

A non-invasive and convenient pregnancy outcome prediction model for URSA: based on the characteristics of maternal peripheral blood inflammatory cytokines

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Abstract

Introduction: Recurrent unexplained miscarriage (URSA), which accounts for over 50% of recurrent abortion cases, is closely associated with maternal immune dysregulation, particularly an imbalance of inflammatory cytokines. However, the prognostic value of peripheral blood cytokine profiles in predicting outcomes of URSA remains incompletely elucidated. This study aimed to construct and validate a prediction model based on peripheral blood inflammatory cytokine levels to non-invasively assess the risk of pregnancy outcomes in patients with unexplained recurrent spontaneous abortion (URSA).

Methods: A retrospective study design was adopted, enrolling 138 URSA patients and 124 healthy controls from the reproductive medicine outpatient department of Fujian Provincial Maternity and Children's Hospital between January 2020 and December 2024. By detecting the levels of 12 serum inflammatory cytokines, differences were compared between healthy controls and URSA patients, as well as between URSA patients with good and poor prognosis. LASSO analysis and logistic regression analysis were used to develop the model, which was validated in a training set (70%) and an internal validation set (30%). The model's performance, discriminative ability, and clinical applicability were assessed using receiver operating characteristic (ROC) curves, calibration curves, and decision curve analysis (DCA). Additionally, the model was visualized by constructing nomograms.

Results: Significant differences in pro-inflammatory cytokines TNF- α , IFN- γ , IL10, IL6, and IL8 were observed among the normal control group, the URSA group, and the two URSA outcome groups. Among these, URSA patients with elevated TNF- α and IFN- γ levels and decreased IL10 levels had a significantly increased risk of miscarriage. A prediction model was developed based on these findings, with an AUC value of 0.887 (95% CI: 0.784–0.991).

Conclusion: The pregnancy outcomes of URSA patients are closely related to the levels of peripheral blood inflammatory cytokines TNF- α , IFN- γ , and IL10. Elevated IFN- γ and TNF- α , along with decreased IL-10, are important markers of a poor prognosis.

INTRODUCTION

Recurrent spontaneous abortion (RSA) is a distressing reproductive disorder that can affect approximately 1–4% of women of childbearing age [1]. Guidelines from the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) define recurrent miscarriage as two or more clinically recognized pregnancy losses before 20–24 weeks of gestation, including clinical and biochemical pregnancy losses, but excluding ectopic pregnancies, molar pregnancies, and implantation failures [2, 3]. The pathophysiological mechanisms of RSA include embryonic chromosomal abnormalities, uterine structural and environmental abnormalities, genitourinary infections, endocrine and autoimmune diseases [4, 5]. However, in over 50% of RSA patients, these causes cannot be identified even after multiple clinical examinations [6], which is defined as unexplained recurrent spontaneous abortion (URSA). URSA patients cannot benefit from targeted treatments guided by a clear etiology. Furthermore, as there is still a lack of global consensus on the

definition, diagnostic evaluation, and potential therapies for URSA, it is difficult for clinicians to recommend preventive treatments based on laboratory results, severely affecting the physical and mental health of these patients.

Women with URSA often have maternal immune abnormalities [7]. Studies have shown that such patients may exhibit a shift in the T helper cell Th1/Th2 immune balance towards Th1, which is considered potentially detrimental to pregnancy maintenance [8]. In a normal pregnancy state, the maternal immune response tends to be dominated by Th2-type cytokines, whereas in spontaneous abortion, it manifests as a Th1-type immune dominance. This immune shift phenomenon is summarized by academia as the "Th1/Th2 balance paradigm" in pregnancy maintenance and miscarriage [9]. Th1 immunity primarily mediates cytotoxic responses against intracellular pathogens [10], while Th2 immunity tends to promote humoral immunity and the formation of immune tolerance [11]. Cytokines can be classified into pro-inflammatory (Th1) and anti-inflammatory (Th2) types based on their immune functions. Typical Th1-type pro-inflammatory cytokines include tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and interleukin (IL)-1 β ; Th2-type cytokines mainly include IL-4, IL-5, IL-10, and IL-13 [12, 13]. Moderate pro-inflammatory cytokines can induce a local inflammatory environment during embryo implantation, promoting trophoblast cell proliferation, invasion, and endometrial angiogenesis, thereby facilitating successful embryo implantation [14]. However, long-term exposure to a high-level pro-inflammatory cytokine environment may reduce uterine embryo tolerance, interfere with endometrial blood supply, and even trigger miscarriage.

The ESHRE RSA guideline recommends using the predictive models of Brigham et al. [15] or Lund et al. [16] to estimate the live birth chance for RSA couples, but these models do not distinguish between URSA and RSA. Currently, there are few prognostic models focusing primarily on URSA. A study from Saudi Arabia relied on demographic and routine blood test data as predictors, using various machine learning algorithms to predict the miscarriage rate in URSA patients [17], without considering immunological factors. The study by Li et al. [18] examined up to 49 clinical indicators, including immunological and metabolic parameters (but not inflammatory cytokines), and constructed multiple models to predict pregnancy outcomes in URSA patients. Although their article reported good predictive performance, it has been proven that increasing the number of features not only fails to guarantee continuous improvement in model performance but can also lead to performance degradation due to the introduction of irrelevant or redundant information [19]. Furthermore, some studies considering immunological factors use results from clinically invasive examinations, which greatly hinders the implementation and dissemination of the models. Given the above limitations and the importance of inflammatory cytokines in the mechanism of URSA development, it is necessary to develop a decision support tool for clinicians to accurately assess the miscarriage risk in women with URSA, thereby laying the foundation for systematic pregnancy health management.

This study adopted a retrospective analysis, comparing and analyzing cytokines among normal individuals, URSA patients, and different prognostic groups of URSA patients, aiming to construct and

validate a model based on pre-treatment peripheral blood cytokine test results as a non-invasive and convenient tool to predict pregnancy outcomes in URSA patients for clinical assistance.

MATERIAL AND METHODS

Patient population

In this retrospective study, patients were recruited from the reproductive medicine outpatient department of Fujian Provincial Maternity and Children's Hospital, China, between January 2020 and December 2024. All enrolled patients underwent comprehensive medical history, laboratory tests, and imaging evaluations (chromosomes, endocrine function, infections, immune diseases, and examinations of various organs, especially reproductive organs) conducted by reproductive and gynecological doctors before the patient became pregnant. Diagnostic examinations primarily followed the latest ESHRE guidelines (2022), focusing on genetic, immune, and anatomical factors. Inclusion criteria: (1) Consecutive occurrence of ≥ 2 spontaneous abortions; (2) Normal chromosomes in both spouses; (3) Normal reproductive organs shown by gynecological and B-ultrasound examinations; (4) No genetic diseases; (5) No history of thrombosis or anemia. Exclusion criteria: (1) Presence of autoimmune diseases; (2) Presence of endocrine diseases such as diabetes, thyroid disorders, luteal phase deficiency; (3) Comorbid organic diseases of the heart, liver, kidney, etc.; (4) Having received drug treatments, and patients with missing data. The patient selection flowchart is shown in Fig. 1.

Pregnancy outcome definition and follow-up

The primary clinical outcome of interest in this study was pregnancy status, specifically clinical pregnancy and miscarriage. These outcomes were determined through comprehensive review of medical records and telephone follow-up 2 years after the outpatient visit. According to current medical standards in China, clinical pregnancy is defined as the presence of at least one gestational sac in the uterus and detection of fetal heartbeat after 28 weeks of gestation. URSA patients who were in a state beyond 28 weeks of gestation or had delivered a live birth at follow-up were classified into the good prognosis group. Miscarriage included spontaneous miscarriage and biochemical loss. Spontaneous miscarriage was defined as termination of pregnancy before 28 weeks with a fetal weight below 1000 grams. Biochemical loss was characterized by elevated levels of human chorionic gonadotropin (HCG) in blood or urine, despite the absence of a gestational sac confirmed by gynecological color Doppler ultrasound. URSA patients who were not pregnant and had experienced another miscarriage at follow-up were classified into the poor prognosis group.

Data collection

Our data came from 138 URSA patients and 124 normal women. The attributes in the dataset were numerical and categorical. Features included basic clinical information, routine blood tests, and serum inflammatory cytokine test results. These features were collected at the patient's first visit, before any treatment.

Statistical analyses

The dataset was randomly split into a training cohort and a validation cohort in a 7:3 ratio, the former for model development and the latter for internal validation. Data are presented as median (interquartile range). The Shapiro-Wilk test was used to assess the distribution of data, while the Chi-square or Fisher's exact test was used to compare categorical variables. The t-test was applied to compare continuous variables that conformed to a normal distribution, while the Mann-Whitney U test was used for those that did not. LASSO regression analysis was used for predictor selection. Univariate and multivariate logistic regression analyses were both used to identify predictive factors and develop the model. Evaluation metrics included the area under the curve (AUC), receiver operating characteristic (ROC) curve, and calibration curve. The confusion matrix was used to evaluate the model's performance by comparing predicted values with true values. We further plotted decision curve analysis (DCA) to visualize the net benefit of clinical decisions. Finally, we constructed dynamic and static nomograms to visualize the model. All analyses were performed using R software (version 4.3.0) and SPSS software (version 24.0). R packages included glmnet, rms, pROC, regplot, and DymNon. A p-value < 0.05 was considered statistically significant.

Results

We first listed the clinical characteristics of the registered participants, including routine blood test results (Table 1). The number of patients recruited in the URSA and control groups were 138 and 124, respectively. Except for gravidity and number of abortions, there were no significant differences in age and BMI between the two groups, indicating that the two populations were comparable. There were no significant differences in routine blood indicators such as WBC count, Neutrophil%, and Lymphocyte%, indicating that neither group was in an acute inflammatory phase.

Table 1
Clinical characteristics

Characteristic ¹	Control (N = 124)	URSA (N = 138)	p value ²
Age	32.00 (29.00, 34.00)	31.00 (28.00, 33.00)	0.051
BMI	22.13 (19.84, 24.34)	21.22 (19.86, 23.44)	0.101
Gravidity	1.00 (0.00, 1.00)	3.00 (2.00, 3.00)	< .001
Parity	0.00 (0.00, 0.00)	0.00 (0.00, 1.00)	0.003
number of abortions	1.00 (0.00, 1.00)	2.00 (2.00, 3.00)	< .001
WBC	6.13 (5.31, 7.74)	6.39 (5.53, 7.48)	0.436
Neutrophil%	59.65 (53.27, 64.75)	58.95 (52.68, 62.18)	0.262
Lymphocyte%	31.40 (28.05, 37.65)	33.35 (28.77, 38.15)	0.238
Note: 1, median (interquartile range); ² , Mann-Whitney test			

Abbreviations: URSA: Unknown recurrent spontaneous abortion; BMI, body mass index; WBC, white blood cell.

To investigate whether inflammatory cytokines play a role in URSA patients with poor prognosis, URSA patients were further divided into Good Prognosis and Poor Prognosis subgroups based on follow-up results. In the comparison of individual cytokines, IFN- γ , IL6, IL8, and TNF- α showed significant differences and gradually increased in the process from the control group -> Good Prognosis (URSA) group -> Poor Prognosis (URSA) group, while IL5 and IL10 were significantly lower in the Poor Prognosis (URSA) group (Table 2).

Table 2.Comparison of Inflammatory Cytokines Between Groups

Variables ¹	Control	Patients with URSA		p value ²
		Good Prognosis	Poor Prognosis	
IFN α	1.70 (1.11,2.28)	1.64 (0.94,2.39)	1.77 (1.22,2.46)	$p_{1,2,3} = 0.395$ $p_{1,2} = 0.670$ $p_{1,3} = 0.286$ $p_{2,3} = 0.184$
IFN γ	0.85 (0.20,1.52)	1.21 (0.64,1.94)	2.37 (1.64,3.67)	$p_{1,2,3} = <0.001$ $p_{1,2} = 0.003$ $p_{1,3} = <0.001$ $p_{2,3} = <0.001$
IL-1 β	1.71 (0.81,2.47)	1.42 (0.61,2.17)	1.57 (1.12,2.35)	$p_{1,2,3} = 0.357$ $p_{1,2} = 0.278$ $p_{1,3} = 0.688$ $p_{2,3} = 0.167$
IL-2	1.42 (0.64,2.17)	1.15 (0.48,2.03)	1.56 (0.97,2.07)	$p_{1,2,3} = 0.14$ $p_{1,2} = 0.160$ $p_{1,3} = 0.534$ $p_{2,3} = 0.046$
IL4	1.99 (0.02,5.64)	1.27 (0.53,2.87)	1.50 (0.80,2.25)	$p_{1,2,3} = 0.723$ $p_{1,2} = 0.489$ $p_{1,3} = 0.663$ $p_{2,3} = 0.554$
IL5	1.36 (0.69,1.94)	0.67 (0.30,1.21)	0.76 (0.41,1.37)	$p_{1,2,3} = <0.001$ $p_{1,2} = <0.001$ $p_{1,3} = 0.002$ $p_{2,3} = 0.395$
IL6	1.46 (0.88,2.11)	2.08 (1.17,3.04)	3.43 (2.33,4.20)	$p_{1,2,3} = <0.001$ $p_{1,2} = <0.001$ $p_{1,3} = <0.001$

					$p_{2,3} = <0.001$
IL8	1.31 (0.65,1.92)	2.91 (1.79,4.70)	4.83 (3.04,7.08)	$p_{1,2,3} = <0.001$	
				$p_{1,2} = <0.001$	
				$p_{1,3} = <0.001$	
				$p_{2,3} = <0.001$	
IL10	1.65 (1.30,3.10)	1.79 (1.11,2.40)	1.27 (0.71,1.78)	$p_{1,2,3} = <0.001$	
				$p_{1,2} = 0.456$	
				$p_{1,3} = <0.001$	
				$p_{2,3} = 0.95$	
IL-12 p70	2.09 (1.11,3.10)	1.67 (0.82,2.98)	2.02 (1.20,3.43)	$p_{1,2,3} = 0.187$	
				$p_{1,2} = 0.095$	
				$p_{1,3} = 0.927$	
				$p_{2,3} = 0.146$	
IL-17A	3.64 (2.34,4.99)	5.46 (1.78,7.74)	3.71 (0.22,7.23)	$p_{1,2,3} = 0.260$	
				$p_{1,2} = 0.164$	
				$p_{1,3} = 0.474$	
				$p_{2,3} = 0.201$	
TNF α	1.06 (0.16,2.15)	1.31 (0.73,1.85)	2.71 (1.94,4.42)	$p_{1,2,3} = <0.001$	
				$p_{1,2} = 0.154$	
				$p_{1,3} = <0.001$	
				$p_{2,3} = <0.001$	

Note: ¹, variables are given as median (interquartile range). ², Kruskal-Wallis rank sum test

Abbreviations: IFN- α , interferon-alpha; IFN- γ , interferon-gamma; IL-1 β , interleukin-1 β eta; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-17, interleukin-17; TNF- α , tumor necrosis factor alpha.

To explore the possibility of further constructing a prediction model and validating its efficacy, the total population was split into two groups in a 7:3 ratio based on a random seed number, designated as the training set (183 cases) and the validation set (79 cases). There were no significant differences between them in terms of age, BMI, gravidity, parity, number of abortions, including cytokine test results (Table 3). Subsequently, comparisons between the Good Prognosis group and the Poor Prognosis group within the

training set and validation set respectively showed that IFN- γ , IL6, IL8, IL10, and TNF- α had significant differences, similar to the previous results (Table 4).

Table 3. Comparison of Clinical Characteristics Between Training cohort and Validation cohort Groups

Variables ¹	Training cohort	Validation cohort	p value
	N = 183	N = 79	
Age	31.0 (29.0, 34.0)	32.0 (30.0, 34.0)	0.129
BMI	21.57 (19.83, 24.12)	21.63 (19.98, 23.24)	0.549
Gravidity	2.00 (1.00, 3.00)	2.00 (1.00, 3.00)	0.558
Parity	0.00 (0.00, 0.00)	0.00 (0.00, 0.50)	0.231
number of abortions	2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	0.88
WBC	6.18 (5.30, 7.56)	6.56 (5.52, 7.63)	0.455
Neutrophil%	59.10 (52.75, 63.10)	59.90 (52.60, 65.70)	0.299
Lymphocyte%	32.70 (28.70, 38.18)	32.60 (27.20, 37.05)	0.312
IFN α	1.66 (0.96, 2.28)	1.78 (1.22, 2.51)	0.129
IFN γ	1.23 (0.40, 2.23)	1.26 (0.67, 2.14)	0.661
IL-1 β	1.48 (0.88, 2.35)	1.77 (0.85, 2.62)	0.194
IL-2	1.37 (0.67, 2.11)	1.54 (0.68, 2.32)	0.714
IL4	1.4 (0.3, 3.4)	1.7 (0.4, 3.3)	0.76
IL5	0.97 (0.42, 1.58)	1.02 (0.53, 1.73)	0.309
IL6	2.00 (1.12, 2.96)	1.88 (1.13, 2.65)	0.686
IL8	1.93 (1.06, 4.10)	2.06 (1.13, 4.24)	0.729
IL10	1.59 (1.08, 2.45)	1.66 (1.23, 2.09)	0.452
IL-12 p70	1.95 (1.01, 3.16)	1.89 (0.86, 3.12)	0.633
IL-17A	3.9 (1.7, 6.7)	4.0 (2.0, 6.7)	0.537
TNF α	1.46 (0.52, 2.52)	1.41 (0.89, 2.30)	0.712

Note: ¹, variables are given as median (interquartile range). ², Kruskal-Wallis rank sum test

Abbreviations: BMI, body mass index; IFN- α , interferon-alpha; IFN- γ , interferon-gamma; IL-1 β , interleukin-1 β ; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-17, interleukin-17; TNF- α , tumor necrosis facto ralpha.

Table 4. Comparison of Inflammatory Cytokines Between Different Prognostic groups into Training cohort and Validation cohort.

Variables	Training cohort			Validation cohort		
	Good Prognosis	Poor Prognosis	p	Good Prognosis	Poor Prognosis	p value
	N = 59	N = 38	value	N = 25	N = 16	
IFN α	1.57 (0.81, 2.20)	1.68 (1.20, 2.34)	0.214	1.78 (1.18, 2.67)	1.99 (1.40, 2.74)	0.584
IFN γ	1.20 (0.47, 2.01)	2.52 (1.63, 3.90)	<0.001	1.26 (1.00, 1.92)	2.24 (1.62, 3.09)	0.002
IL-1 β	1.42 (0.88, 2.10)	1.52 (1.10, 1.85)	0.471	1.23 (0.46, 2.39)	1.98 (1.13, 2.77)	0.142
IL-2	1.16 (0.40, 2.02)	1.38 (0.94, 2.04)	0.115	1.11 (0.68, 2.15)	1.72 (1.04, 2.49)	0.219
IL4	1.25 (0.39, 2.80)	1.50 (0.70, 2.25)	0.579	1.30 (0.74, 2.90)	1.56 (1.14, 2.29)	0.82
IL5	0.66 (0.24, 1.29)	0.68 (0.32, 1.23)	0.935	0.67 (0.34, 1.02)	1.08 (0.71, 1.74)	0.112
IL6	2.19 (1.22, 3.17)	3.82 (2.15, 4.24)	<0.001	1.61 (1.06, 2.73)	3.02 (2.34, 3.74)	0.003
IL8	3.00 (1.85, 5.85)	4.37 (2.60, 6.92)	0.023	2.70 (1.61, 4.24)	5.38 (4.40, 7.14)	<0.001
IL10	1.75 (1.08, 2.52)	1.17 (0.69, 1.75)	0.002	1.88 (1.54, 2.06)	1.48 (0.83, 1.83)	0.048
IL-12 p70	1.69 (1.00, 2.99)	2.02 (1.06, 3.44)	0.297	1.65 (0.48, 2.51)	2.23 (1.22, 3.32)	0.31
IL-17A	4.9 (0.9, 7.4)	2.7 (0.0, 7.2)	0.233	6.3 (2.5, 9.0)	4.5 (2.0, 8.3)	0.584
TNF α	1.37 (0.52, 2.02)	2.66 (1.93, 4.72)	<0.001	1.28 (1.00, 1.73)	2.96 (2.03, 3.78)	<0.001

Note: ¹, variables are given as median (interquartile range). ², Kruskal-Wallis rank sum test

Abbreviations: BMI, body mass index; IFN- α , interferon-alpha; IFN- γ , interferon-gamma; IL-1 β , interleukin-1 β ; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-17, interleukin-17; TNF- α , tumor necrosis factor alpha.

To identify important variables related to URSA prognosis, seven cytokines (TNF- α , IFN- γ , IL6, IL10, IFN- α , IL17A, IL5) were screened using LASSO regression analysis in the training set (Fig. 2A, B), and their ROC curves were plotted. The area under the curve (AUC) values were 0.818, 0.811, 0.746, 0.685, 0.575, 0.572, and 0.495, respectively (Fig. 2C).

Subsequently, univariate and multivariate logistic regression analyses (Table 5) were used to finally screen out three cytokines—IFN- γ , TNF- α , and IL10—for constructing a risk prediction model for poor prognosis in URSA (Fig. 3A). The model was applied to the training set and internal validation set for performance testing. The ROC curves (Fig. 3B) showed AUC values of 0.865 (0.790-0.941) and 0.887 (0.784-0.991), respectively, demonstrating good ability to distinguish patients with different outcomes. Calibration curves and decision curve analysis (DCA) were then plotted for the models in the training and validation cohorts. The calibration curves were almost diagonal, indicating good consistency between the model's predicted probabilities and the actual probabilities. The DCA plots indicated good overall net benefit for clinical interventions guided by this prediction model (Fig. 4).

Table 5. Univariable and multivariable logistic regression analysis of URSA predictors.

Variables	Univariable			Multivariable		
	OR	95% CI	p-value	OR	95% CI	P value
IFN α	0.99	0.75, 1.26	0.932			
IFN γ	3.13	2.01, 5.34	<0.001	2.47	1.35, 5.00	0.006
IL-1 β	1.03	0.84, 1.26	0.754			
IL-2	1.1	0.82, 1.50	0.504			
IL4	0.91	0.72, 1.11	0.413			
IL5	0.87	0.49, 1.43	0.592			
IL6	1.98	1.42, 2.91	<0.001	1.34	0.88, 2.22	0.203
IL8	1.19	1.04, 1.39	0.015	0.89	0.67, 1.17	0.418
IL10	0.48	0.28, 0.78	0.006	0.39	0.14, 0.90	0.049
IL-12 p70	1.17	0.87, 1.60	0.299			
IL-17A	0.94	0.85, 1.03	0.22			
TNF α	2.49	1.71, 4.00	<0.001	2.57	1.40, 5.55	0.007

Note: ¹, variables are given as median (interquartile range). ², Kruskal-Wallis rank sum test

Abbreviations: BMI, body mass index; IFN- α , interferon-alpha; IFN- γ , interferon-gamma; IL-1 β , interleukin-1 β eta; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-17, interleukin-17; TNF- α , tumor necrosis facto ralpha.

DISCUSSION

This study was the first to construct and validate a predictive model based on specific inflammatory cytokines in maternal peripheral blood for evaluating the pregnancy outcomes of patients with unexplained recurrent spontaneous abortion (URSA). The direct association between the Th1/Th2 cytokine imbalance (increased FN γ and TNF α , decreased IL10) and the adverse pregnancy outcome of URSA was clarified. Through machine learning algorithms (such as LASSO regression), the most predictive core associated factor combinations were screened out from the 12 factors. A non-invasive, simple and highly predictive (AUC 0.887) clinical prediction tool was developed, which was visualized through a nomogram and had good potential for clinical transformation.

The three core factors focused in this study play a key role in maternal-fetal immunity. TNF- α and IFN- γ are Th1-type cytokines. Under normal levels, TNF- α can induce the trophoblast to secrete vascular endothelial growth factor (VEGF), which helps the embryo implant in the uterus and promotes trophoblast invasion [20,21]. However, most studies have shown that the levels of TNF- α in the serum and decidua tissue of women with URSA are higher than those of normal pregnant women [22,23,24,25], which is the same as our research results. It is reported that TNF- α can induce fetal membrane degradation by increasing the expression of pro-apoptotic genes on the surface of the embryonic membrane [61], while inhibiting TNF- α can reduce stress-induced abortion in mice [26]. IFN- γ has been found in animal models to promote implantation through vascular recast, vascular development at the implantation site, and maintaining decidua tissue during placental development [32]. However, excessive IFN- γ is harmful to the embryo. It can inhibit the production of granulocyte-macrophage colony-stimulating factor (GM-CSF), which is essential for successful pregnancy [33]. Most literature reports that the expression of IFN- γ in the peripheral blood of patients with URSA significantly increases [22,27,28]. However, some studies have not found any changes in IFN- γ . In the study by Peng et al. [29], there was no significant difference in IFN- γ alone between patients with URSA and healthy women. However, they found that the ratio of IFN- γ /IL-4 cytokines was relatively high and recommended this to help diagnose URSA. This might be due to the differences in the environment among different regions and ethnic groups. Studies have shown that URSA is related to gene polymorphism [30]. Elevated levels of IFN- γ mRNA and protein can also be detected in the endometrium and decidua of URSA patients [31]. Moderate levels of TNF- α and IFN- γ are beneficial for normal pregnancy, but high levels of both can hinder embryo growth by inducing trophoblast cell apoptosis [34], which may be the reason why they are observed to change in patients with URSA. IL-10 is produced by activated Th2 cells and can induce the generation of tolerant dendritic cells in the mother, which is crucial for maintaining maternal and fetal immune tolerance to promote embryonic development [35]. Multiple studies have found that healthy pregnancy is associated with high levels of IL-10, and the levels of IL-10 in the peripheral blood of women with URSA are significantly decreased [27,22,36,28], which is the same as our research results. Similar changes were also observed at the decidual tissue level [31,37]. In animal experiments, Robertson et al. found that compared with wild-type mice, IL10-deficient mice were prone to fetal loss and accompanied by growth restriction of the remaining surviving fetuses [38,39]. Some other researchers found that using IL-10 on mice prone to miscarriage could reduce embryo loss [40].

IL6 and IL8 both showed significant increases in patients with URSA and those with adverse pregnancy outcomes in our previous analysis and univariate logistic regression analysis. However, after multivariate logistic regression analysis, these two factors were excluded from establishing the predictive model. It is indicated that although IL6 and IL8 can to some extent suggest the possibility of a poor prognosis in patients with URSA, there is no specific association between them and the pregnancy outcome of URSA after considering the overall condition of the patients and controlling confounding factors. The research results of Peng et al. are consistent with ours [29]. Compared with URSA patients, pregnant women have lower levels of IL6 in peripheral blood, but IL6 is not recommended for the diagnosis or monitoring of URSA. Because IL6 is a non-specific biomarker in clinical practice, it will increase when multiple pathogens infect the body or the patient is in different pathological states [41]. The increase here may be due to an undiagnosed underlying disease. However, some studies in the uterine decidua tissue have reached opposite conclusions. Some experiments [42,43] have found that the content of IL-6 and related mRNA in the decidua tissue of patients with URSA increases. However, the study by M.J et al. [44] indicated that the level of IL-6 in the decidua tissue of RSA patients was relatively low, which was inconsistent with our research results. Nevertheless, the number of cases in this study was relatively small (9 in the experimental group and 12 in the control group), and its representativeness might be insufficient. Few studies have reported the changes of IL8 in patients with URSA. Gu et al. [45,46] confirmed through qRT-PCR and ELISA experiments that the levels of IFN- γ and IL-8 in the peripheral blood of URSA patients were higher than those in the control group, which was the same as our research results.

The main advantage of this study lies in the fact that all the predictors used in the model are derived from peripheral serum samples of pregnant women, which are easily accessible and non-invasive in clinical practice. Moreover, the cost of this examination is not expensive, which makes the model highly applicable and popular in clinical practice. This study employed LASSO regression to effectively screen for variables, combined with multivariate regression to control confounding factors, and comprehensively evaluated the model's performance and clinical efficacy through training set/validation set segmentation, ROC curve, calibration curve, and decision curve analysis (DCA). The research method and process were relatively rigorous, and the prediction model was relatively reliable. In addition, all the data we collected for model construction were pre-treatment data of patients. Compared with other related studies, this avoids inaccurate statistical results caused by changes in predictor content after different treatment methods.

This research also has certain limitations. Firstly, this study is a single-center, retrospective one with a relatively limited sample size. Moreover, the research subjects come from a specific regional population, which may lead to selection bias and limit the extrapolation of the results. Secondly, this study did not conduct a detailed analysis of the patients' previous fertility-related treatment history. The absence of this factor makes it difficult for us to completely rule out the influence of the treatment history on the research conclusion. The research mainly focused on correlation analysis and model construction, but failed to conduct in-depth experimental exploration of how the specific interaction networks among the selected key cytokines and their downstream signaling pathways jointly lead to miscarriage.

Therefore, in the future, it is necessary to conduct external validation of the model in larger sample sizes, multi-center, prospective cohorts to confirm its robustness in different populations. Animal models or in vitro cell experiments can be combined to deeply explore the specific molecular mechanisms by which the increase of TNF- α and IFN- γ and the decrease of IL-10 synergistically lead to the occurrence of URSA. And verify whether these key cytokines can serve as intervention targets, providing evidence-based evidence for immunomodulatory therapy (such as the use of anti-TNF - α drugs or IL-10 enhancers), and further clarifying its clinical significance.

CONCLUSION

This retrospective study found that the pregnancy outcomes of URSA patients are closely related to peripheral blood inflammatory cytokine levels, among which elevated IFN- γ and TNF- α and decreased IL-10 are important markers of a poor prognosis. The prediction model constructed based on these three cytokines demonstrated high accuracy and clinical applicability, capable of helping doctors identify high-risk patients before treatment and providing a basis for personalized intervention.

Declarations

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Ethics declarations

Ethics approval and consent to participate

This retrospective study was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital (No. 2025KY291). Subject's personal information is confidential, participation in the study is entirely voluntary.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Contributors

Xite Lin designed and conducted data analysis and wrote the manuscript. Xiaodan Mao contributed to critically revising the article for important intellectual content and supervised the study. Maotong Zhang conducted data collection, organization and wrote the manuscript. Qingyang Hu conducted data

collection and preliminary organization. Xiaoqi Wu, Jingxuan Ye, Yashi Shi, Nan Wang and Jing Ye conducted some of the data acquisition and participated in valuable discussions. Shuqiong He contributed to critically revising the article for important intellectual content. All the authors gave their final approval of the version to be submitted and agreed to be accountable for all aspects of the work. Guarantor: Xiaodan Mao is the guarantor of this study.

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Figures

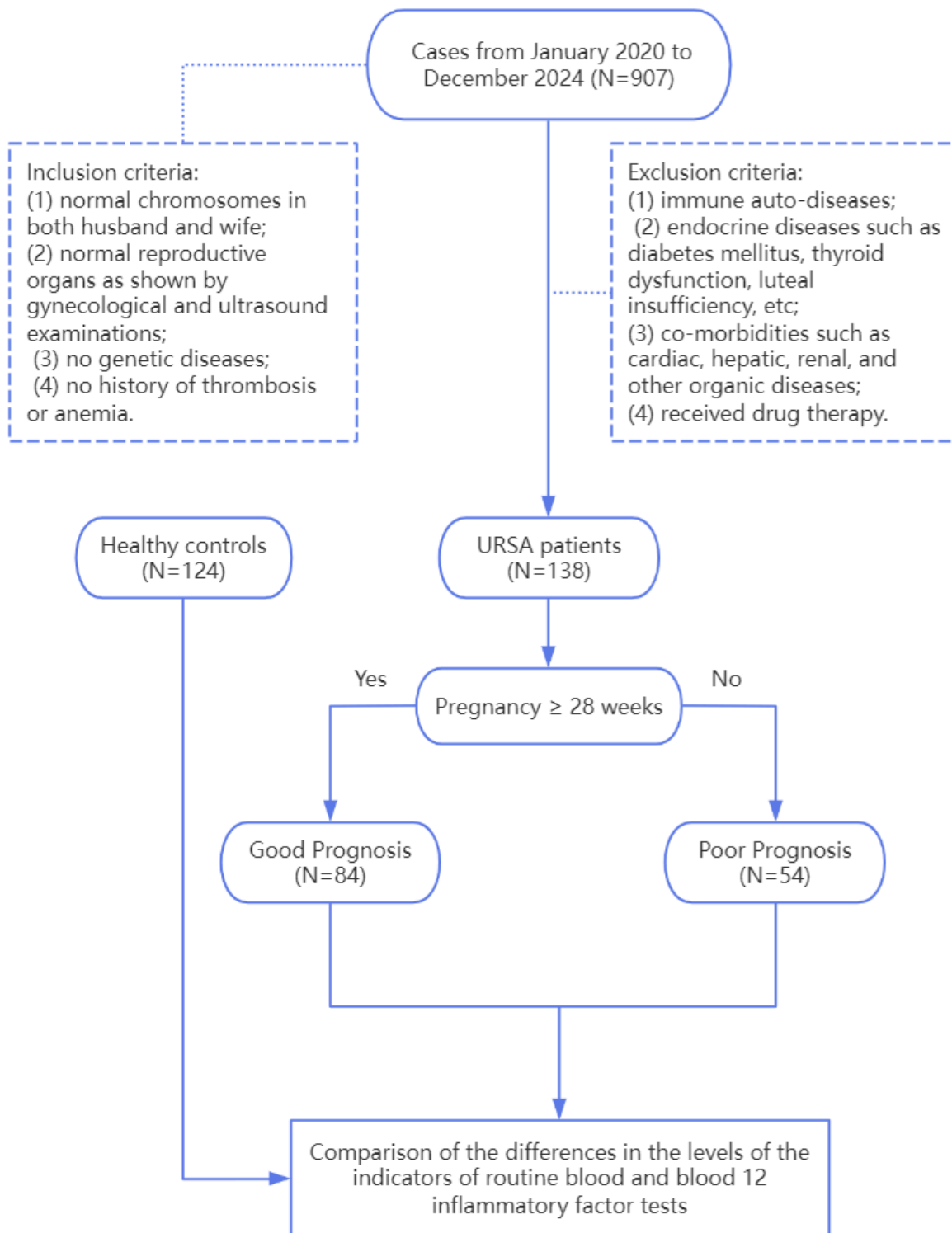


Figure 1

Flow Chart of patient selection

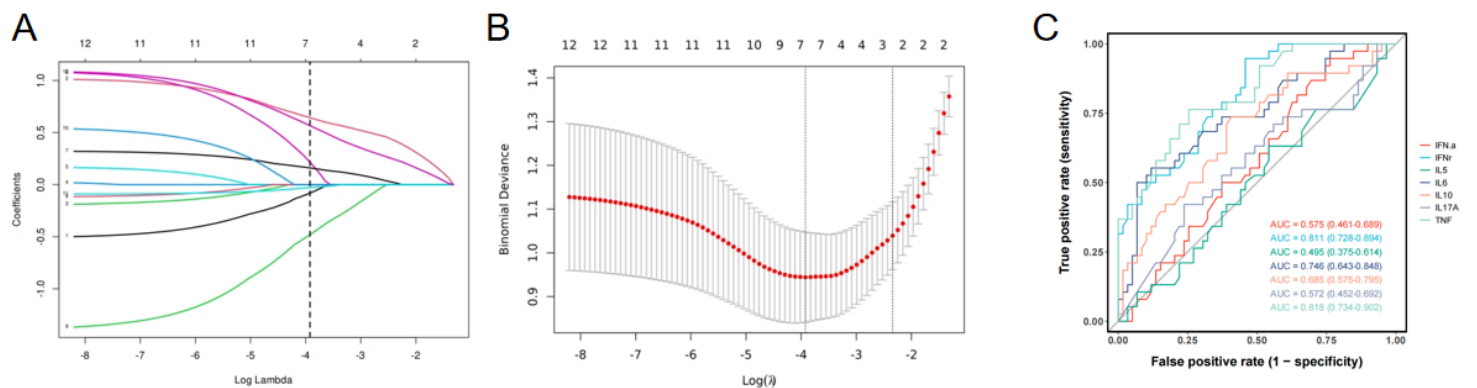


Figure 2

The results of Lasso regression analysis and 10- fold cross-validation and The receiver operating characteristic curve (ROC). (A) A coefficient profile was plotted against a logarithmic sequence of lambda. (B) Selection of the tuning parameter (λ) for deviance in Lasso regression was based on two criteria: the minimum criterion (represented by the left dotted line) and the 1-SE criterion (represented by the right dotted line). Lasso, least absolute shrinkage and selection operator; SE, standard error. ROC of Seven cytokines

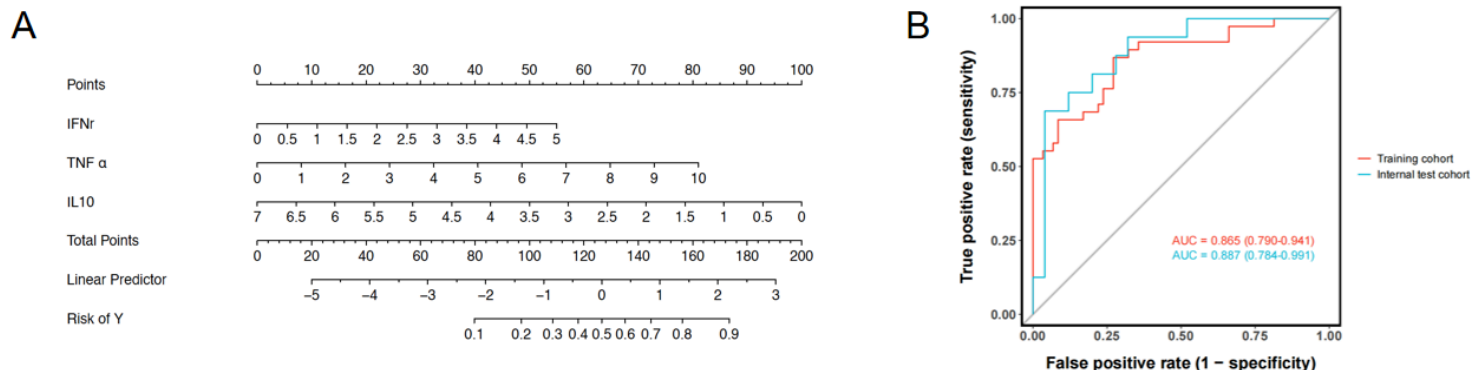


Figure 3

Nomogram and ROC of prediction model. A Nomogram was constructed using three predictive cytokines: IFN γ , TNF α , and IL10. B The receiver operating characteristic curve (ROC) performance of the model in predicting pregnancy outcomes of unexplained recurrent miscarriage (URSA). Two different color lines depict the performance of the model on the training set (red) and the validation set (blue).

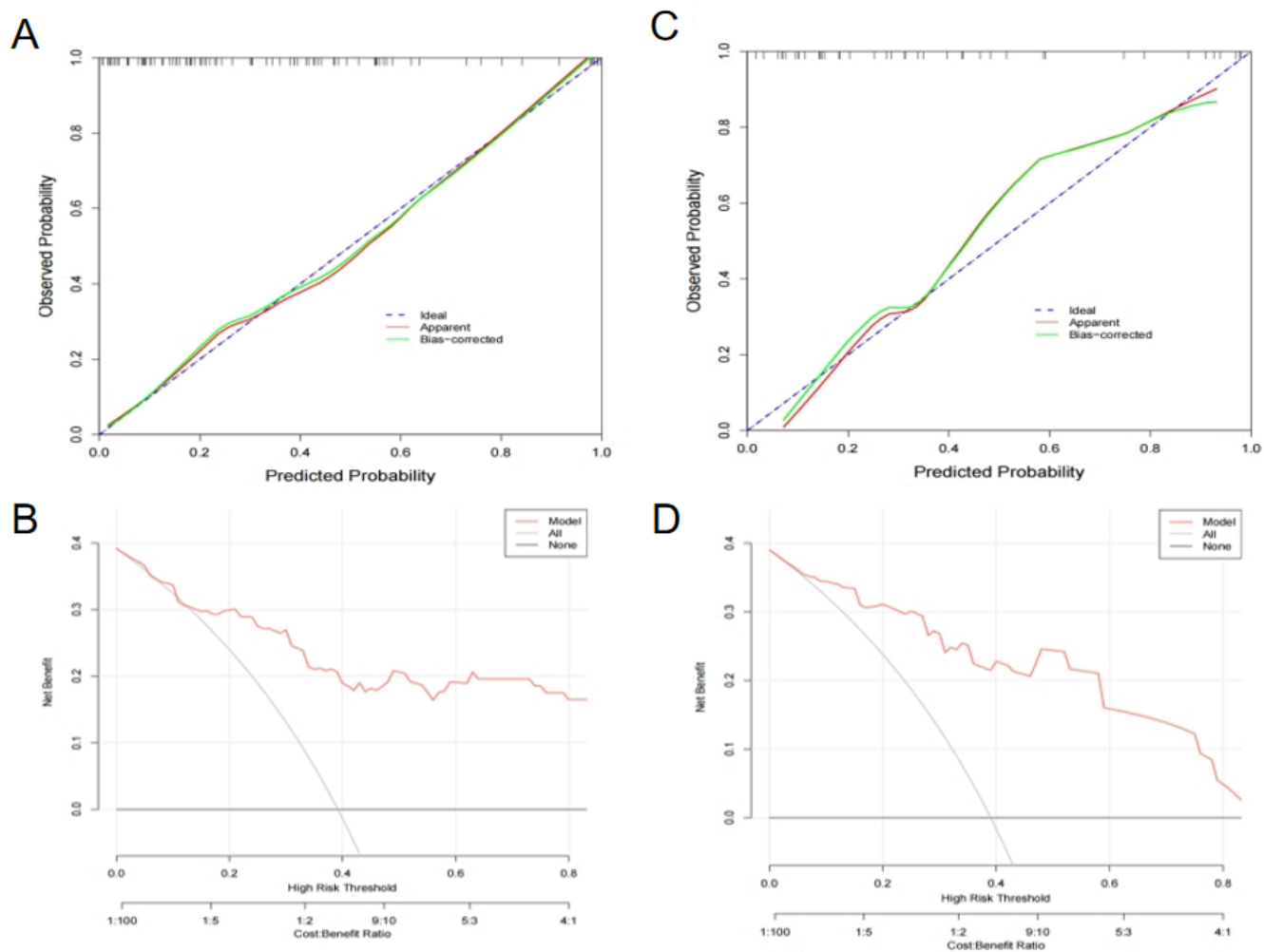


Figure 4

The calibration curve and the decision curve analysis (DCA) of the models in predicting URSA pregnancy outcomes. (A,B) The training set. (C,D) The validation set. The decision curve compares the net clinical benefits of predicting the pregnancy outcomes of unexplained recurrent pregnancy loss (URSA) patients under three scenarios: perfect prediction model (gray line), no screening (horizontal black solid line), and screening based on models (red solid line).