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# Doppler-based predictive model for methotrexate resistance in low-risk gestational trophoblastic neoplasia with myometrial invasion: prospective study of 147 patients

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**KEYWORDS:** Doppler; low-risk gestational trophoblastic neoplasia; methotrexate resistance; predictive model; time-averaged mean velocity

## CONTRIBUTION

*What are the novel findings of this work?*
We have developed a predictive model for methotrexate (MTX) resistance in low-risk gestational trophoblastic neoplasia (GTN) with myometrial invasion, which combines a Doppler marker of tumor vascularity with the International Federation of Gynecology and Obstetrics (FIGO) score, thus improving the predictive power of the latter.

*What are the clinical implications of this work?*
The Doppler-based predictive model achieved a specificity of 96.6% and stratified patients with low-risk GTN into low (< 10%), intermediate (10–90%) and high (> 90%) probability of MTX resistance. Thus, it has the potential to guide patient counseling and subsequent management.

## ABSTRACT

***Objectives*** *This prospective clinical study aimed to evaluate the vascularization characteristics of low-risk gestational trophoblastic neoplasia (GTN) using Doppler imaging and to develop a predictive model for resistance to methotrexate (MTX).*

***Methods*** *Patients with low-risk GTN receiving primary MTX treatment were enrolled from the Women's Hospital, Zhejiang University School of Medicine, Hangzhou, China, from September 2012 to August 2018. The primary endpoint was to develop and internally validate a predictive model for resistance to MTX therapy in these patients. In the training set, clinical features and Doppler hemodynamic parameters before MTX therapy were analyzed using logistic regression to identify independent predictors of MTX resistance, which were integrated into the model. The predictive performance of the model was evaluated by leave-one-out cross-validation in the training dataset and internal validation in an independent-sample test dataset.*

***Results*** *The entire imaging protocol was completed by 147 eligible patients, of which 110 comprised the training set and 37 the test set. In the training set, cases with myometrial invasion (81.8%; 90/110) showed vascular-enriched areas in the myometrium and high velocity and low impedance ratios of the uterine artery (UtA) compared to cases without myometrial invasion (18.2%; 20/110). On multivariate logistic regression analysis, time-averaged mean velocity in UtA (UtA-TAmean) and the International Federation of Gynecology and Obstetrics (FIGO) score were identified as independent predictors (*$P = 0.009$ *and* $P = 0.043$*, respectively) of MTX resistance. The Doppler-based predictive model, developed based on the 90 cases with myometrial invasion, was* $y = -2.95332 + 0.41696 \times FIGO\ score + 0.03551 \times UtA\text{-}TAmean$*. The model showed an area under the curve of 0.757 (95% CI, 0.653–0.862) and the optimal cut-off value was 0.50622, which had 45.2% sensitivity and 96.6% specificity. The model stratified*

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*patients with low-risk GTN into low (< 10%), intermediate (10–90%) and high (> 90%) probability of MTX resistance, based on the threshold values of −1.59544 and 0.10046. The model had an accuracy of 74.4% (95% CI, 64.5–82.3%) in the cross-validation and 72.7% (95% CI, 55.8–84.9%) in the internal validation.*

***Conclusions*** *The Doppler-based predictive model, combining a non-invasive marker of tumor vascularity with the FIGO scoring system, can differentiate cases with low from those with high probability of developing MTX resistance and therefore has the potential to guide treatment options in patients with low-risk GTN and myometrial invasion.* 

## INTRODUCTION

Gestational trophoblastic neoplasia (GTN) is a group of disease processes originating from the placenta with local invasion and metastasis[1]. GTN can be treated successfully by cytotoxic chemotherapy. The chemotherapeutic regimen is selected based on the International Federation of Gynecology and Obstetrics (FIGO) 2000 prognostic scoring system[2]. This system differentiates quantitatively patients as having low risk (FIGO score $\leq 6$) or high risk (FIGO score $\geq 7$) of potentially developing resistance to single-agent chemotherapy. Commonly, methotrexate (MTX) is the agent of choice due to its advantages of easy administration and relatively low cost[1,3–7]. However, the FIGO score fails to provide a clear prediction between low-risk patients that will subsequently become MTX resistant and those that will respond well[8]. Thus, approximately 30–40% of patients with low-risk GTN require alternative therapeutic regimens following the development of resistance to MTX[1,4,5,7,9,10]. Changing drugs substantially prolongs treatment duration, increases the accumulation of drug side effects and thus increases the risk of morbidities[3]. Therefore, efforts to refine this prognostication system are needed for GTN management[11].

Tumor vascularization is known to be correlated significantly with drug resistance[12–14]. Doppler ultrasound is a non-invasive, radiation-free, high temporal resolution tool that assesses vascular characteristics of tumors in real time[15–21]. Several (mostly retrospective) studies on GTN have evaluated the usefulness of Doppler in predicting chemotherapy resistance; however, their conclusions remain controversial[22–26]. Such discrepancies could be attributed to the variation in the MTX regimens (e.g. 5-day, 8-day or weekly) and the small sample sizes. Furthermore, since the existing papers selected different ultrasound parameters, these results were dependent mostly on the candidate parameters.

This cohort study recruited prospectively a relatively large number of subjects with low-risk GTN who were treated with the same regimen. The study aimed to evaluate the clinical features and vascularization characteristics, based on Doppler imaging, of low-risk GTN and to develop a predictive model for MTX resistance in these patients.

## METHODS

### Patients

This prospective study was conducted in compliance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Women's Hospital, Zhejiang University School of Medicine (Reference: 20120023). Study subjects with low-risk GTN were recruited consecutively between September 2012 and August 2018.

Eligible patients met the following inclusion criteria: the first-line chemotherapy was single-agent MTX; the complete course of treatment was undertaken in our hospital; and written informed consent was obtained. Exclusion criteria were: change of treatment regimen due to MTX side effects (serositis or allergy); transcatheter arterial embolization before chemotherapy; and histologic diagnosis of placental-site trophoblastic tumor or epithelioid trophoblastic tumor.

All patients were followed up for at least 1 year after the last course of chemotherapy[27].

### Endpoints

The primary endpoint was to develop and internally validate a predictive model for resistance to MTX therapy in low-risk GTN patients.

The secondary endpoints were: (1) to describe the vascularization characteristics of low-risk GTN at the baseline ultrasound examination, focusing on the uterine artery (UtA) and the tumor central vessels (TCV), particularly with respect to the differences between cases with and those without myometrial invasion; (2) to assess the intraobserver, interobserver and interdevice reliability of Doppler measurements in the UtAs; and (3) to assess the correlation between UtA Doppler indices and actinomycin-D resistance after MTX resistance.

### Sample size calculation

In accordance with the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) statement, the subjects were separated into the training set for development of the predictive model in the first phase, and the test set for independent validation of the model in the second phase[28]. The sample size of the training set was calculated based on preliminary data of 45 cases in our hospital (16 cases with MTX resistance and 29 MTX responsive), which showed that the overall rate of MTX response was about 65% (allocation ratio; $0.35/0.65 = 0.538$) and revealed that the area under the curve (AUC) of the Doppler-based model for MTX resistance was estimated to be 0.70. In order to achieve the AUC of 0.70 with the

Doppler-based predictive model, assuming a SD of the AUC of 0.05, with an alpha of 5% and a power of 90%, a minimum of 31 cases of MTX resistance and 57 cases of MTX responsive, i.e. a total of 88 cases with low-risk GTN, were required to develop the multivariate logistic regression model. For the test set, the sample size was based on an alpha of 10% and a power of 80% in order to meet the minimal amount of data, i.e. the estimated sample size of 33 cases, for independent validation.

### Chemotherapy and response evaluation

Patients were treated initially with MTX administered as an intramuscular injection at a dose of 0.4 mg/kg/day (maximum of 25 mg) for 5 days, repeated every 2 weeks[10]. Their response to chemotherapy was monitored by weekly measurement of serum human chorionic gonadotropin (hCG). MTX resistance was defined as a plateau or rise in serum hCG level that continued for at least two consecutive cycles of chemotherapy or development of new metastases or both, indicating a need for salvage therapy[29–32].

Patients who developed MTX resistance were changed to treatment with actinomycin-D (intravenous injection, 10 µg/kg/day for 5 days, every other week). Patients who developed resistance to actinomycin-D therapy were then treated with an etoposide, MTX, actinomycin-D, vincristine and cyclophosphamide (EMA/CO) intravenous injection as a weekly alternating schedule. Treatment was continued in all patients for two cycles of consolidation chemotherapy after their hCG levels fell to normal (< 5.3 IU/L).

### Ultrasound and data collection

Participants underwent a transvaginal ultrasonographic examination within 3 days before chemotherapy. Doppler assessments were performed using Voluson 730 Expert or Voluson E8 (version number 1 1.4.2, BT 14.0.6; GE Healthcare, Zipf, Austria) equipped with a 5–9-MHz transvaginal probe.

The patient was examined in the lithotomy position and with an empty bladder. The ultrasound probe was gently introduced into the vagina. The following variables were measured: (1) uterine volume; (2) bilateral UtA diameters and hemodynamic parameters (Figure S1); and (3) tumor volume and hemodynamic parameters of TCV in cases with detectable myometrial invasion.

Uterine volume was calculated using the prolate ellipsoid formula: uterine volume ($cm^3$) = L (cm) × AP (cm) × W (cm) × 0.523, where L is length, AP is anteroposterior diameter and W is width.

For measurement of the UtA diameters and hemodynamic parameters, the image was magnified sufficiently to include only the isthmus and the lower segment of the uterus. The UtA diameters were measured on the segment before bifurcation of the UtA into cervical and corporeal branches in order to ensure that the UtA, rather than the arcuate artery, was being examined. The calipers were placed at the innermost edge of the echogenic lines so that the crossbars coincided with the inner border of the echogenic lines (on–on) to measure the artery diameter. The following seven hemodynamic parameters were measured twice in each UtA on pulsed Doppler: peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAmax), time-averaged mean velocity (TAmean), systolic/diastolic flow velocity ratio (S/D), resistance index (RI) and pulsatility index (PI). The sample volume had to include the whole internal diameter of the vessel and the position of the transvaginal probe was adjusted to keep the angle of insonation below 30°. The highest velocity values (UtA-PSV, UtA-EDV, UtA-TAmax and UtA-TAmean) and the lowest impedance ratios (UtA-RI, UtA-PI and UtA-S/D) recorded were used for analysis, as described previously[33,34].

In patients with detectable myometrial invasion, the length, width and anteroposterior diameter of the tumor were measured in the cross-sectional plane, and the tumor volume was calculated using the prolate ellipsoid formula as mentioned above. The TCV hemodynamic parameters were acquired in four different central vessels, which were defined as vessels located at least 5 mm from the tumor's outer margin[34]. For each central vessel, the same seven hemodynamic parameters as for the UtAs were captured using pulsed Doppler. The highest TCV-PSV, TCV-EDV, TCV-TAmax and TCV-TAmean measurements and the lowest TCV-RI, TCV-PI and TCV-S/D measurements were used for analysis.

### Development of predictive model

Variables that were identified as significant on univariate logistic regression analysis were included in multivariate logistic regression analysis to assess which variables provided an independent prediction of MTX resistance. Independent predictors were incorporated into a predictive model. The performance of the model was evaluated by cross-validation in the training dataset and internal validation in the test dataset.

### Statistical analysis

The sample size was estimated using a one receiver-operating-characteristics (ROC) curve power calculation. Continuous data were tested for normality using the Kolmogorov–Smirnov test. Student's *t*-test was used for comparison of normally distributed data and Mann–Whitney *U*-test for non-normally distributed continuous data. All contingency tables were assessed using the chi-square test, or Fisher's exact test if the number of subjects was less than five. Univariate and forward stepwise multivariate logistic regressions were performed to determine the predictive factors for MTX resistance. ROC curves were used to test the discriminative potential of significant factors selected by logistic regression. An AUC with a lower limit of 95% CI > 0.5 was considered to be significant discrimination.

*Z*-test was used for pairwise comparison of ROC curves. Leave-one-out cross-validation was used to reduce selection bias, in which the model was trained using data from $n-1$ patients and the remaining case was used as the validation set. This process was repeated $n$ times, so every case was left out once. The classification accuracy for each process was used to estimate how well the model performed[35]. Bland–Altman analysis and intraclass correlation coefficient (ICC) were used to evaluate intraobserver, interobserver and interdevice repeatability of the UtA Doppler measurements (detailed methods are provided in Appendix S1). $P < 0.05$ (two-tailed) was considered statistically significant. Analyses were carried out using SPSS version 19.0 (IBM Corp., Armonk, NY, USA) and R statistical package for Mac (R Foundation for Statistical Computing, Vienna, Austria) and PASS 15.0 for Windows XP (NCSS LLC., Kaysville, UT, USA).

## RESULTS

Of 211 patients presenting with low-risk GTN during the study period, 147 patients (110 in the training set and 37 in the test set) who received chemotherapy were eligible for inclusion and completed the entire imaging protocol. Figure 1 illustrates the inclusion of participants into this study. Table 1 summarizes the clinical characteristics of the included patients. There were no significantly different characteristics between the training and the test datasets (all $P > 0.05$).

In the training set, the median age of patients was 33 (range, 15–56) years. The overall primary complete-response rate to single-agent MTX chemotherapy was 64.5% (71/110). The 39 patients with MTX resistance were switched to treatment with single-agent actinomycin-D, and 76.9% (30/39) of them achieved complete remission. The remaining 23.1% (9/39) cases were changed to an EMA/CO regimen. No cases had recurrence and the overall survival was 100% at least 1 year after the last course of chemotherapy. The patients underwent an average of 6.7 (range, 2–13) treatment cycles.

### Ultrasound findings in training dataset

Among the 110 low-risk GTN cases in the training dataset, uterine myometrial invasion was detected by transvaginal ultrasound in 81.8% (90/110) cases and was not detected in 18.2% (20/110) cases. In the 90 cases with myometrial invasion, the mean tumor volume was 17.26 (range, 0.15–113.03) $cm^3$ and hypervascular areas were observed in the myometrium on color Doppler (Figure 2a,b). In the 20 cases without myometrial invasion, no vascular-enriched areas were noted (Figure 2c,d). Patients with myometrial invasion showed significantly higher UtA-PSV, UtA-EDV, UtA-TAmax and UtA-TAmean and significantly lower UtA-S/D, UtA-RI and UtA-PI compared with those without myometrial invasion ($P < 0.01$ for all) (Table S1). Since the abnormal Doppler signal was not detected within the lesion in the 20 GTN

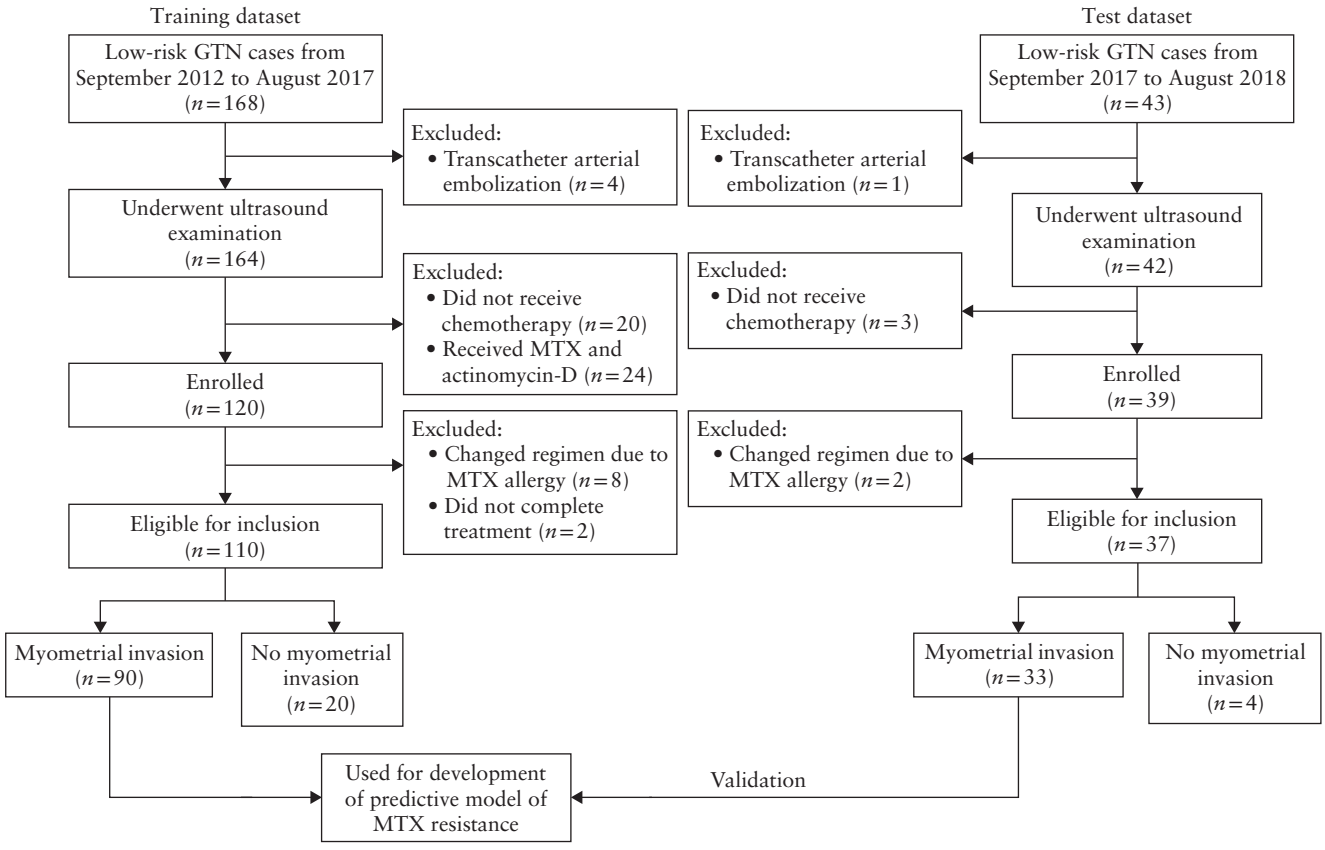


**Figure 1** Flowchart showing inclusion of patients with low-risk gestational trophoblastic neoplasia (GTN) in training and test datasets for development of predictive model of resistance to methotrexate (MTX).

cases without myometrial invasion, they were excluded from further analysis of the Doppler-based predictive model (Table S2).

Analysis of repeatability of Doppler measurement of the seven UtA hemodynamic parameters showed that the ICC for intraobserver repeatability ranged from 0.855 to 0.985 (95% CI, 0.773–0.990), the ICC for interobserver repeatability ranged from 0.797 to 0.952 (95% CI, 0.677–0.969) and the ICC for interdevice repeatability ranged from 0.790 to 0.902 (95% CI, 0.653–0.937) (Tables S3–S5 and Figures S2–S4).

## Predictive factors for MTX resistance based on training dataset

The presence or absence of uterine myometrial invasion on ultrasound was not associated significantly with MTX resistance ($P = 0.639$).

**Table 1** Clinical characteristics of patients with low-risk gestational trophoblastic neoplasia included in training and test datasets

| *Characteristic* | *Training dataset (n = 110)* | *Test dataset (n = 37)* | P |
|---|---|---|---|
| Age | | | 0.409 |
| < 40 years | 82 (74.5) | 25 (67.6) | |
| ≥ 40 years | 28 (25.5) | 12 (32.4) | |
| Antecedent pregnancy | | | 0.818 |
| Hydatidiform mole | 98 (89.1) | 32 (86.5) | |
| Spontaneous miscarriage | 7 (6.4) | 2 (5.4) | |
| Term pregnancy | 4 (3.6) | 2 (5.4) | |
| Unknown | 1 (0.9) | 1 (2.7) | |
| Interval from antecedent pregnancy | | | 0.723 |
| < 4 months | 104 (94.5) | 35 (94.6) | |
| 4 to < 7 months | 1 (0.9) | 1 (2.7) | |
| 7 to < 13 months | 2 (1.8) | 0 (0.0) | |
| ≥ 13 months | 3 (2.7) | 1 (2.7) | |
| Initial clinical symptom | | | 0.656 |
| Abnormal serum hCG | 97 (88.2) | 30 (81.1) | |
| Irregular vaginal bleeding | 9 (8.2) | 4 (10.8) | |
| Abnormal imaging | 3 (2.7) | 2 (5.4) | |
| Other | 1 (0.9) | 1 (2.7) | |
| Pretreatment serum hCG | | | 0.703 |
| < 1000 IU/L | 25 (22.7) | 6 (16.2) | |
| 1000–10 000 IU/L | 44 (40.0) | 16 (43.2) | |
| 10 000–100 000 IU/L | 41 (37.3) | 15 (40.5) | |
| Myometrial invasion | | | 0.294 |
| No | 20 (18.2) | 4 (10.8) | |
| Yes | 90 (81.8) | 33 (89.2) | |
| Detectable lesion on imaging* | | | 0.815 |
| No | 5 (4.5) | 2 (5.4) | |
| Yes | 105 (95.5) | 35 (94.6) | |
| FIGO stage | | | 0.221 |
| I | 38 (34.5) | 13 (35.1) | |
| III | 72 (65.5) | 23 (62.2) | |
| IV | 0 (0.0) | 1 (2.7) | |
| FIGO score | | | 0.098 |
| 0–2 | 61 (55.5) | 13 (35.1) | |
| 3–4 | 38 (34.5) | 18 (48.6) | |
| 5–6 | 11 (10.0) | 6 (16.2) | |

Data are given as *n* (%). *Imaging included pelvic Doppler ultrasound, chest X-ray and computed tomography and brain magnetic resonance imaging. FIGO, International Federation of Gynecology and Obstetrics; hCG, human chorionic gonadotropin.

Based on univariate logistic regression analysis, in patients with myometrial invasion, all seven UtA hemodynamic parameters (PSV, EDV, TAmax, TAmean, S/D, PI and RI) were associated significantly with MTX resistance (all $P < 0.01$), as were the three impedance ratios of TCV

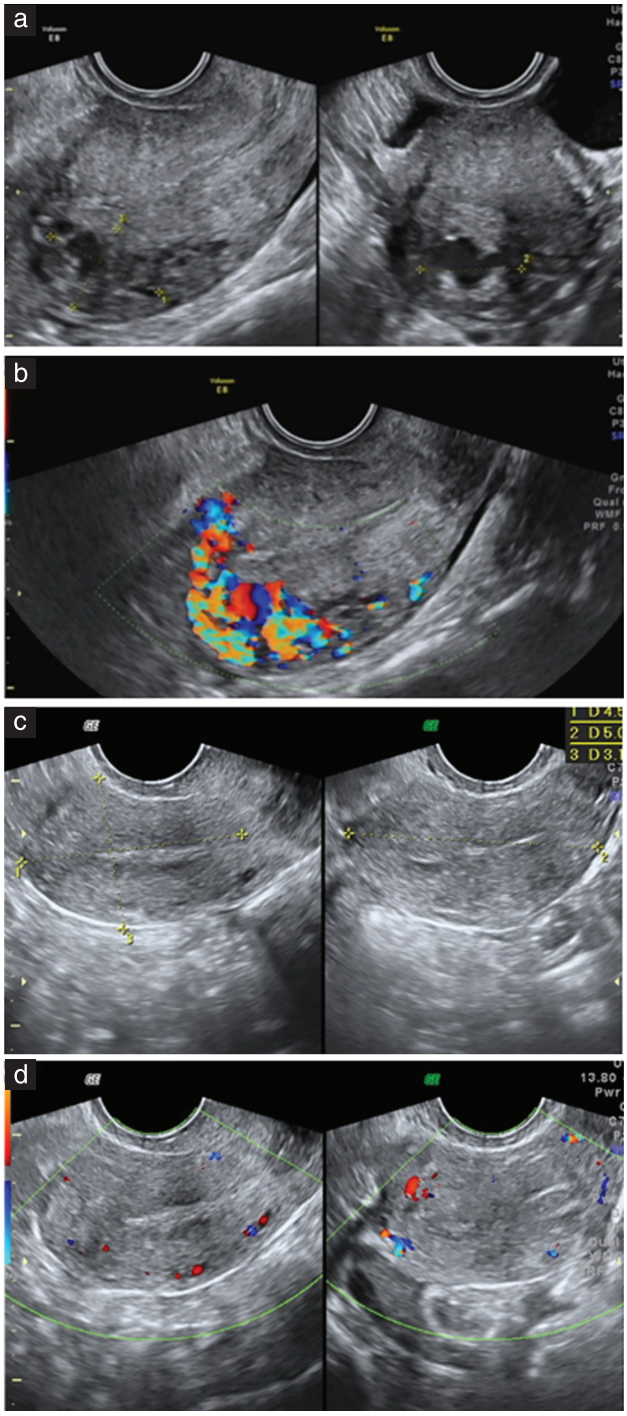


**Figure 2** Difference in uterine vascularization between patient with low-risk gestational trophoblastic neoplasia (GTN) and myometrial invasion (a,b) and patient with low-risk GTN without myometrial invasion (c,d). In the case with detectable myometrial invasion, heterogeneous mass is seen on grayscale ultrasound (a) and myometrial vascular-enriched mass on color Doppler (b). In patient without invasion, myometrium appears normal on both grayscale ultrasound (c) and color Doppler (d).

(S/D, PI and RI; all $P < 0.05$). Clinical characteristics including the FIGO score, pretreatment serum hCG level and uterine lesion volume, were identified as significant predictors for MTX resistance (all $P < 0.01$) (Table 2).

On multivariate logistic regression analysis, the FIGO score and UtA-TAmean were identified as independent predictors of MTX resistance. Both predictors were significantly higher in the MTX-resistance group compared with the MTX-responsive group ($P = 0.043$ and $P = 0.009$, respectively) (Table 2). On ROC curve analysis, the UtA-TAmean and FIGO score had an AUC of 0.765 (95% CI, 0.663–0.867) and 0.726 (95% CI, 0.611–0.841), respectively, indicating that they had discriminatory potential (Figure 3). The optimal cut-off value for UtA-TAmean was 25.9 cm/s, with 71.0% sensitivity, 69.5% specificity, 55.0% positive predictive value (PPV) and 82.0% negative predictive value (NPV). Patients with high UtA-TAmean (≥ 25.9 cm/s) had a 5.57-fold (95% CI, 2.147–14.443) increased risk for MTX resistance compared to those with low (< 25.9 cm/s) UtA-TAmean. The optimal cut-off value for FIGO score was 4, with 51.6% sensitivity, 86.4% specificity, 66.7% PPV and 77.3% NPV. Patients with FIGO score ≥ 4 had a 6.80-fold (95% CI, 2.539–18.963) increased risk for MTX resistance compared to those with low (< 4) FIGO score.

The univariate logistic regression analysis of possible predictors of resistance to single-agent actinomycin-D following MTX resistance is presented in Table S6. None of the evaluated clinical or ultrasound parameters obtained before MTX treatment was predictive of resistance to actinomycin-D as a second-line treatment.

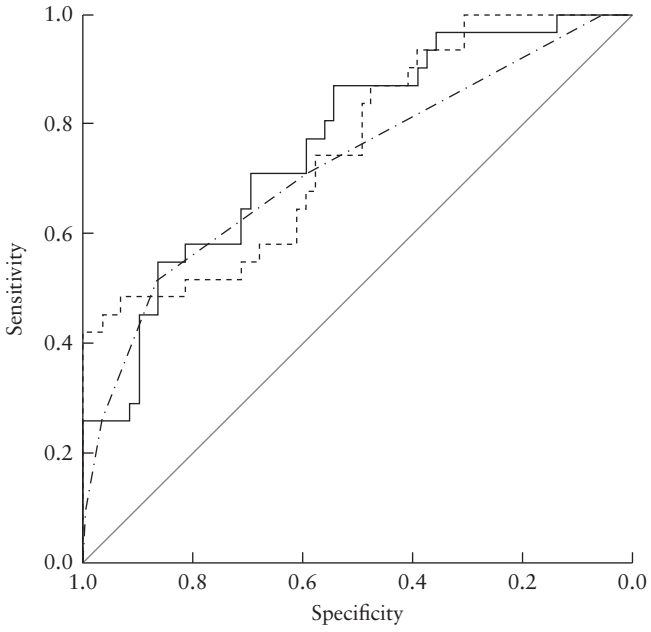


**Figure 3** Receiver-operating-characteristics curves for prediction of methotrexate resistance by uterine artery time-averaged mean velocity (——), International Federation of Gynecology and Obstetrics (FIGO) score (– · –) and the predictive model combining these two variables (- - - -), in patients with low-risk gestational trophoblastic neoplasia and myometrial invasion.

### Development of predictive model for MTX resistance in cases with myometrial invasion

FIGO score and UtA-TAmean were integrated into the predictive model as $y = -2.95332 + 0.41696 \times \text{FIGO score} + 0.03551 \times \text{UtA-TAmean}$. The model had an AUC of 0.757 (95% CI, 0.653–0.862) for the prediction of MTX resistance (Figure 3). The optimal cut-off value was 0.50622, on the maximal Youden's index, which had 45.2% sensitivity, 96.6% specificity, 87.5% PPV and 77.0% NPV. The odds ratio was 2.718 (95% CI, 1.665–4.438). Figure 4 shows ultrasound findings in two patients with model-predicted probability of MTX resistance above and below this cut-off, respectively.

As shown in Figure 5a, the Doppler-based predictive model is able to differentiate cases into low (< 10%), intermediate (10–90%) and high (> 90%) predicted probability of MTX resistance, based on the threshold values of −1.59544 (the 10$^{th}$ centile) and 0.10046 (the 90$^{th}$ centile). In the low-probability group, 14.3% (4/28; 95% CI, 5.7–31.5%) and 85.7% (24/28; 95% CI, 68.5–94.3%) of patients were MTX resistant and MTX responsive, respectively. In the high-probability group, 77.8% (14/18; 95% CI, 54.8–91.0%) and 22.2% (4/18; 95% CI, 9.0–45.2%) of patients were MTX resistant and MTX responsive, respectively. However, using FIGO score only, there was significant overlap of risk scores between the two groups (Figure 5c).

Using leave-one-out cross-validation for the training dataset ($n = 90$), 22 cases were predicted to be MTX resistant, of which 68.2% (15/22) became resistant, while 68 cases were predicted to be MTX responsive, of which 76.5% (52/68) responded well to MTX treatment. Therefore, the predictive model had a sensitivity of 48.4% (95% CI, 32.0–65.2%), specificity of 88.1% (95% CI, 77.5–94.1%) and accuracy of 74.4% (95% CI, 64.5–82.3%).

### Internal validation in the independent-sample test set

The clinical and ultrasound features of the 37 patients included in the test dataset are presented in Table 1. Among them, 33 patients had detectable uterine myometrial invasion, including 20 MTX-responsive cases and 13 MTX-resistant cases. All four cases (4/4; 100%) with calculated probability values for MTX resistance above the optimal cut-off value of 0.50622 had MTX resistance. Based on the validation dataset, the prediction model had a sensitivity of 30.8% (95% CI, 12.7–57.6%), specificity of 100% (95% CI, 84.8–100%) and accuracy of 72.7% (95% CI, 55.8–84.9%). Based on the thresholds established from the training set, there were no cases (0/6) of MTX resistance in the group with low probability of resistance and no cases (0/6) of MTX response in the group with high probability of MTX resistance (Figure 5b).

## DISCUSSION

Vascularization is a significant feature of tumor development that is associated with drug resistance[12–14].

However, tumor vascularity is not included in the current clinical evaluation system for GTN, such as the FIGO prognostic scoring system. In this study, by adding a Doppler marker of tumor vascularity to improve the predictive power of the FIGO score, we developed a predictive model for MTX resistance in low-risk GTN before treatment. This Doppler-based predictive model achieved 96.6% specificity and can stratify patients with low-risk GTN into low, intermediate and high probability of MTX resistance. In accordance with

**Table 2** Univariate and multivariate logistic regression analysis of possible predictors of methotrexate (MTX) resistance in 90 patients in training set with low-risk gestational trophoblastic neoplasia and myometrial invasion

| *Parameter* | *Total (n = 90)* | *MTX responsive (n = 59)* | *MTX resistant (n = 31)* | *Univariate analysis (OR (95% CI))* | *Multivariate analysis (OR (95% CI))* |
|---|---|---|---|---|---|
| Age (years) | 32.9 (15–56) | 33.1 (20–56) | 32.5 (15–53) | 0.993 (0.953–1.035) | |
| Antecedent pregnancy | | | | 0.783 (0.354–1.731) | |
| Hydatidiform mole | 82 (91.1) | 55 (93.3) | 27 (87.1) | | |
| Spontaneous miscarriage | 5 (5.6) | 1 (1.7) | 4 (12.9) | | |
| Term pregnancy | 2 (2.2) | 2 (3.4) | 0 (0.0) | | |
| Unknown | 1 (1.1) | 1 (1.7) | 0 (0.0) | | |
| Interval from antecedent pregnancy (days) | 71.3 (12–913) | 45.2 (12–180) | 118.7 (19–913) | 1.008 (0.998–1.019) | |
| FIGO stage | | | | 1.112 (0.713–1.733) | |
| I | 33 (36.7) | 23 (39.0) | 10 (32.3) | | |
| III | 57 (63.3) | 36 (61.0) | 21 (67.7) | | |
| FIGO score | 2.59 (0–6) | 2.12 (0–5) | 3.39 (1–6) | 1.857 (1.303–2.646) | 1.517 (1.014–2.271) |
| Pretreatment hCG (IU/L) | 14 395.92 (131–77 634) | 10 467.58 (131–57 563) | 21 516.04 (280–77 634) | 2.245 (1.115–4.518)* | |
| Uterine volume ($cm^3$) | 105.45 (26.72–406.56) | 93.98 (26.72–366.04) | 126.24 (39.89–406.56) | 1.009 (1.001–1.016) | |
| Tumor volume ($cm^3$) | 17.26 (0.15–113.03) | 10.95 (0.37–43.41) | 28.69 (0.15–113.03) | 1.044 (1.017–1.072) | |
| TCV-PSV (cm/s) | 48.87 (6.47–185.66) | 48.03 (6.47–183.77) | 50.40 (9.15–185.66) | 1.002 (0.989–1.014) | |
| TCV-EDV (cm/s) | 28.40 (2.89–109.20) | 27.66 (2.93–109.20) | 29.74 (2.89–87.77) | 1.004 (0.984–1.024) | |
| TCV-TAmax (cm/s) | 36.30 (4.02–136.98) | 35.29 (4.02–136.98) | 38.14 (5.23–130.38) | 1.003 (0.987–1.020) | |
| TCV-TAmean (cm/s) | 14.20 (1.41–68.24) | 14.00 (1.41–68.24) | 14.57 (3.05–33.34) | 1.003 (0.967–1.040) | |
| TCV-S/D | 1.66 (1.15–3.86) | 1.73 (1.15–3.86) | 1.53 (1.16–2.33) | 0.197 (0.044–0.892) | |
| TCV-PI | 0.51 (0.14–1.51) | 0.55 (0.14–1.51) | 0.44 (0.15–0.92) | 0.092 (0.010–0.841) | |
| TCV-RI | 0.37 (0.13–0.74) | 0.39 (0.13–0.74) | 0.32 (0.14–0.57) | 0.005 (0.000–0.295) | |
| UtA-PSV (cm/s) | 96.18 (19.46–255.59) | 80.82 (19.46–211.5) | 124.02 (40.75–255.59) | 1.016 (1.009–1.024) | |
| UtA-EDV (cm/s) | 39.35 (2.10–184.44) | 29.53 (2.10–108.2) | 57.15 (4.93–184.44) | 1.024 (1.009–1.038) | |
| UtA-TAmax (cm/s) | 56.36 (7.20–223.78) | 44.01 (7.20–142.86) | 78.74 (14.11–223.78) | 1.021 (1.009–1.033) | |
| UtA-TAmean (cm/s) | 31.47 (2.55–137.35) | 22.32 (2.55–65.43) | 46.23 (8.25–137.35) | 1.044 (1.019–1.069) | 1.036 (1.009–1.064) |
| UtA-S/D | 3.43 (1.30–11.78) | 3.79 (1.30–11.78) | 2.80 (1.30–8.27) | 0.717 (0.536–0.961) | |
| UtA-PI | 1.27 (0.26–2.99) | 1.40 (0.27–2.99) | 1.03 (0.26–2.63) | 0.365 (0.168–0.793) | |
| UtA-RI | 0.62 (0.23–0.92) | 0.66 (0.23–0.92) | 0.56 (0.23–0.88) | 0.024 (0.002–0.363) | |
| UtA diameters (mm) | 2.05 (1.1–3.5) | 1.97 (1.1–3.4) | 2.18 (1.40–3.50) | 2.878 (1.070–7.744) | |

Continuous data are given as mean (range) and categorical data as *n* (%). Odds ratio (OR) and 95% CI were calculated using univariate/multivariate logistic regression analysis, comparing data between MTX-responsive and MTX-resistant patients. *For human chorionic gonadotropin (hCG), OR and 95% CI were obtained by log transformation. EDV, highest end-diastolic velocity; FIGO, International Federation of Gynecology and Obstetrics; PI, lowest pulsatility index; PSV, highest peak systolic velocity; RI, lowest resistance index; S/D, lowest systolic/diastolic flow velocity ratio; TAmax, highest time-averaged maximum velocity; TAmean, highest time-averaged mean velocity; TCV, tumor central vessel; UtA, uterine artery.

the TRIPOD statement, its predictive performance was tested by both cross-validation and internal validation on an independent-sample test set. To our knowledge, we are the first (in our presentation at the Radiological Society of North America (RSNA) meeting in 2016) to develop and validate a model to predict drug resistance based on the classification of low-risk GTN cases, with or without uterine myometrium invasion. These two distinct groups (with and without myometrial invasion) of patients with low-risk GTN had been shown to have very different uterine vascularization characteristics.

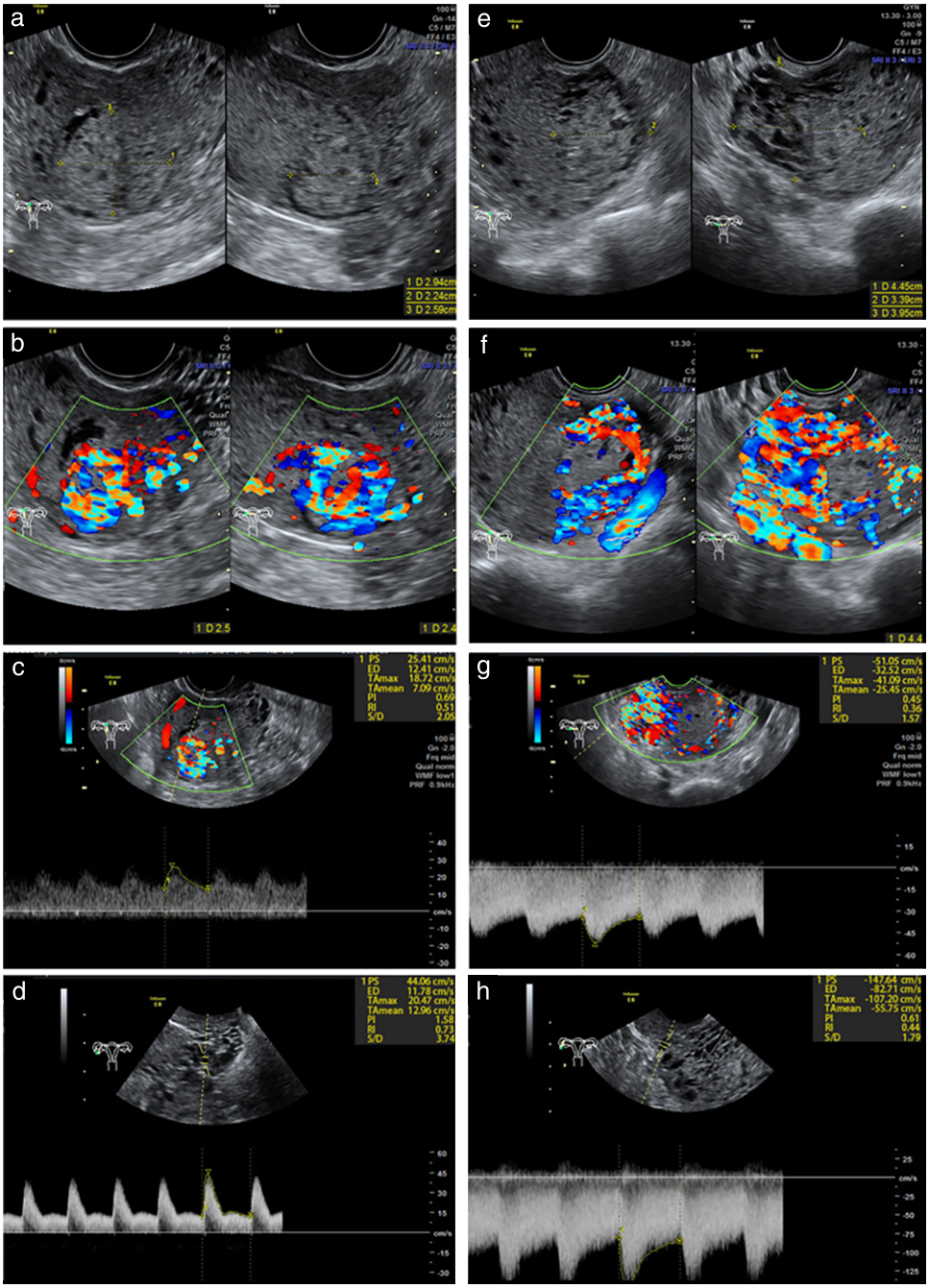


**Figure 4** Grayscale (a,e) and Doppler (b–d, f–h) images and measurement of hemodynamic parameters of tumor central vessels (c,g) and of uterine artery (d,h) in a patient with low-risk gestational trophoblastic neoplasia (GTN) who responded to methotrexate (MTX) treatment (a–d) and in a patient with low-risk GTN with MTX resistance (e–h). (a–d) This 28-year-old woman (International Federation of Gynecology and Obstetrics (FIGO) Stage I and FIGO score 1) was predicted to be MTX responsive by the Doppler-based model (calculated value of −2.0762). Before MTX therapy, her serum human chorionic gonadotropin (hCG) was 4441 IU/L, the uterine lesion measured 2.9 × 2.6 × 2.2 cm and time-averaged mean velocity in the uterine artery (UtA-TAmean) was 12.96 cm/s. After five cycles of MTX, her serum hCG declined to 2.52 IU/L. (e–h) This 44-year-old woman (FIGO Stage III and FIGO score 4) was predicted to be MTX resistant by the Doppler-based model (value of 0.69420). Before MTX therapy, her serum hCG was 54 288 IU/L, the uterine lesion measured 4.5 × 4.0 × 3.4 cm and UtA-TAmean was 55.75 cm/s. During the first three cycles, her serum hCG fluctuated on a plateau level. She was then changed to actinomycin-D treatment.

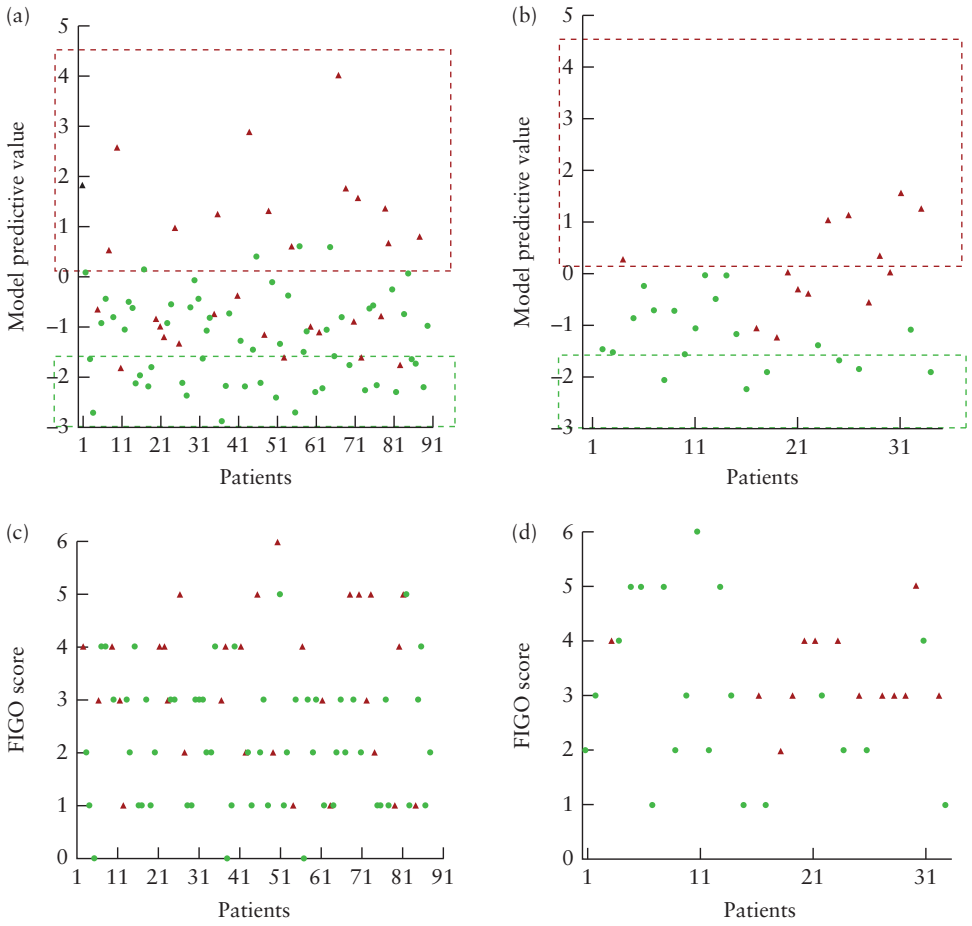


**Figure 5** Scatterplots showing distribution of probability of methotrexate (MTX) resistance as calculated by the Doppler-based predictive model (a,b) and distribution of International Federation of Gynecology and Obstetrics (FIGO) score (c,d) in MTX-responsive (●) and MTX-resistant (▲) patients in the training set (a,c) and in the test set (b,d). High-probability (- - - -) and low-probability (- - - -) cases are marked in (a) and (b).

Several ultrasound parameters have been reported to predict MTX resistance in patients with low-risk GTN, such as a sizeable myometrial invasion ($\geq 4$ cm diameter)[26], a low TCV-RI ($\leq 0.28$)[25] and a low UtA-PI ($\leq 1.0$ or 1.1)[23,24]. In this study, we analyzed 17 ultrasound features using univariate logistic regression analysis and identified 12 significant predictors of MTX resistance. Some of these predictors have been reported previously, but others, such as UtA-PSV, UtA-EDV, UtA-TAmax, UtA-TAmean, UtA-S/D, UtA-RI, TCV-S/D and TCV-PI, were shown for the first time to be prognostic markers. Furthermore, UtA-TAmean was identified on multivariate logistic regression analysis as a significant independent predictor of MTX resistance. Therefore, our results are not only consistent with previous research[23–26], but also demonstrate new Doppler predictors for MTX resistance.

UtA-TAmean might be a new marker for MTX resistance. It consists of volumetric blood-flow parameters representing intensity-weighted mean frequency obtained with all ranges of velocities along the cardiac cycle. UtA-TAmean is adaptable for capturing parabolic flow, in which flow in the center of the vessel is faster and flow along the periphery of the vessel is slower[36]. In the UtA, which is a peripheral artery vessel, higher TAmean, suggestive of increased volumetric blood flow, is due perhaps to increased perfusion in the downstream microvasculature in a uterine lesion. Pathologically, high UtA-TAmean is associated potentially with increased neoangiogenesis, enlarged vessels and severe arteriovenous shunting, which are inherent features of GTN[37]. Thus, a high UtA-TAmean is a potential marker associated with increased severity of the disease and an increased risk of MTX resistance. The measurement of UtA-TAmean was determined to be an objective and reproducible method for the assessment of blood vessel flow.

The Doppler-based predictive model for MTX resistance has the potential to guide counseling and subsequent management. For instance, cases with a model-calculated value of $< -1.59544$ ($10^{th}$ centile of the predicted probability) would be advised to receive MTX treatment, whereas cases with a model value of $> 0.10046$ ($90^{th}$ centile of the predicted probability) would be advised to receive other treatment. However, further investigation through randomized controlled trials is required to identify the most appropriate alternative chemotherapy treatment for patients who are

identified by our prediction model to be MTX resistant. In the absence of alternative chemotherapy treatment, patients with intermediate probability of MTX resistance (predicted values between −1.59544 and 0.10046) should be counseled regarding the risk of non-response to MTX (29.5% (13/44) in our cohort).

One potential limitation of this study is that the patient cohort (both the training and the test datasets) was derived from a single institution, allowing for the possibility of selection bias of studied subjects. Women recruited in the study, however, represented a consecutive series of low-risk GTN cases. Our hospital is the Center for Uterine Cancer Diagnosis & Therapy Research of Zhejiang Province. As a consequence, our results were unlikely to be subject to referral/selection bias compared with community hospitals. The future direction of this project is to obtain data from multicenter clinical studies and to validate the robustness of our Doppler-based predictive model for predicting chemoresistance in an external independent dataset.

In conclusion, we have developed a Doppler-based predictive model, which includes a non-invasive marker of tumor vascularity and the FIGO prognostic scoring system, for the prediction of MTX resistance in patients with low-risk GTN and myometrial invasion. This model has the potential to guide treatment options in these patients. External validation of the model with multi-center data is needed to confirm our findings to allow its clinical utility.

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## REFERENCES

1. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet* 2010; **376**: 717–729.
2. Ngan HY, Bender H, Benedet JL, Jones H, Montruccoli GC, Pecorelli S, FIGO Committee on Gynecologic Oncology. Gestational Trophoblastic Neoplasia, FIGO 2000 staging and classification. *Int J Gynaecol Obstet* 2003; **83** (Suppl 1):175–177.
3. Alazzam M, Tidy J, Osborne R, Coleman R, Hancock BW, Lawrie TA. Chemotherapy for resistant or recurrent gestational trophoblastic neoplasia. *Cochrane Database Syst Rev* 2016; **2016**: CD008891.
4. Seckl MJ, Sebire NJ, Fisher RA, Golfier F, Massuger L, Sessa C. Gestational trophoblastic disease: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2013; **24** (Suppl 6): vi39–50.
5. Lawrie TA, Alazzam M, Tidy J, Hancock BW, Osborne R. First-line chemotherapy in low-risk gestational trophoblastic neoplasia. *Cochrane Database Syst Rev* 2016; **2016**: CD007102.
6. Chalouhi GE, Golfier F, Soignon P, Massardier J, Guastalla JP, Trillet-Lenoir V, Schott AM, Raudrant D. Methotrexate for 2000 FIGO low-risk gestational trophoblastic neoplasia patients: efficacy and toxicity. *Am J Obstet Gynecol* 2009; **200**: 643e1–6.
7. Miller CR, Chappell NP, Sledge C, Leath CA 3rd, Phippen NT, Havrilesky LJ, Barnett JC. Are different methotrexate regimens as first line therapy for low risk gestational trophoblastic neoplasia more cost effective than the dactinomycin regimen used in GOG 0174? *Gynecol Oncol* 2017; **144**: 125–129.
8. McNeish IA, Strickland S, Holden L, Rustin GJ, Foskett M, Seckl MJ, Newlands ES. Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000. *J Clin Oncol* 2002; **20**: 1838–1844.
9. Aghajanian C. Treatment of low-risk gestational trophoblastic neoplasia. *J Clin Oncol* 2011; **29**: 786–788.
10. Lurain JR. Gestational trophoblastic disease II: classification and management of gestational trophoblastic neoplasia. *Am J Obstet Gynecol* 2011; **204**: 11–18.
11. Verri D, Pasciuto T, Epstein E, Fruscio R, Mascilini F, Moro F, Scambia G, Valentin L, Testa AC. GestaTIonal TrophoblAstic NeoplasIa Ultrasound assessMent: TITANIUM study. *Int J Gynecol Cancer* 2019; **29**: 1216–1220.
12. Yang J, Liu X, Nyland SB, Zhang R, Ryland LK, Broeg K, Baab KT, Jarbadan NR, Irby R, Loughran TP Jr. Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. *Blood* 2010; **115**: 51–60.
13. Linderholm BK, Hellborg H, Johansson U, Skoog L, Lehtio J. Vascular endothelial growth factor receptor 2 and downstream p38 mitogen-activated protein kinase are possible candidate markers of intrinsic resistance to adjuvant endocrine treatment in steroid receptor positive breast cancer. *Breast Cancer Res Treat* 2011; **125**: 457–465.
14. Carmo CR, Lyons-Lewis J, Seckl MJ, Costa-Pereira AP. A novel requirement for Janus kinases as mediators of drug resistance induced by fibroblast growth factor-2 in human cancer cells. *PLoS One* 2011; **6**: e19861.
15. Allen SD, Lim AK, Seckl MJ, Blunt DM, Mitchell AW. Radiology of gestational trophoblastic neoplasia. *Clin Radiol* 2006; **61**: 301–313.
16. Jain KA. Gestational trophoblastic disease: pictorial review. *Ultrasound Q* 2005; **21**: 245–253.
17. Oguz S, Sargin A, Aytan H, Kelekci S, Dumanli H. Doppler study of myometrium in invasive gestational trophoblastic disease. *Int J Gynecol Cancer* 2004; **14**: 972–979.
18. Kani KK, Lee JH, Dighe M, Moshiri M, Kolokythas O, Dubinsky T. Gestational trophoblastic disease: multimodality imaging assessment with special emphasis on spectrum of abnormalities and value of imaging in staging and management of disease. *Curr Probl Diagn Radiol* 2012; **41**: 1–10.
19. Bidzinski M, Lemieszczuk B, Drabik M. The assessment of value of transvaginal ultrasound for monitoring of gestational trophoblastic disease treatment. *Eur J Gynaecol Oncol* 1997; **18**: 541–543.
20. Lin LH, Bernardes LS, Hase EA, Fushida K, Francisco RP. Is Doppler ultrasound useful for evaluating gestational trophoblastic disease? *Clinics (Sao Paulo)* 2015; **70**: 810–815.
21. Huang SC, Chou CY. The role of transvaginal ultrasonography in the management of gestational trophoblastic tumor. *Am J Obstet Gynecol* 1995; **172**: 1063–1064.
22. Agarwal R, Harding V, Short D, Fisher RA, Sebire NJ, Harvey R, Patel D, Savage PM, Lim AK, Seckl MJ. Uterine artery pulsatility index: a predictor of methotrexate resistance in gestational trophoblastic neoplasia. *Br J Cancer* 2012; **106**: 1089–1094.
23. Agarwal R, Strickland S, McNeish IA, Patel DC, Foskett M, Boultbee JE, Newlands ES, Seckl MJ. Doppler ultrasonography of the uterine artery and the response to chemotherapy in patients with gestational trophoblastic tumors. *Clin Cancer Res* 2002; **8**: 1142–1147.
24. Long MG, Boultbee JE, Langley R, Newlands ES, Begent RH, Bagshawe KD. Doppler assessment of the uterine circulation and the clinical behaviour of gestational trophoblastic tumours requiring chemotherapy. *Br J Cancer* 1992; **66**: 883–887.
25. Oguz S, Sargin A, Aytan H, Kelekci S, Dumanli H. Doppler study of myometrium in invasive gestational trophoblastic disease. *Int J Gynecol Cancer* 2004; **14**: 972–979.
26. Epstein E, Joneborg U. Sonographic characteristics of post-molar gestational trophoblastic neoplasia at diagnosis and during follow-up, and its relation to methotrexate resistance. *Ultrasound Obstet Gynecol* 2020; **56**: 759–765.
27. Wang X. Gestational trophoblastic disease. In *Gynecology and Obstetrics*, Xie X, Kong B, Duan T (eds). People's Medical Publishing House: Beijing, 2018; 324–332.
28. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD): the TRIPOD Statement. *Br J Surg* 2015; **102**: 148–158.
29. Feng F, Xiang Y. Surgical management of chemotherapy-resistant gestational trophoblastic neoplasia. *Expert Rev Anticancer Ther* 2010; **10**: 71–80.
30. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. *Gynecol Oncol* 2009; **112**: 654–662.
31. Morgan JM, Lurain JR. Gestational trophoblastic neoplasia: an update. *Curr Oncol Rep* 2008; **10**: 497–504.
32. Cao Y, Xiang Y, Feng F, Wan X, Yang X. Surgical resection in the management of pulmonary metastatic disease of gestational trophoblastic neoplasia. *Int J Gynecol Cancer* 2009; **19**: 798–801.
33. Testa AC, Ferrandina G, Distefano M, Fruscella E, Mansueto D, Basso D, Salutari V, Scambia G. Color Doppler velocimetry and three-dimensional color power angiography of cervical carcinoma. *Ultrasound Obstet Gynecol* 2004; **24**: 445–452.
34. Alcazar JL, Castillo G, Martinez-Monge R, Jurado M. Transvaginal color Doppler sonography for predicting response to concurrent chemoradiotherapy for locally advanced cervical carcinoma. *J Clin Ultrasound* 2004; **32**: 267–272.
35. Molinaro AM, Simon R, Pfeiffer RM. Prediction error estimation: a comparison of resampling methods. *Bioinformatics* 2005; **21**: 3301–3307.
36. Blanco P. Volumetric blood flow measurement using Doppler ultrasound: concerns about the technique. *J Ultrasound* 2015; **18**: 201–204.
37. Lurain JR. Gestational trophoblastic disease I: epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. *Am J Obstet Gynecol* 2010; **203**: 531–539.

## SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

**Table S1** Uterine artery hemodynamic parameters in patients with low-risk gestational trophoblastic neoplasia, according to whether they had myometrial invasion

**Table S2** Univariate logistic regression analysis of possible predictors of methotrexate resistance in 20 patients in training set with low-risk gestational trophoblastic neoplasia and no myometrial invasion

**Tables S3–S5** Intraobserver (Table S3), interobserver (Table S4) and interdevice (Table S5) repeatability of uterine artery hemodynamic measurements

**Table S6** Univariate logistic regression analysis of possible predictors of resistance to single-agent actinomycin-D after methotrexate resistance in 90 patients in training set with low-risk gestational trophoblastic neoplasia and myometrial invasion

**Appendix S1** Intraobserver, interobserver and interdevice repeatability of Doppler measurements in uterine arteries

**Figure S1** Doppler assessment of uterine artery. (a) Uterine artery was visualized on grayscale ultrasound at level of uterine isthmus. (b) Hemodynamic parameters of uterine artery were measured using pulsed Doppler.

**Figure S2** Bland–Altman plots of agreement between two measurements by the same observer (intraobserver reproducibility) of the uterine artery peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAmax), time-averaged mean velocity (TAmean), systolic/diastolic flow velocity ratio (S/D), pulsatility index (PI) and resistance index (RI) using GE Voluson E10. Solid line indicates mean difference (PSV, −0.34; EDV, −0.08; TAmax, −0.39; TAmean, −0.43; S/D, 0.11; PI, 0.08; RI, 0.00) and dashed lines mark 95% limits of agreement.

**Figure S3** Bland–Altman plots of agreement between two measurements by two different observers (interobserver reproducibility) of the uterine artery peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAmax), time-averaged mean velocity (TAmean), systolic/diastolic flow velocity ratio (S/D), pulsatility index (PI) and resistance index (RI) using GE Voluson E10. Solid line indicates mean difference (PSV, −0.41; EDV, −0.09; TAmax, −0.58; TAmean, 0.06; S/D, −0.04; PI, 0.02; RI, 0.00) and dashed lines mark 95% limits of agreement.

**Figure S4** Bland–Altman plots of two measurements by the same observer using GE Voluson E10 and Philips EPIQ 5 (interdevice reproducibility) of the uterine artery peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged maximum velocity (TAmax), time-averaged mean velocity (TAmean), systolic/diastolic flow velocity ratio (S/D), pulsatility index (PI) and resistance index (RI). Solid line indicates mean difference (PSV, −0.43; EDV, 0.64; TAmax, 0.88; TAmean, 0.83; S/D, −0.30; PI, −0.16; RI, −0.01) and dashed lines mark 95% limits of agreement.