Research in Molecular Medicine

Research Paper

Study of Factors Affecting the Failure of Methotrexate Treatment in Patients Diagnosed With Tubal Ectopic Pregnancy

Afsaneh Bashiri[1], Zinatossadat Bouzari[1, 2*], Mehrzad Netadj[3], Shahla Yazdani[1, 2], Soraya Khafri[4]

1. Department of Obstetrics and Gynecology, Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
2. Clinical Research Development Unit, Rohani Hospital, Babol University of Medical Sciences, Babol, Iran.
3. Department of Sociology, Mississippi State University, Starkville, United States of America.
4. Social Determinants of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.

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Citation Bashiri A, Bouzari Z, Netadj M, Yazdani Sh, Khafri S. Study of Factors Affecting the Failure of Methotrexate Treatment in Patients Diagnosed With Tubal Ectopic Pregnancy. Research in Molecular Medicine. 2026; 14(2):89-102. http://dx.doi.org/10.22034/rmm.14.2.1462.1

http://dx.doi.org/10.22034/rmm.14.2.1462.1

Article Type:
Research Paper

Article info:
Received: 26 Dec 2025
Revised: 21 Feb 2026
Accepted: 25 Feb 2026

Keywords:
Ectopic pregnancy, Methotrexate (MTX), Treatment failure, Inflammatory markers, Neutrophil-to-lymphocyte ratio

ABSTRACT

Background: Methotrexate (MTX) is the first-line treatment for stable tubal ectopic pregnancy (EP); however, its failure can lead to serious complications. This study aimed to identify predictive factors for MTX treatment failure to improve patient selection.

Materials and Methods: In this retrospective cross-sectional study, 371 patients with EP treated with a single-dose MTX regimen (50 mg/m²) at Rouhani Hospital, Babol City, Iran (2013-2024), were analyzed. Demographic, clinical (beta–human chorionic gonadotropin, pain), sonographic (mass size, free fluid), and inflammatory indices (neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and systemic immune-inflammation index [SII]) data were extracted from medical records. Treatment failure was defined as the need for surgery. Univariate and multivariate logistic regression were used to identify independent predictors, with receiver operating characteristic (ROC) curves assessing their accuracy.

Results: The treatment failure rate was 9.7% (n=36). Multivariate analysis identified a larger mass size (OR=1.32; 95% CI, 1.15%, 1.52%; P<0.001) and a higher SII (OR=1.03; 95% CI, 1%, 1.06%; P=0.036) as significant independent predictors of failure. A higher red blood cell count and platelet distribution width were protective factors. Receiver operating characteristic (ROC) analysis demonstrated high predictive accuracy for mass size (area under the curve [AUC]=0.85) and SII (AUC=0.82) in distinguishing treatment outcomes.

Conclusion: EP mass size ≥23 mm and elevated SII are strong predictors of MTX treatment failure. These readily available parameters can serve as valuable tools for clinical decision-making, suggesting that primary surgery may be a preferable option for patients with these risk factors to avoid the complications of failed medical therapy.

*** Corresponding Author:**
Zinatossadat Bouzari, MD.
Address: *Clinical Research Development Unit, Rohani Hospital, Babol University of Medical Sciences, Babol, Iran.*
Phone: *+98 (920) 4238994*
E-mail: *z_b412003@yahoo.com*

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Introduction

Ectopic pregnancy (EP) is a life-threatening gynecological emergency and a leading cause of maternal mortality during the first trimester, accounting for approximately 6% of pregnancy-related deaths [1, 2]. It occurs when a fertilized ovum implants outside the endometrial cavity, most commonly in the fallopian tubes (95% of cases). If left undiagnosed or untreated, EP can lead to tubal rupture, severe intra-abdominal hemorrhage, and catastrophic outcomes. Over the past two decades, the incidence of EP has risen significantly, likely due to increased risk factors such as pelvic inflammatory disease (PID), tubal surgery, assisted reproductive technologies, and smoking [3]. The medical management of tubal EP with methotrexate (MTX) represents a significant advancement in gynecological care, offering a non-surgical alternative to appropriately selected patients. However, the effectiveness of single-dose MTX therapy varies considerably, with reported failure rates ranging from 7% to 30%, necessitating additional doses or emergency surgery, which increases patient morbidity and healthcare burdens [4]. This variability makes the identification of reliable predictors crucial for clinical decision-making and optimal patient selection. Multiple studies have investigated various factors that could influence treatment outcomes, ranging from biochemical markers to imaging findings and hematological parameters. The most consistently identified predictors of MTX treatment failure center around endocrine and ultrasonographic features. Elevated initial serum beta–human chorionic gonadotropin (β-hCG) levels, particularly those exceeding 5,000 IU/L, have been strongly associated with reduced treatment success across multiple investigations [5, 6]. The presence of embryonic cardiac activity demonstrates a particularly strong correlation with treatment failure, with odds ratios indicating substantially increased risk [7, 8]. Similarly, the visualization of a yolk sac and the presence of an adnexal mass greater than 3.5-4 cm on ultrasound examination have been significantly linked to poorer outcomes [5, 7, 9, 10]. Other sonographic findings, including increased endometrial thickness and the presence of hemoperitoneum, have also shown predictive value [5, 7, 9]. The pattern of β-hCG change following MTX administration provides valuable prognostic information. Studies have indicated that an insufficient decrease in β-hCG levels (typically defined as <15% reduction) between days one and four after treatment strongly predicts therapeutic failure [7, 11]. Some researchers have proposed specific threshold values for initial β-hCG levels that may help identify optimal candidates for medical management, with one study establishing 1,375 IU/mL as a significant cut-off point [11]. Additionally, the rate of β-hCG increase in the forty-eight hours preceding treatment has been identified as a significant predictor [7]. The potential role of inflammatory hematological markers as predictors has yielded conflicting results. Several studies have examined various ratios, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocytetolymphocyte ratio, and systemic immune-inflammation index (SII) as novel indicators of treatment response, with inconsistent findings [12, 13]. While some investigations found no significant differences in these parameters between successful and unsuccessful treatment groups [14], others reported lower values in patients who responded successfully to MTX therapy [6]. The delta neutrophil index has emerged as a promising predictor in some recent studies, potentially offering superior predictive capability compared to traditional parameters [15]. Demographic and clinical factors also appear to influence treatment outcomes. Younger maternal age has been associated with higher success rates, while the presence of pelvic pain at presentation may indicate a reduced likelihood of successful medical management [8]. Historical factors, including previous ectopic pregnancies and PID, have also been identified as significant predictors [7]. The considerable variation in reported predictive factors underscores the complex nature of EP and its response to medical treatment. While certain parameters demonstrate consistent predictive value across multiple studies, others require further validation through larger, prospective investigations. The integration of multiple predictive factors rather than reliance on single parameters may provide the most accurate assessment of treatment prognosis. Future research should develop integrated prediction models combining clinical, sonographic, and laboratory parameters. Region-specific validation studies remain crucial for optimizing patient selection across diverse populations. In Iran, where access to advanced healthcare may vary, identifying low-cost, readily available predictors such as routine blood tests and ultrasound findings could significantly improve clinical decision-making and reduce adverse outcomes. This study evaluated the demographic, clinical, and laboratory factors associated with MTX treatment failure in EP patients. The research was conducted at a tertiary care center in Babol, Iran, between 2013 and 2024. The purpose of this study was to identify and evaluate predictive factors for MTX treatment failure in EP. This encompassed an analysis of systemic inflammatory markers (NLR, PLR, SII), hematologic parameters (hemoglobin, platelet count, platelet distribution width [PDW]), key

sonographic features (gestational mass size, free fluid), and relevant demographic factors (age, parity, history of abortion) to determine their association with treatment outcomes and their utility in clinical decision-making.

Materials and Methods

Study design

This study was designed as a retrospective, cross-sectional, analytical investigation aimed at identifying predictive factors for MTX treatment failure in patients diagnosed with EP. The study utilized secondary data extracted from the medical records of patients treated at Ayatollah Rouhani Hospital in Babol City, Iran, between 2013 and 2024.

Study population and sampling

The study population included all women diagnosed with EP who received single-dose MTX (50 mg/m² intramuscularly) as first-line treatment, with a census sampling method employed to review all eligible medical records from this period. The inclusion criteria required a confirmed diagnosis of tubal EP based on either serum β-hCG levels above the discriminatory zone (≥1161 IU/L) with no intrauterine gestational sac on transvaginal ultrasound, or plateauing/suboptimal β-hCG rise (<50% over 48 hours), along with complete medical record availability. The exclusion criteria included patients with hematologic disorders affecting cell counts, heterotopic pregnancy, presence of fetal cardiac activity on ultrasound, initial β-hCG >10,000 IU/L, ectopic mass size ≥4 cm, cesarean scar pregnancy, known hepatic or renal disorders, contraindications to MTX, or concurrent use of potentially hepatotoxic or nephrotoxic drugs, in accordance with the center's treatment protocol.

Sample size determination and data collection methodology

This study employed a rigorous methodology to examine factors influencing MTX treatment outcomes in EP cases. The sample size determination was based on preliminary data showing a mean NLR difference of 0.8 between treatment success and failure groups, with calculations indicating a minimum requirement of 180 patients to achieve 80% power at a 95% confidence level. Ultimately, the research team included all 371 eligible cases identified between 2013 and 2024 to ensure adequate statistical power and account for potential missing data. Data collection was conducted systematically using a detailed, structured checklist that captured comprehensive patient information across multiple categories. Demographic variables, including patient age in years and body mass index (BMI) in kg/m², were recorded for all participants. Medical history documentation focused on three key factors: Previous genital surgery (categorized as yes/no), history of intrauterine device use (yes/no), and prior diagnosis of PID (yes/no). Laboratory parameters formed a crucial component of the data collection, with particular attention given to complete blood count indices, such as NLR, PLR, and systemic immune-inflammation index, along with platelet count measurements in $\times 10^3/\mu L$ and PDW values. Initial serum β-hCG levels in mIU/mL and hemoglobin concentrations in g/dL were also carefully documented. Ultrasound findings provided important diagnostic information, including precise measurements of ectopic mass dimensions in millimeters, the presence or absence of free pelvic fluid, endometrial thickness in millimeters, and visualization of a gestational sac or yolk sac. Clinical presentation details incorporated gestational age calculations in weeks based on last menstrual period or ultrasound dating, along with documentation of presenting symptoms, including abdominal pain, vaginal bleeding, and current smoking status, all recorded as yes/no responses. Treatment outcomes were categorized as either success or failure, with failure defined by the need for surgical intervention. Obstetric history variables included parity count, number of previous miscarriages, and history of infertility. All data were extracted from patient medical records with meticulous attention to detail, ensuring the reliability and completeness of the dataset for subsequent statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS software, version 22, including descriptive statistics (Mean±SD, frequencies), comparative tests (t-tests, Mann-Whitney, chi-square), and predictive modeling (univariate/multivariate logistic regression, receiver operating characteristic [ROC] curve analysis) with significance at $P<0.05$. Variables with $P<0.1$ in the univariable regression analysis, as well as those considered clinically important, were entered into the multivariable logistic regression model.

Results

This study investigated the factors associated with MTX treatment failure in patients with EP. According to the study protocol, it is important to note that the total number of patients in this study was 418. According to the variable of EP location, 205(49.05%) were right tube, 164(39.23%) were left tube, 4(0.95%) were right breech,

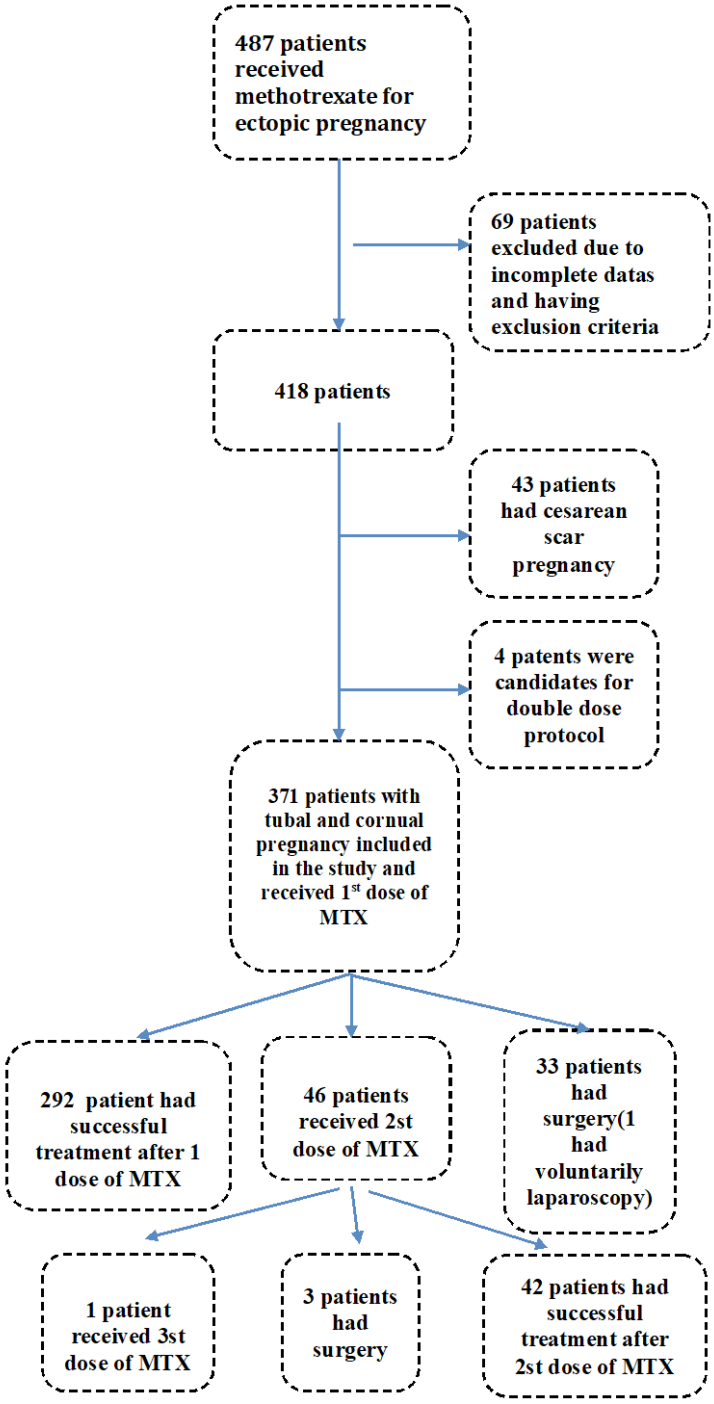


Figure 1. Patient flowchart and treatment outcomes

2(0.47%) were left breech, and 43(10.28%) were previous cesarean scar sites. Therefore, the 43 patients whose EP location was the same as the previous cesarean scar site and the 4 patients who were candidates for the two-dose MTX regimen from the beginning were excluded from the analysis; therefore, the total number of patients on this basis was 371 (Figure 1).

Baseline characteristics of the study population

The demographic and clinical characteristics of the 371 patients included in this study are summarized in Table 1. The cross-sectional study had a mean age of 30.73±6.07 years. The mean BMI was 26.64±4.24 kg/m², calculated from a mean height of 163.84±5.92 cm and a mean weight of 71.53±12.11 kg. Regarding the

Table 1. Demographic and clinical characteristics of the study (n=371)

Variables	Mean±SD
Age (y)	30.73±6.07
Height (cm)	163.84±5.92
Weight (kg)	71.53±12.11
BMI (kg/m²)	26.64±4.24
Initial β-hCG (IU/L)	1790.94±2164.92
β-hCG at treatment (IU/L)	1889.46±2232.03
EP mass size (mm)	24.81±10.12

Abbreviations: EP: Ectopic pregnancy; β-hCG: β–human chorionic gonadotropin; BMI: Body mass index.

key clinical parameters for EP, the mean initial serum β-hCG level was 1790.94±2164.92 IU/L. At the time of MTX administration, the mean β-hCG level was slightly higher, at 1889.46±2232.03 IU/L. The mean size of the EP mass, as measured by ultrasonography, was 24.81±10.12 mm. Based on the non-normal distribution of β-hCG levels, these variables were reported as median (interquartile range). The median initial β-hCG level was 907 (407–2346) IU/L, and the median β-hCG level at the time of drug administration was 989 (477–2375) IU/L.

Table 2 presents the laboratory parameters for the study. Hematological analysis revealed a mean white blood cell (WBC) count of 8187.87±1737.10 /µL. The mean red blood cell (RBC) count was 4.11±0.54 million/µL, with a corresponding mean hemoglobin level of 11.63±2.05 g/dL and a mean hematocrit of 34.14±3.41%. The mean platelet count was 238,641.51±53,342.02 /µL, with a mean platelet volume (MPV) of 10.16±2.31 fL and a PDW of 12.55±2.45%. The calculated systemic inflammatory indices, which are of primary interest in this study, showed the following mean values: The NLR was 2.14±0.69, the PLR was 8.41±3.03, and the SII was 515.22±208.86.

Clinical history, presentation, and treatment outcomes

This study of 371 patients with EP was predominantly Rh-positive (89.8%) and had a blood group distribution typical of the general population. The majority were multiparous, with a history of at least one pregnancy (75.5%) and one delivery (61.5%) (Table 3), and nearly two-thirds had no history of abortion (64.3%). The past medical history was notable for a high rate of pelvic surgery (48.2%) but low rates of infertility, endometriosis, and smoking (Table 4). Upon presentation, most patients reported vaginal bleeding (74.1%) and abdominal pain (62.3%). The EP was most frequently located in the right tube (55.3%) at a gestational age of 7-8 weeks (62.6%). Sonographic findings showed that the presence of a gestational sac (10.0%), fetal pole (6.5%), or yolk sac (6.7%) was rare, while free fluid was present in a third of cases (33.2%). The primary treatment was a single-dose MTX regimen (78.7%), which was successful in avoiding surgery for the vast majority of patients (90.3%) (Table 5).

Comparison of the performance of different indices in predicting treatment outcomes

The comparative graph of ROC curves shows that the ectopic mass size variable (area under the curve [AUC] =0.8499) has the best performance and has a high ability to discriminate the results. Based on ROC curve analysis and using the Youden index, the optimal cut-off point for the SII was determined to be approximately 300, which had a sensitivity of approximately 92.5% and a specificity of approximately 15% in predicting MTX treatment failure. The RBC variable (AUC=0.4298) has poor performance, and the AUC close to 0.5 indicates that the model does not have a significant predictive ability. The PDW variable (AUC=0.4159) has the weakest performance and is not suitable for prediction. Ectopic mass size and SII were identified as the best indicators in this model (Figure 2).

Results of univariate logistic regression analysis

Univariate logistic regression analysis was performed to identify factors associated with MTX treatment failure in patients with EP. The analysis revealed several significant predictors while also identifying variables showing clinically relevant trends.

Table 2. Laboratory parameters of the study (n=371)

Variables		Mean±SD
Hematology	WBC (count/µL)	8187.87±1737.1
	RBC (million/µL)	4.11±0.54
	Hemoglobin (g/dL)	11.63±2.05
	Hematocrit (%)	34.14±3.41
Red cell indices	MCV (fL)	106.44±442.63
	MCH (pg)	28.15±4.75
	MCHC (g/dL)	33.26±1.72
Platelet parameters	Platelets (count/µL)	238641.51±53342.02
	MPV (fL)	10.16±2.31
	PDW (%)	12.55±2.45
Differential count	PMN (%)	62.41±10.48
	Lymph (%)	31.06±13.7
Inflammatory indices	NLR	2.14±0.69
	PLR	8.41±3.03
	SII	515.22±208.86
Liver and renal function	AST (U/L)	21.04±8.53
	ALT (U/L)	18.8±9.73
	BUN (mg/dL)	12.32±6.6
	Creatinine (mg/dL)	0.86±1.39

Abbreviations: PLR: Platelet-to-lymphocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; SII: Systemic immune-inflammation index; PDW: Platelet distribution width; WBC: White blood cell; RBC: Red blood cell; MPV: Mean platelet volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PMN: Polymorphonuclear; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen.

Patient age demonstrated a significant association with treatment failure (odds ratio [OR]=1.07; 95% confidence interval [CI], 1.01-1.13; P=0.023), indicating a 7% increase in failure odds with each additional year of age. Previous cesarean delivery (OR=2.16; 95% CI, 1.10%, 4.27%; P=0.026) and history of abortion (OR=2.14; 95% CI, 1.07%, 4.27%; P=0.031) were both significantly associated with increased treatment failure risk, with odds ratios exceeding 2.0. The number of live births also showed a significant association (OR=1.70; 95% CI, 1.12%, 2.58%; P=0.012), indicating a 70% increase in failure odds with each additional live birth. Both initial β-hCG levels (OR=1.00; 95% CI, 1%, 1%; P<0.001) and β-hCG levels at treatment initiation (OR=1.00; 95% CI, 1%, 1%; P<0.001) showed significant associations with treatment failure. Inflammatory markers demonstrated particularly strong associations, with NLR showing the strongest effect (OR=7.26; 95% CI, 4%, 13.18%; P<0.001), indicating a more than seven-fold increase in failure odds with elevated NLR. PLR (OR=1.37; 95% CI, 1.22%, 1.54%; P<0.001) and SII (OR=1.01; 95% CI, 1%, 1.01%; P<0.001) also showed significant positive associations. Polymorphonuclear leukocyte count (PMN) (OR=1.05; 95% CI, 1.01%, 1.08%; P=0.016) was positively associated, while lymphocyte count showed a protective effect (OR=0.83; 95% CI, 0.78%, 0.89%; P<0.001).

EP mass size demonstrated a significant association (OR=1.15; 95% CI, 1.11%, 1.2%; P<0.001), with each

Table 3. Demographic and basic obstetric history (n=371)

Variables	Category	No. (%)
Blood group	A	93(25.1)
	B	114(30.7)
	O	136(36.7)
	AB	28(7.5)
RH status	Positive	333(89.8)
	Negative	38(10.2)
Gravidity	1	91(24.5)
	2	132(35.6)
	3	87(23.5)
	4	39(10.5)
	≥5	22(5.9)
Parity	0	143(38.5)
	1	148(39.9)
	2	70(18.9)
	≥3	10(2.7)
Number of live births	0	143(38.5)
	1	149(40.2)
	2	72(19.4)
	3	7(1.9)

RH: Rhesus.

millimeter increase corresponding to a 15% increase in failure odds. The presence of fetal pole (OR=3.52; 95% CI, 1.3%, 9.54%; P=0.013) and yolk sac (OR=4.25; 95% CI, 1.64%, 11.02%; P=0.003) were both strongly associated with treatment failure, with odds ratios exceeding 3.5. Several variables showed clinically relevant trends despite not reaching statistical significance in univariate analysis. These included parity (OR=1.49, P=0.051), history of stillbirth (OR=4.76, P=0.208), abdominal pain (OR=1.65, P=0.198), female infertility history (OR=2.08, P=0.207), smoking (OR=2.76, P=0.217), RBC count (OR=0.53, P=0.120), hematocrit (OR=0.92, P=0.064), and PDW (OR=0.85, P=0.089).

Results of multivariate logistic regression analysis

Multivariate logistic regression analysis was performed to identify independent predictors of MTX treatment failure. A history of previous cesarean sections was not significantly associated with treatment failure (OR=6.27; 95% CI, 0.7%, 56.51%; P=0.102). However, an odds ratio greater than 1 suggests a potential increase in the risk of treatment failure among patients with a history of cesarean delivery, which may warrant further investigation in future studies with larger sample sizes. RBC count demonstrated a significant protective association with treatment failure (OR=0.05; 95% CI, 0%, -0.81%; P=0.035). This indicates that with each unit increase in RBC count, the odds of treatment failure decrease significantly, highlighting the importance of hematological parameters in predicting treatment outcomes. The PMN count showed a non-significant association with treatment failure (OR=0.79; 95% CI, 0.61%, 1.03%; P=0.079), though the trend suggests that higher PMN levels might be associated with reduced risk of treatment failure. Similarly, the PLR showed a non-significant as-

Table 4. Detailed past medical and surgical history (n=371)

Variables	Category	No. (%)
Number of abortions	0	238(64.3)
	1	85(23)
	2	37(10)
	3	10(2.7)
History of stillbirth	No	368(99.2)
	Yes	3(0.8)
Number of stillbirths	0	364(98.1)
	1	7(1.9)
History of abortion	No	237(63.9)
	Yes	134(36.1)
History of normal delivery	No	274(73.9)
	Yes	97(26.1)
History of C-section	No	260(70.1)
	Yes	111(29.9)
History of female infertility	No	348(93.8)
	Yes	23(6.2)
History of male infertility	No	359(96.8)
	Yes	12(3.2)
History of IVF	No	367(98.9)
	Yes	4(1.1)
History of IUI	No	366(98.7)
	Yes	5(1.3)
History of endometriosis	No	367(98.9)
	Yes	4(1.1)
History of PID	No	361(97.3)
	Yes	10(2.7)
History of EP	No	358(96.5)
	Yes	13(3.5)
History of pelvic surgery	No	192(51.8)
	Yes	179(48.2)
History of IUD use	No	370(99.7)
	Yes	1(0.3)

Variables	Category	No. (%)
Smoking	No	362(97.6)
	Yes	9(2.4)
Emergency contraceptive use	No	331(89.2)
	Yes	40(10.8)

Abbreviations: PID: Pelvic inflammatory disease; IUD: Intrauterine device; IUI: Intrauterine insemination; IVF: In vitro fertilization.

sociation (OR=0.2; 95% CI, 0.03%, 1.22%; P=0.081), with values suggesting a potential protective effect against treatment failure. The systemic SII emerged as a significant predictor of treatment failure (OR=1.03; 95% CI, 1%, 1.06%; P=0.036). Each unit increase in SII was associated with a 3% increase in the odds of treatment failure, emphasizing the importance of inflammatory status assessment. PDW showed a significant protective association (OR=0.44; 95% CI, 0.25%, 0.76%; P=0.004), indicating that each unit increase in PDW was associated with an approximately 56% reduction in the odds of treatment failure. Finally, EP mass size demonstrated the strongest association with treatment failure (OR=1.32; 95% CI, 1.15%, 1.52%; P<0.001). Each millimeter increase in mass size was associated with a 32% increase in the odds of treatment failure, establishing it as a crucial prognostic factor in clinical decision-making.

Discussion

This comprehensive study elucidates the multifactorial nature of MTX treatment failure in EP, confirming that a complex interplay of sonographic, inflammatory, hematological, and demographic factors dictates therapeutic

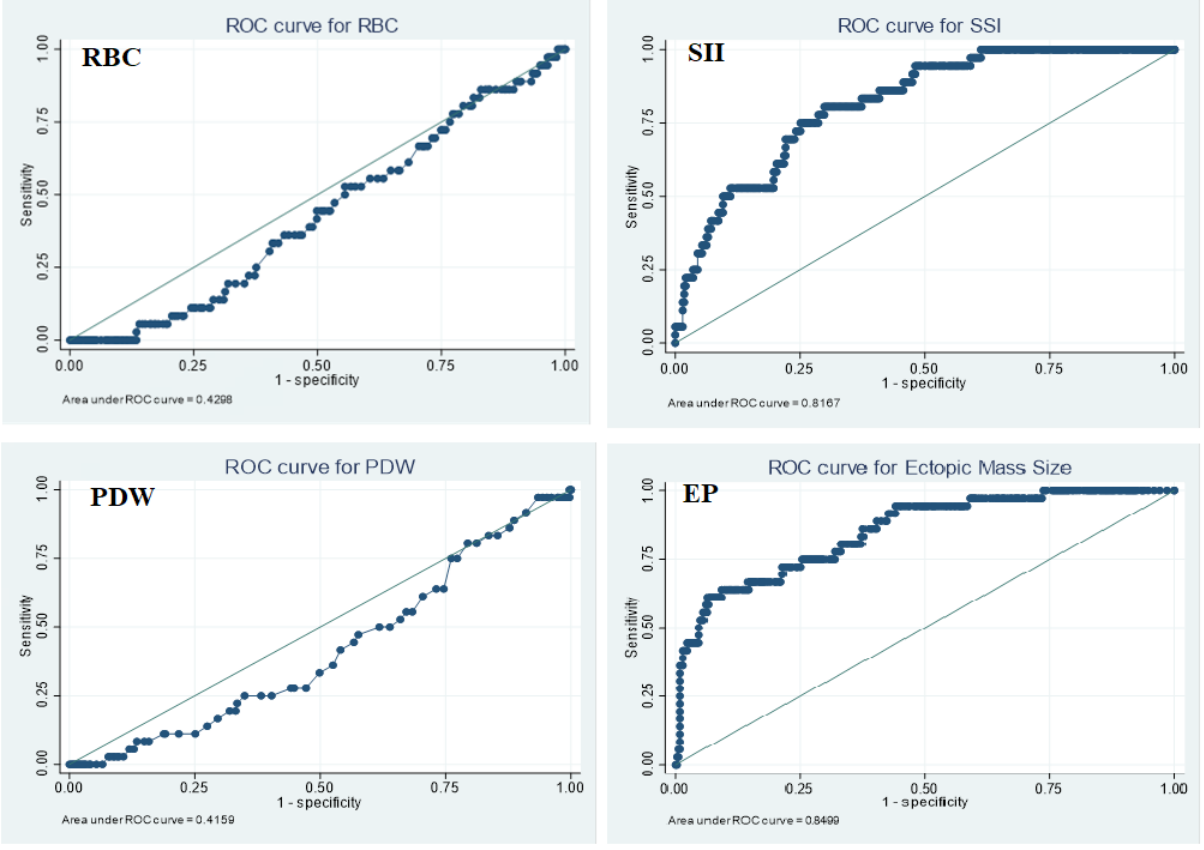


Figure 2. Comparison of the performance of different indices in predicting treatment outcomes

Table 5. Presenting symptoms, clinical characteristics, and treatment outcomes of current EP (n=371)

Variables		Category	No. (%)
Presenting symptoms	Abdominal pain	No	140(37.7)
		Yes	231(62.3)
	Vaginal bleeding	No	96(25.9)
		Yes	275(74.1)
Clinical characteristics	Gestational age (weeks)	5	4(1.1)
		6	49(13.2)
		7	113(30.5)
		8	119(32.1)
		9	47(12.7)
		10	31(8.4)
		11	5(1.3)
		12	3(0.8)
	Presence of gestational sac	No	334(90)
		Yes	37(10)
	EP location	Right tube	205(55.3)
		Left tube	160(43.1)
		Right cornual	4(1.1)
		Left cornual	2(0.5)
		Previous C-section scar	0(0)
	Presence of fetal pole	No	347(93.5)
		Yes	24(6.5)
	Presence of yolk sac	No	346(93.3)
		Yes	25(6.7)
	Presence of free fluid	No	248(66.8)
		Yes	123(33.2)
Treatment and outcome	MTX regimen	Single dose	292(78.7)
		Two dose	46(12.4)
		Three dose	1(0.3)
	Underwent surgery	No	335(90.3)
		Yes	36(9.7)

EP: Ectopic pregnancy.

success. Our findings align with the established literature, notably the seminal work by Barnhart [16], which first emphasized the composite predictive nature of these variables. The discordant outcomes reported across various studies, including ours, underscore the biological complexity of EP and the critical influence of specific patient and disease characteristics on MTX pharmacokinetics and pharmacodynamics.

The most robust predictor identified in our analysis was the size of the ectopic mass. We found a clear positive correlation between increasing mass diameter and the odds of treatment failure, a result strongly supported by a significant body of evidence [6, 17, 18]. The biological rationale for this is sound: Larger masses often possess a more extensive vascular network, enhancing nutrient delivery to the trophoblastic tissue and promoting its survival. Conversely, MTX, an antimetabolite that targets rapidly dividing cells by inhibiting DNA synthesis, may achieve subtherapeutic concentrations in larger volumes of tissue due to inadequate distribution, a phenomenon known as pharmacokinetic failure [19]. This explains why a mass size exceeding a certain threshold (often cited around 30-35 mm) is frequently incorporated into clinical guidelines as a relative contraindication to medical management.

However, the literature is not entirely unanimous. Several studies have found no statistically significant link between mass size and treatment outcome [8, 20, 21]. This discrepancy may arise from variations in study populations, differences in the sonographic measurement techniques, or the overriding influence of other, more potent confounding factors, such as very high initial β-hCG levels in some studies. Nevertheless, the preponderance of evidence, including our data, solidifies mass size as a cornerstone of clinical decision-making, urging caution when considering MTX for large ectopic masses.

Our investigation into inflammatory indices, particularly the SII and NLR, revealed their significant prognostic value. We found that elevated SII and NLR were independently associated with a higher risk of MTX failure. This aligns with a growing understanding that the host's inflammatory response is a critical determinant of disease progression and treatment response.

The SII, a composite marker reflecting neutrophil, platelet, and lymphocyte counts, offers a holistic view of the systemic inflammatory state. Our results are consistent with studies linking high SII to deeper trophoblastic invasion and a consequently increased risk of tubal rupture [22] and overall treatment failure [6]. The proposed mechanism involves a pro-inflammatory environment that may directly stimulate trophoblastic proliferation and invasion, while simultaneously impairing the local immune response necessary for containing the ectopic gestation. Similarly, a high NLR signifies a shift towards innate immunity (neutrophilia) and away from adaptive regulatory responses (lymphopenia). Neutrophils can inflict direct tissue damage via enzymes, such as elastase and reactive oxygen species (ROS), exacerbating tubal inflammation and damage. Furthermore, a systemic inflammatory state may indirectly induce resistance to MTX by disrupting folate metabolism, mirroring mechanisms observed in oncology where high NLR is a marker of poor prognosis and drug resistance [23].

While some studies have not found this association [14, 24], the differences may be attributed to the timing of blood draw relative to symptom onset or treatment initiation, variations in treatment protocols, or population heterogeneity. However, the collective evidence strongly suggests that these easily calculable indices provide invaluable insight into the patient's inflammatory status and should be considered part of the pretreatment risk stratification process.

The roles of RBC count and PDW are more complex and less consistently defined in the literature. Contrary to several studies that found no significant association between RBC parameters and treatment outcome [13, 15, 25], our analysis identified a low RBC count as a risk factor for failure. This finding is supported by Madenli and Öztürk [18], who reported a similar link with low hemoglobin. The pathophysiological connection may involve chronic anemia leading to tissue hypoxia, which can upregulate vascular growth factors, such as vascular endothelial growth factor, thereby stimulating trophoblastic growth and invasion. Additionally, iron deficiency, a common cause of anemia, can impair the function of cytochrome P450 enzymes in the liver, potentially altering the metabolism and efficacy of MTX [26]. The frequent conflation of RBC count, hemoglobin, and iron studies in previous research represents a significant knowledge gap, highlighting the need for future studies to evaluate these parameters independently and mechanistically.

Conversely, we identified a higher PDW as a protective factor. PDW measures the variability in platelet size, with higher values indicating a greater proportion of young, reactive platelets. This finding contradicts some reports [13, 25] but can be explained by the beneficial roles of activated platelets beyond coagulation. Activated platelets release a plethora of growth factors (platelet-derived growth factor, transforming growth factorbeta)

and cytokines that are crucial for initiating and modulating tissue repair processes and controlling inflammation [27]. Therefore, a robust platelet response, indicated by a high PDW, might facilitate the healing and resolution of the tubal injury caused by the ectopic implantation, creating a more favorable environment for MTX to act. The contradictory results across studies suggest that PDW's role may be nuanced and influenced by other clinical factors, warranting further investigation.

Patient age emerged as a significant factor, with advanced maternal age correlating with higher failure rates. This is supported by several studies [8, 28, 29] and can be attributed to age-related physiological changes: Diminished tubal motility and ciliary function, reduced tissue integrity, a higher prevalence of pelvic adhesions from previous infections or surgeries, and a general decline in regenerative capacity [30, 31]. While some studies found no independent association [11, 32, 33], age likely interacts with other variables, such as ovarian reserve and comorbid conditions, making it a valuable component of a multifactorial risk assessment model.

Our analysis also found a history of cesarean section and abortion to be significant risk factors. A prior cesarean section increases the odds of failure 2.16-fold, likely due to the presence of a scarred uterine niche that can harbor chronic, subclinical inflammation. This inflammatory milieu, characterized by elevated cytokines, such as tumor necrosis factor-α and interleukin6, may promote trophoblastic survival and growth, reducing MTX efficacy [34]. Similarly, a history of abortion, particularly recurrent loss, was associated with a 2.14-fold increase in risk. This may be due to underlying endometrial pathology such as chronic endometritis or intrauterine adhesions (Asherman's syndrome), which create a receptive environment for abnormal implantation and may reflect a broader systemic dysregulation of immune tolerance that also affects tubal implantation sites [35]. Although not all studies agree on these historical factors [13, 17], their significant effect in our large study suggests they should be carefully considered during patient counseling and treatment planning.

Conclusion

In conclusion, this study reinforces that MTX treatment failure is not a random event but a predictable outcome based on a constellation of patient-specific factors. The size of the ectopic mass remains the most reliable sonographic predictor. Furthermore, we highlight the critical, yet often underexplored, role of systemic inflammation, as captured by indices like SII and NLR. Easily derived from routine complete blood counts, these indices provide a powerful glimpse into the host's response and should be integrated into clinical decision-making algorithms. The conflicting results found in the literature regarding factors, such as RBC, PDW, and patient history, are not merely discrepancies but reflections of the complex, multifactorial pathophysiology of EP. They underscore the necessity for a personalized medicine approach rather than a one-size-fits-all protocol. Based on our findings, we recommend that patients presenting with high-risk features, such as a large mass (>3.5 cm), elevated inflammatory indices (SII, NLR), advanced maternal age, a history of cesarean section or abortion, and anemia, should be counseled extensively on the higher risk of MTX failure. For these patients, primary surgical management or a multi-dose MTX regimen with very close monitoring should be strongly considered. Future research should focus on developing a validated risk-stratification score that incorporates these demographic, sonographic, and laboratory variables to guide clinicians towards the most effective and safest first-line treatment for each patient.

This study's limitations include a relatively small sample size (371 patients), which may have reduced its statistical power to detect significant associations, particularly for rare exposures, such as smoking. The analysis did not investigate potential interactions between variables, and the exclusion of 43 patients with cesarean scar pregnancies may limit the generalizability of the results. Data were also collected from a single center, and some potential confounding factors were not fully controlled. Although the odds ratio for a history of cesarean section was elevated, this variable did not reach statistical significance in the multivariate model; therefore, these findings should be interpreted with caution and warrant further investigation in future studies with larger sample sizes. For future research, larger, multi-center prospective studies are needed to improve statistical power and generalizability. Advanced statistical analyses should be employed to examine interactions between predictive factors. Future work should also include cesarean scar ectopic pregnancies, develop integrated clinical prediction models, and investigate the efficacy of novel or combination therapies for high-risk patients.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Babol University of Medical Sciences, Babol, Iran (Code: IR.MUBABOL.REC.724134276), and patient

confidentiality was maintained through data anonymization.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors contribution's

Conceptualization: Afsaneh Bashiri and Zinatossadat Bouzari; Methodology: Zinatossadat Bouzari and Soraya Khafri; Formal analysis and statistical analysis: Mehrzad Netadj and Soraya Khafri; Investigation, resources, and validation: Shahla Yazdani; Data curation and writing the original draft: Afsaneh Bashiri and Mehrzad Netadj; Review and editing: Zinatossadat Bouzari and Soraya Khafri; Supervision and project administration: Zinatossadat Bouzari; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors express their sincere gratitude to the Clinical Research Development Unit of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran, for their support and assistance with data collection. We also thank all the patients whose medical records were used in this study.

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