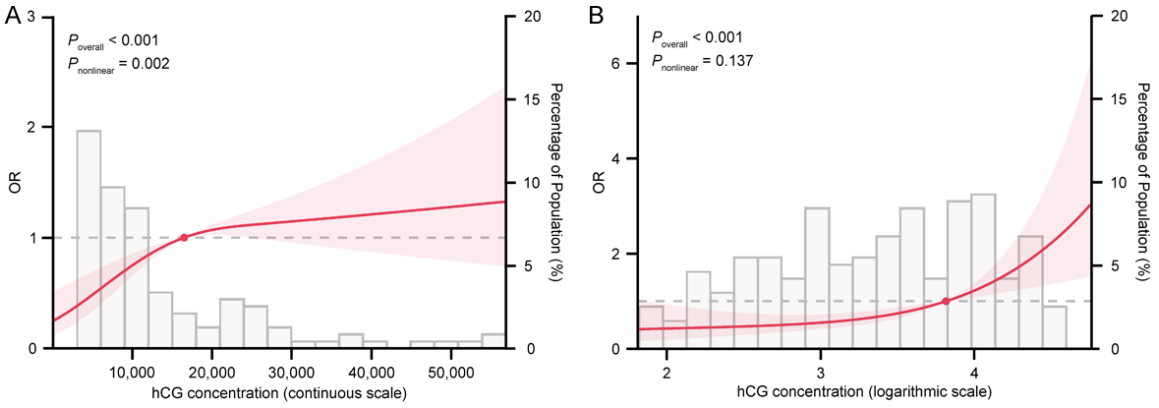

Figure 2. Restricted cubic spline plots demonstrating the relationship between the risk of MTX resistance and pretreatment hCG concentration on a continuous scale (A) and logarithmic scale (B). The red lines and shaded areas represented ORs and 95% CIs, respectively. The grey dashed lines showed the reference level. $P_{nonlinear}$ < 0.05 indicated a nonlinear relationship.

experience of McGrath S et al. suggested that drug resistance was related to a pretreatment hCG concentration higher than 400,000 IU/L [25]. Though many studies did not provide a precise pretreatment hCG threshold to determine whether patients would resist single-

Table 3. Univariable and multivariable logistic regression analysis of risk factors associated with MTX resistance in GTN patients with a FIGO score of 0-4

Variables	OR (univariable)	OR (multivariable)
Age	0.97 (0.94-1.00, *P* = 0.024)	0.97 (0.94-1.00, *P* = 0.062)
Clinicopathologic diagnosis		
Postmolar GTN	Ref	Ref
Postmolar CCA	12.75 (3.62-44.96, *P* < 0.001)	17.18 (4.64-63.70, *P* < 0.001)
Postab/term CCA	1.35 (0.56-3.24, *P* = 0.501)	1.19 (0.47-3.00, *P* = 0.720)
Log-hCG concentration	13.43 (3.29-54.87, *P* < 0.001)	18.11 (3.72-88.15, *P* < 0.001)
Metastatic disease status	1.27 (0.74-2.18, *P* = 0.376)	-
Largest tumor mass diameter (cm)	1.76 (1.09-2.84, *P* = 0.021)	1.45 (0.86-2.45, *P* = 0.160)

OR = odds ratio. ref = reference. Log-hCG concentration = hCG concentration on a logarithmic scale.

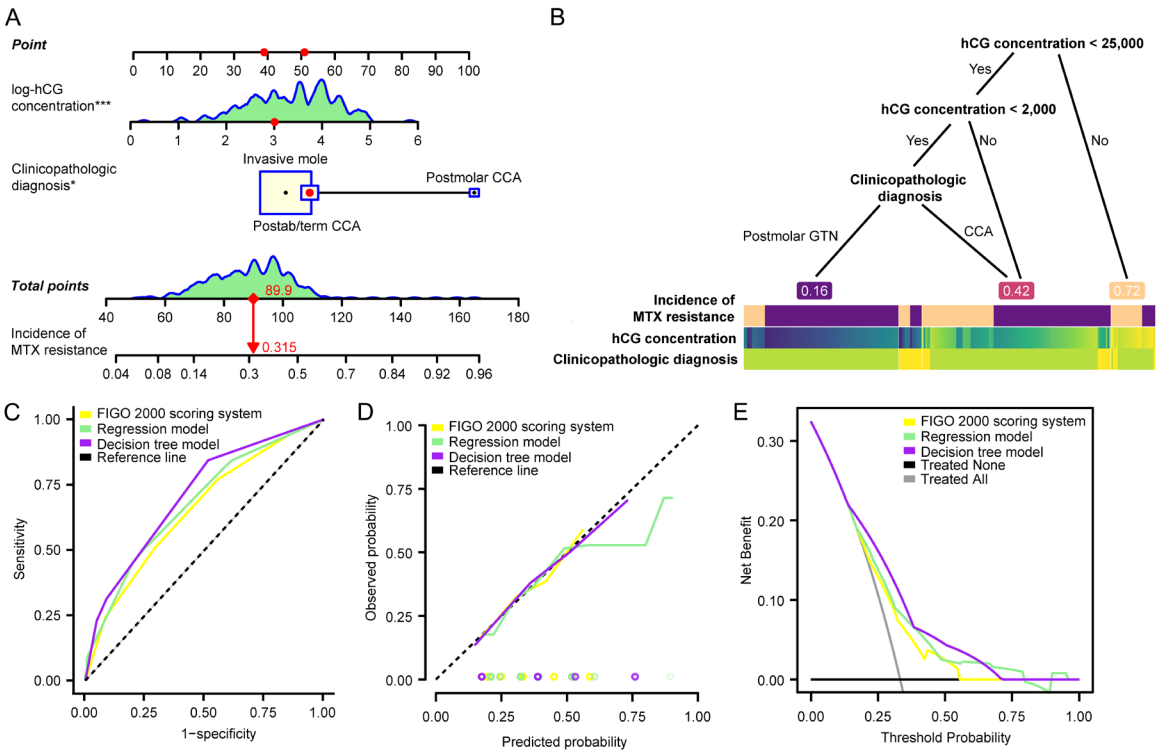

Figure 3. Construction of the regression model (A) and the decision tree model (B) to identify the individual incidence of MTX resistance among GTN patients with a FIGO score of 0-4. Performance comparison of the two novel models and the FIGO 2000 scoring system, including the receiver operating characteristic (ROC) curves (C), the calibration curves (D) and the decision curves (E).

Table 4. A decision tree model was developed to identify the clinical characteristics and estimate the risk difference in resistance to MTX chemotherapy among subgroups in GTN patients with a FIGO score of 0-4

Groups	Variables	Population (n = 265)	Incidence of MTX resistance
High-risk	hCG ≥ 25,000 IU/L	29 (11.0%)	72.4%
Medium-risk	2,000 ≤ hCG < 25,000 IU/L or hCG < 2,000 IU/L and CCA	100 (37.7%)	41.9%
Low-risk	hCG < 2,000 IU/L and postmolar GTN	136 (51.3%)	16.0%

Table 5. The two novel models and their performance with the FIGO 2000 scoring system

Models	AUROC (95% CI)	True-Positive	True-Negative	False-Positive	False-Negative	Accuracy	Sensitivity	Specificity	PPV	NPV
Decision tree model	0.71 (0.65, 0.77)	37	154	17	57	0.72	0.86	0.48	0.47	0.86
Regression model	0.68 (0.61, 0.75)	20	165	6	74	0.69	0.55	0.75	0.55	0.75
FIGO 2000 scoring system	0.65 (0.58, 0.72)	23	157	14	71	0.68	0.53	0.71	0.50	0.74

AUROC = area under the receiver operating characteristic curve. PPV = positive prognostic value. NPV = negative prognostic value.

agent chemotherapy, they suggested a correlation between increased pretreatment hCG concentration and the likelihood of single-agent chemotherapy resistance [26, 27].

Our study is one of the most extensive studies of the association between the clinicopathologic diagnosis of choriocarcinoma and MTX resistance. Although the FIGO 2000 scoring system does not include clinicopathologic diagnosis, previous studies proved that the clinicopathologic diagnosis of choriocarcinoma was the factor associated with resistance to single-agent chemotherapy [5, 10]. Previous studies suggested that compared with postmolar GTN, those with chemoresistance had a more frequent diagnosis of choriocarcinoma [8, 28]. However, when we further divided the patients with antecedent pregnancy, we found that postmolar choriocarcinoma was at greater risk of developing chemoresistance, suggesting this variable should be taken more seriously.

After 20 years of utilizing the globally acknowledged FIGO 2000 scoring system, many low-risk patients exhibiting resistance to MTX chemotherapy prompts us to explore potential enhancements for the FIGO 2000 scoring system's efficacy [9, 29]. Employing the decision tree model, a simplified model with pretreatment hCG concentration and clinicopathologic diagnosis, resulted in an identical risk stratification for GTN patients with a FIGO score of 0-4. Notably, among GTN patients with a FIGO score of 0-4, those with a pretreatment hCG concentration of 25,000 IU/L or more, an increased likelihood of developing MTX resistance was observed. Conversely, patients with a clinicopathologic diagnosis of postmolar GTN and pretreatment hCG concentration < 2,000 IU/L demonstrated heightened sensitivity to MTX chemotherapy.

One potential concern of using only a few predictive variables is the risk of underperformance in this new simplified model. The decision tree model resulted in similar and even better discriminations for MTX resistance risk prediction than other models and overall fair agreement between observed and predicted risk (calibration). The analysis of decision curves showed that the decision tree model provided better net benefit. We avoided complex artificial intelligence methods and used easily accessible clinical variables in our decision model, ensuring simplicity and ease of adoption. While histopathologic assessment is recommended for GTN workups, challenges in obtaining specimens led us to prioritize clinicopathologic diagnoses, which are more readily accessible in clinical settings.

In discussing the limitations of this study, it is crucial to acknowledge the inherent challenges posed by the low incidence rate of GTN, leading to difficulties in sample collection. The limited number of patients with postmolar choriocarcinoma included in this study restricts our ability to conduct a comprehensive investigation and draw generalized conclusions about this rare cancer. Another limitation is that the decision tree model can assess patients' risk of developing MTX resistance but can not provide information on which patients are more suitable for multi-agent chemotherapy or surgical treatment. Thus, further validations and prospective clinical research are needed to validate these findings.

Conclusion

The study concentrated on low-risk GTN patients with a FIGO score of 0-4, revealing that higher pretreatment hCG concentration and a clinicopathologic diagnosis of postmolar choriocarcinoma were the independent risk factors associated with MTX resistance. With the expectation that 65% of patients with a FIGO score of 0-4 will enter remission with primary MTX chemotherapy, introducing a decision tree model might help maximize the ability of predictions of patients' initial responses to MTX che-

motherapy. This study highlights the need for personalized treatment approaches and further research validation.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Hui Wang and Lili Chen, Department of Gynecologic Oncology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, Zhejiang, China. Tel: +86-13995688338; Fax: +86-0571-87061501; E-mail: wang71hui@zju.edu.cn (HW); Tel: +86-13958138597; Fax: +86-0571-89992142; E-mail: 5197004@zju.edu.cn (LLC); Dr. Yan Hu, Department of Gynecology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. Tel: +86-13958138597; Fax: +86-0577-55578100; E-mail: 627830566@qq.com

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