British Journal of Hospital Medicine

Br. J. Hosp. Med. (Lond) **2026**; 87(8): 56970
https://doi.org/10.31083/BJHM56970

Article

Diagnostic Performance of Serum Lactate Dehydrogenase and Immune-Inflammatory Markers in Recurrent Spontaneous Abortion: A Retrospective Study

Jianfeng Wen[1,*], Wenjun Cheng[1], Shanshan Hu[1], Shengnan Xia[1], Lingmei Shi[1]

[1]Department of Gynaecology, Yongkang Women and Children's Health Hospital, 321300 Jinhua, Zhejiang, China
*Correspondence: wjf960825@126.com (Jianfeng Wen)
Academic Editor: John Alcolado
Submitted: 13 October 2025 Revised: 10 March 2026 Accepted: 29 March 2026 Published: 12 August 2026

Abstract

Aims/Background: Recurrent spontaneous abortion (RSA) may contribute to intrauterine adhesions and lead to female infertility. This study aims to analyze serum lactate dehydrogenase (LDH) levels and immune-inflammatory markers in patients with recurrent abortion and to evaluate their diagnostic value in RSA. **Methods:** A total of 120 patients with RSA admitted between January 2023 and December 2024 were included as the study group, and 80 pregnant women with normal prenatal examination findings during the same period were selected as controls. Serum and hematological assays were performed in both groups, and levels of LDH, immune-inflammatory markers, autoimmune antibodies, and lymphocyte subsets were compared. Factors associated with RSA were analyzed using logistic regression, and the diagnostic performance of each index was assessed using a receiver operating characteristic (ROC) curve. **Results:** Compared with the control group, serum LDH levels, immune-inflammatory markers [interleukin (IL)-6, IL-10, and interferon gamma (IFN-γ)], antiphospholipid antibodies, and T lymphocyte subsets were significantly different in the RSA group ($p < 0.05$). Serum LDH, IFN-γ, antiphospholipid antibodies (APA) positive rate and cluster of differentiation (CD)4^+ T-cell levels were significantly higher in the RSA group than in controls ($p < 0.05$), whereas IL-6, IL-10, and $CD8^+$ T-cell levels were significantly lower ($p < 0.05$). Binary logistic regression analysis showed that elevated serum LDH, reduced IL-6 level, increased proportion of $CD4^+$ cells, and decreased proportion of $CD8^+$ cells were independent risk factors for recurrent abortion in RSA patients (all $p < 0.05$). ROC curve analysis showed that the combined detection of serum LDH, IL-6, $CD4^+$, and $CD8^+$ yielded an area under the curve (AUC) of 0.908, with a sensitivity of 0.875 and specificity of 0.823. The combined model demonstrated superior diagnostic performance compared with individual markers. **Conclusion:** Combined assessment of serum LDH and selected immune-inflammatory markers (IL-6, $CD4^+$, $CD8^+$) shows good predictive and diagnostic value for RSA and may facilitate early identification of high-risk patients in clinical practice.

Keywords: recurrent spontaneous abortion; lactate dehydrogenase; immune-inflammatory markers; lymphocyte subsets; diagnosis

1. Introduction

Recurrent spontaneous abortion (RSA) refers to two or more consecutive pregnancy losses before 28 weeks of gestation in women with the same partner. Most cases occur as early pregnancy losses, while a small proportion present as late abortions. The reported incidence is approximately 1–2% [1]. The etiology of RSA is complex and multifactorial. In early pregnancy, causes are mainly related to embryonic chromosomal abnormalities, familial genetic factors, corpus luteum dysfunction, and insulin resistance. In contrast, late pregnancy losses are more commonly associated with uterine anatomical abnormalities, infectious factors, and environmental or lifestyle influences [2,3]. Clinically, patients with RSA often present with vaginal bleeding and tissue discharge, while some may lack obvious abdominal pain. Therefore, accurate diagnosis requires a comprehensive assessment of reproductive history, combined with appropriate auxiliary physical examinations, to determine the underlying etiology.

Recent evidence indicates that the metabolic profile of patients with RSA is significantly altered, with plasma lactate levels markedly elevated compared with those in normal pregnancy. Moreover, Albright et al. [4] reported that elevated lactate levels in pregnant women are associated with adverse maternal outcomes, suggesting a potential role of lactate in the pathophysiology of RSA. Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate during glycolysis, indicating its crucial role in lactate production. However, direct measurements of serum lactate levels in venous blood samples of patients with RSA remain limited, and few studies have evaluated the diagnostic value of LDH in this context.

Emerging evidence suggests that approximately 50–60% of RSA cases are associated with immune dysfunction [5]. Talukdar et al. [6] reported that RSA is characterized by predominance of T helper 1 (Th1) immune responses and elevated levels of reactive oxygen species (ROS). Activation of immune cells can trigger systemic inflammatory responses, which may impair placental development and lead to pregnancy loss [7]. Lactate metabolism is closely related

to immune regulation, as activated immune cells preferentially use lactate to support their functional demands [8]. At present, most studies have focused on immune cell subsets or immune checkpoint pathways in recurrent abortion. Few studies have examined the combined role of serum LDH levels and immune-inflammatory markers in RSA. Therefore, this study aims to explore the association between serum LDH, immune-inflammatory markers, and recurrent abortion to provide evidence to support improved clinical diagnosis and management.

2. Methods

2.1 Study Population

A total of 120 pregnant women with RSA admitted to Yongkang Women and Children's Health Hospital between January 2023 and December 2024 were selected for the RSA group; 80 healthy women who underwent routine prenatal examinations during the same period were included in the control group. According to the Expert Consensus on the Diagnosis and Treatment of Recurrent Spontaneous Abortion, the inclusion criteria for the RSA group were two or more consecutive spontaneous abortions with the same partner before 28 weeks of gestation [9], together with complete clinical data (including pregnancy history, previous examination records, treatment plans, and follow-up information). As shown in Fig. 1, the inclusion criteria for the control group included at least one normal delivery, no history of spontaneous abortion, and complete clinical data (including pregnancy history, previous examination records, treatment plans, and follow-up information).

Exclusion criteria were as follows: (1) age >45 years; (2) thyroid dysfunction, diabetes mellitus, or polycystic ovary syndrome with significant metabolic abnormalities; (3) cervical insufficiency; (4) receipt of assisted reproductive technology; (5) endometrial polyps or inflammation, submucosal fibroids, or uterine malformations; (6) hypertension or arrhythmia; (7) autoimmune diseases; (8) prior targeted treatment for RSA; (9) severe semen abnormalities in the partner; (10) ectopic pregnancy, hydatidiform mole, or other abnormal intrauterine pregnancies; (11) multiple gestations; (12) incomplete clinical data.

2.2 Detection Methods

2.2.1 Measurement of Serum LDH and Inflammatory Markers

Fasting venous blood samples (5 mL) were collected from all subjects in the morning at the time of presentation and sent to the hospital clinical laboratory for analysis of serum lactate dehydrogenase (LDH), interleukin (IL)-6, IL-10, and interferon gamma (IFN-γ). Samples were centrifuged at 1200 r/min for 5 minutes, and the supernatant serum was collected. Serum levels of LDH (J20930; Wuhan Jilid Biotechnology Co., Ltd., Wuhan, China), IL-6 (J20252; Wuhan Jilid Biotechnology Co., Ltd., Wuhan, China), IL-10 (J20265; Wuhan Jilid Biotechnology Co., Ltd., Wuhan, China), and IFN-γ (J20272; Wuhan Jilid Biotechnology Co., Ltd., Wuhan, China) were measured using enzyme-linked immunosorbent assay (ELISA) with an automated microplate reader (Varioskan LUX; Thermo Fisher Scientific Inc., Waltham, USA).

2.2.2 Autoimmune Antibody Assessment

Fasting peripheral venous blood samples were collected as described above. Antinuclear antibody (ANA; J20559, Wuhan Jilid Biotechnology Co., Ltd., Wuhan, China) positivity was determined by ELISA. Antiphospholipid antibodies (APA), including anticardiolipin antibodies [anti-cardiolipin antibody immunoglobulin G (ACA-IgG): YFXEH00795; anti-cardiolipin antibody immunoglobulin M (ACA-IgM): YFXEH00794; anti-cardiolipin antibody immunoglobulin A (ACA-IgA): YFXEH00793] and anti-β2 glycoprotein I antibodies (β2-GP1 IgG/IgM/IgA, YFXEH00194), were measured using kits from Nanjing Yifeixue Biotechnology Co., Ltd., Nanjing, China.

According to manufacturer instructions and international consensus guidelines [10], APA positivity was defined as medium or high titer [>40 IgG phospholipid/IgM phospholipid (GPL/MPL) units or >99th percentile] for ACA-IgG/IgM, and/or >99th percentile for anti-β2-GP1 antibodies, detected on at least two occasions at least 12 weeks apart. ANA positivity was defined as ANA titer ≥1:100. Due to the retrospective design, single-time-point positivity at enrollment was recorded for comparative analysis.

2.2.3 Analysis of T Lymphocyte Subsets

Fasting peripheral venous blood samples were collected as described in section 2.2.1. The distribution of T lymphocyte subsets, including cluster of differentiation (CD)3^+, $CD4^+$, $CD8^+$, and $CD16^+CD56^+$ cells, was determined using flow cytometry with appropriate reagents (BD FACSCanto II, BD Biosciences, San Jose, CA, USA).

2.3 Outcome Measures

(1) General data: age, body mass index (BMI), gestational age, smoking history, alcohol consumption history, coffee consumption history, family history of spontaneous abortion, number of abortions, history of previous miscarriages, noise exposure, and irregular work and rest patterns were recorded for both groups.

(2) Clinical indicators: serum LDH levels; IL-6, IL-10, IFN-γ; proportions of lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ ratio, $CD16^+CD56^+$); and positivity rates of ANA and APA.

2.4 Statistical Methods

Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to determine normality. Continuous variables with normal distributions are expressed as mean ± standard deviation (SD), and intergroup comparisons were

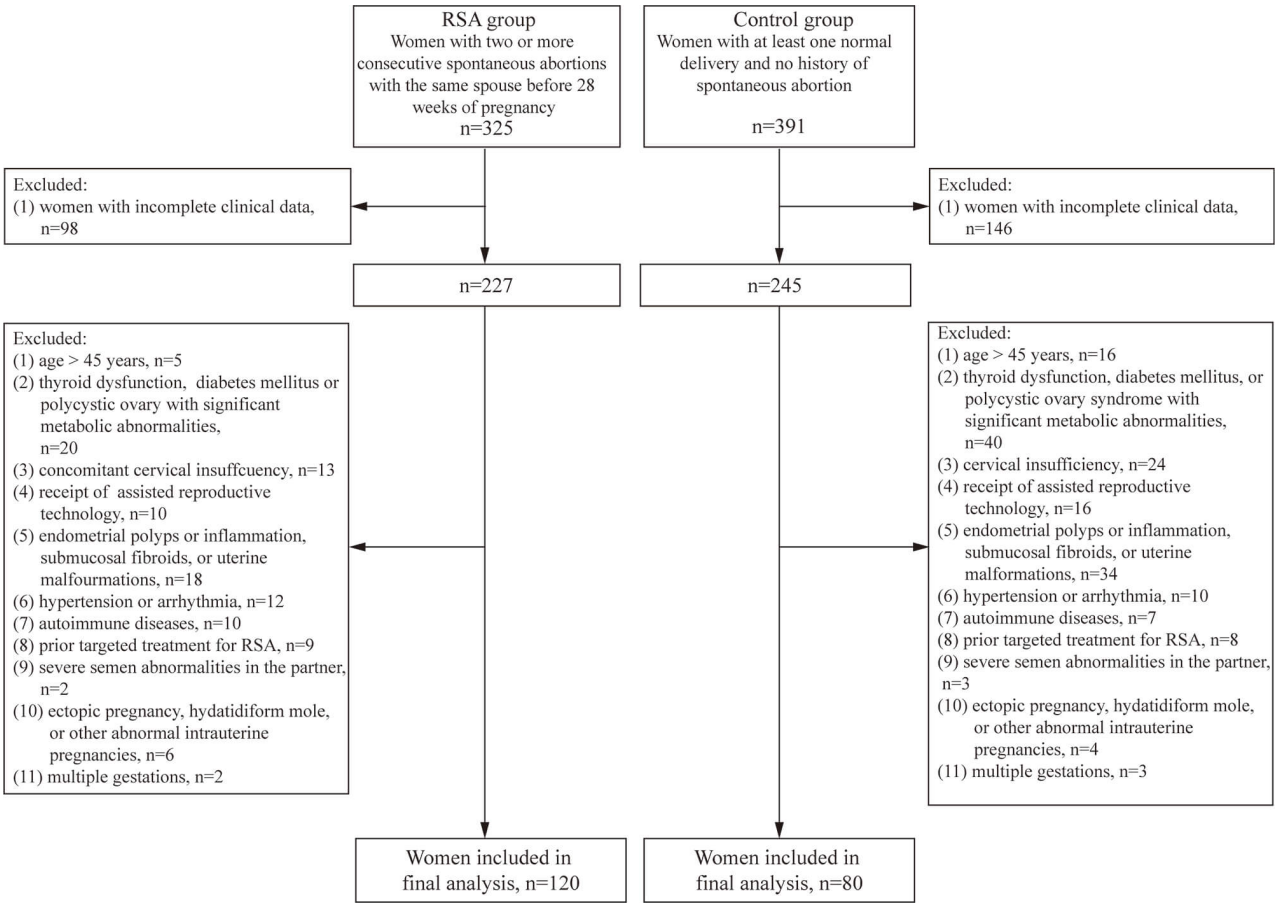

Fig. 1. Flowchart of participant inclusion and exclusion process. RSA, recurrent spontaneous abortion.

performed using the independent-samples *t*-test. Non-normally distributed variables are expressed as median (Q1, Q3), and comparisons between groups were conducted using the Mann–Whitney *U* test. Categorical variables are expressed as the number of cases (%), and comparisons were performed using the chi-square (χ^2) test or the continuity-corrected chi-square test.

Collinearity diagnostics were conducted for variables with statistical significance, and indicators with tolerance >0.1 and variance inflation factor (VIF) <5 were included in binary logistic regression analysis to identify factors associated with RSA. The diagnostic performance of each indicator was evaluated using receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) used to evaluate accuracy. The optimal cut-off value was determined by maximizing the Youden index in the ROC curve. The significance level (α) was set at 0.05 unless otherwise specified.

3. Results

3.1 Comparison of General Characteristics Between the Two Groups

A total of 120 patients with RSA and 80 women in the control group were included in this study. As shown in Table 1, significant difference was observed in number of abortions and previous miscarriages between the two groups ($p < 0.05$). No statistically significant differences were observed between the two groups in age, BMI, gestational age, smoking history, alcohol consumption history, coffee consumption history, family history of spontaneous abortion, noise exposure, or irregular work and rest patterns ($p > 0.05$).

3.2 Differences in Serum LDH and Inflammatory Factor Levels

As shown in Table 2, significant differences in serum LDH and inflammatory markers were observed between the two groups. LDH and IFN-γ levels in the RSA group were significantly higher than those in the control group ($p < 0.05$), while IL-6 and IL-10 levels were significantly lower than those in the control group ($p < 0.05$; Table 2).

3.3 Differences in T Lymphocyte Subsets

As presented in Table 3, significant differences in lymphocyte subsets were identified between the two groups, particularly in $CD4^+$ and $CD8^+$ T-cell proportions ($p < 0.05$). However, no significant differences were observed in $CD3^+$, $CD4^+/CD8^+$ ratio, or $CD16^+CD56^+$ cells ($p > 0.05$).

Table 1. General characteristics of the study population.

Variables	RSA group (n = 120)	Control group (n = 80)	χ^2/Z	*p*-value
Age (years), M (Q1, Q3)	30.00 (28–34)	31.50 (27–35)	0.296	0.767
BMI (kg/m^2), M (Q1, Q3)	24.81 (21.00–27.98)	24.78 (22.26–26.86)	0.323	0.747
Gestational age (days), M (Q1, Q3)	58 (55, 65)	58 (51, 65)	1.074	0.283
Smoking history			0.430	0.512
No (n, %)	101 (84.17)	70 (87.50)		
Yes (n, %)	19 (15.83)	10 (12.50)		
Alcohol consumption history			0.623	0.430
No (n, %)	76 (63.33)	55 (68.75)		
Yes (n, %)	44 (36.67)	25 (31.25)		
Coffee consumption history			0.665	0.415
No (n, %)	65 (54.17)	48 (60.00)		
Yes (n, %)	55 (45.83)	32 (40.00)		
Family history of spontaneous abortion			0.084	0.773
No (n, %)	62 (51.67)	43 (53.75)		
Yes (n, %)	58 (48.33)	37 (46.25)		
Number of abortions, M (Q1, Q3)	3 (3, 3)	0	12.664	<0.001
Gestational age at previous miscarriage (weeks), M (Q1, Q3)	8 (7, 8)	0	12.455	<0.001
Noise exposure			0.079	0.779
No (n, %)	25 (20.83)	18 (22.50)		
Yes (n, %)	95 (79.17)	62 (77.50)		
Irregular work and rest patterns			0.068	0.794
No (n, %)	31 (25.83)	22 (27.50)		
Yes (n, %)	89 (74.17)	58 (75.50)		

BMI, body mass index; M, median.

Table 2. Comparison of serum LDH and inflammatory marker levels between the two groups.

Variables	RSA group (n = 120)	Control group (n = 80)	*Z*	*p*-value
LDH (U/L), M (Q1, Q3)	148.00 (140.75–162.50)	141.00 (131.00–152.00)	3.520	<0.001
IL-6 (pg/mL), M (Q1, Q3)	2.89 (2.30–8.36)	3.10 (2.50–13.00)	2.480	0.013
IL-10 (pg/mL), M (Q1, Q3)	4.52 (1.79–8.26)	6.61 (2.18–10.01)	2.006	0.045
IFN-γ (pg/mL), M (Q1, Q3)	1.91 (1.20–2.69)	1.21 (0.91–2.30)	2.787	0.005

LDH, lactate dehydrogenase; IL, interleukin; IFN-γ, interferon gamma.

Table 3. Comparison of T lymphocyte subsets between the two groups.

Variables	RSA group (n = 120)	Control group (n = 80)	*Z*/*t*	*p*-value
CD3$^+$ (%), M (Q1, Q3)	70.18 (67.21–75.14)	71.02 (68.36–77.24)	1.221	0.222
CD4$^+$ (%), M (Q1, Q3)	41.04 (38.65–45.96)	39.93 (33.48–42.18)	2.682	0.007
CD8$^+$ (%), M (Q1, Q3)	23.87 (20.59–26.03)	26.83 (23.93–31.69)	5.770	<0.001
CD4$^+$/CD8$^+$ ratio, mean ± SD	1.79 ± 0.29	1.70 ± 0.54	1.520	0.130
CD16$^+$CD56$^+$ (%), M (Q1, Q3)	12.75 (10.21, 16.10)	11.88 (9.26, 15.61)	1.299	0.194

CD, cluster of differentiation; SD, standard deviation.

3.4 Differences in Serum Autoimmune Antibody Levels

As shown in Table 4, there was no significant difference in ANA positivity between the two groups ($p > 0.05$). In contrast, APA differed significantly between groups ($p < 0.05$).

3.5 Factors Associated With RSA

Collinearity diagnostics were performed for variables with significant differences. Indicators with VIF less than 5 and tolerance greater than 0.1 were included as independent variables (Table 5), and RSA status was the dependent variable in the multivariable logistic regression model. The analysis showed that LDH, IL-6, CD4$^+$, and CD8$^+$ were significant factors associated with RSA ($p < 0.05$; Table 6).

Table 4. Comparison of autoimmune antibody levels between the two groups.

Variables	RSA group (n = 120)	Control group (n = 80)	χ^2	*p*-value
ANA			0.266	0.606
Negative, n (%)	114 (95.00)	78 (97.50)		
Positive, n (%)	6 (5.00)	2 (2.50)		
APA			6.495	0.011
Negative, n (%)	56 (46.67)	52 (65.00)		
Positive, n (%)	64 (53.33)	28 (35.00)		

ANA, antinuclear antibody; APA, antiphospholipid antibodies.

Table 5. Multicollinearity analysis of variables with significant differences.

Variables	Collinearity statistics	
	Tolerance	VIF
LDH	0.886	1.128
IL-6	0.386	2.589
IL-10	0.361	2.770
IFN-γ	0.788	1.269
$CD4^+$	0.897	1.115
$CD8^+$	0.962	1.040
APA	0.793	1.261

VIF, variance inflation factor.

3.6 Diagnostic Performance of Serum LDH Combined With Immune-Inflammatory Markers in RSA

To evaluate the diagnostic performance of serum LDH and key immune-inflammatory markers for differentiating RSA, ROC curve analysis was performed. The ROC curves for each indicator are shown in Fig. 2, and the corresponding AUC values, optimal cut-off points, sensitivity, and specificity are shown in Table 7. As shown in Table 7 and Fig. 2, $CD8^+$ T cells demonstrated the highest diagnostic performance among individual indicators (AUC = 0.741). The combined model incorporating serum LDH and immune-inflammatory markers yielded an AUC of 0.908, with a sensitivity of 0.875 [95% confidence interval (CI): 0.802–0.928] and specificity of 0.823 (95% CI: 0.721–0.900), indicating superior diagnostic performance compared with any single indicator.

4. Discussion

This study retrospectively analyzed 120 patients with RSA, focusing on serum LDH and multiple immune-inflammatory markers to identify independent risk factors for RSA. The results showed that increased LDH level, increased $CD4^+$ proportion, decreased IL-6 level, and decreased $CD8^+$ proportion were independent risk factors for RSA.

Placental oxygen tension undergoes dynamic changes during pregnancy and is tightly regulated to meet the metabolic demands of the fetus and placenta [11]. During abortion, oxygen tension at the maternal-fetal interface may decrease to approximately 1%, resulting in a state of persistent hypoxia [12]. In the present study, LDH was identified as an independent risk factor for RSA, and its level was significantly higher in RSA patients than in the control group. This may be explained by hypoxia-induced acceleration of glycolysis, which enhances lactate production through LDH. The resulting metabolic shift may influence macrophage polarization at the maternal-fetal interface, promote inflammatory responses, and ultimately contribute to pregnancy loss [13]. In addition, the positive rate of antiphospholipid antibodies was significantly higher in the RSA than in the control group. These antibodies can interact with cellular membranes and proteins, activate platelet function and coagulation cascades, and lead to a hypercoagulable state [14]. This process may result in placental thrombosis, infarction, and reduced fetal blood supply, thereby creating a hypoxic environment that further promotes lactate accumulation through glycolysis and contributes to abortion.

Moreover, LDH-mediated lactate production may exert immunomodulatory effects. Elevated lactate levels can inhibit the proliferation and function of $CD8^+$ T cells. At the maternal-fetal interface in RSA, a similar mechanism may occur. Trophoblast cells under hypoxic stress may exhibit increased LDH accumulation [15]. This microenvironment may suppress $CD8^+$ T-cell subsets that are essential for maintaining immune tolerance during pregnancy, disrupt the balance between $CD4^+$ and $CD8^+$ T cells, and promote a shift toward a pro-inflammatory immune phenotype.

Previous studies have demonstrated that immune dysfunction is an important contributor to RSA [16,17]. T lymphocytes can be broadly classified into helper and suppressor subsets according to phenotype and function. Helper T cells promote immune activation through cytokine secretion, while suppressor T cells exert regulatory effects that limit excessive immune responses. The balance between these subsets is essential for maintaining normal physiological function and immune response. In normal pregnancy, a relative decrease in $CD4^+$ helper T lymphocytes and an increase in $CD8^+$ suppressor T lymphocytes have been reported. In the present study, although the $CD4^+/CD8^+$ ratio in RSA patients was slightly higher than that in the control group, without statistical significance, $CD4^+$ T-cell levels in peripheral blood were significantly elevated, and $CD8^+$ T-

Table 6. Multivariate logistic regression analysis of factors associated with RSA.

Variables	β	SE	Wald	OR	95% CI	*p*-value
LDH (U/L)	0.073	0.018	16.876	1.075	1.039–1.113	<0.001
IL-6 (pg/mL)	–0.195	0.066	8.767	0.823	0.724–0.936	0.003
IL-10 (pg/mL)	–0.109	0.072	2.274	0.897	0.779–1.033	0.132
IFN-γ (pg/mL)	0.179	0.215	0.690	1.196	0.784–1.825	0.406
$CD4^+$ (%)	0.255	0.056	21.006	1.290	1.157–1.438	<0.001
$CD8^+$ (%)	–0.456	0.081	31.409	0.634	0.540–0.743	<0.001
APA (%)	0.796	0.473	2.833	2.217	0.877–5.601	0.092

OR, odds ratio; CI, confidence interval; SE, standard error; LDH, lactate dehydrogenase; IL, interleukin; IFN-γ, interferon gamma; APA, antiphospholipid antibodies.

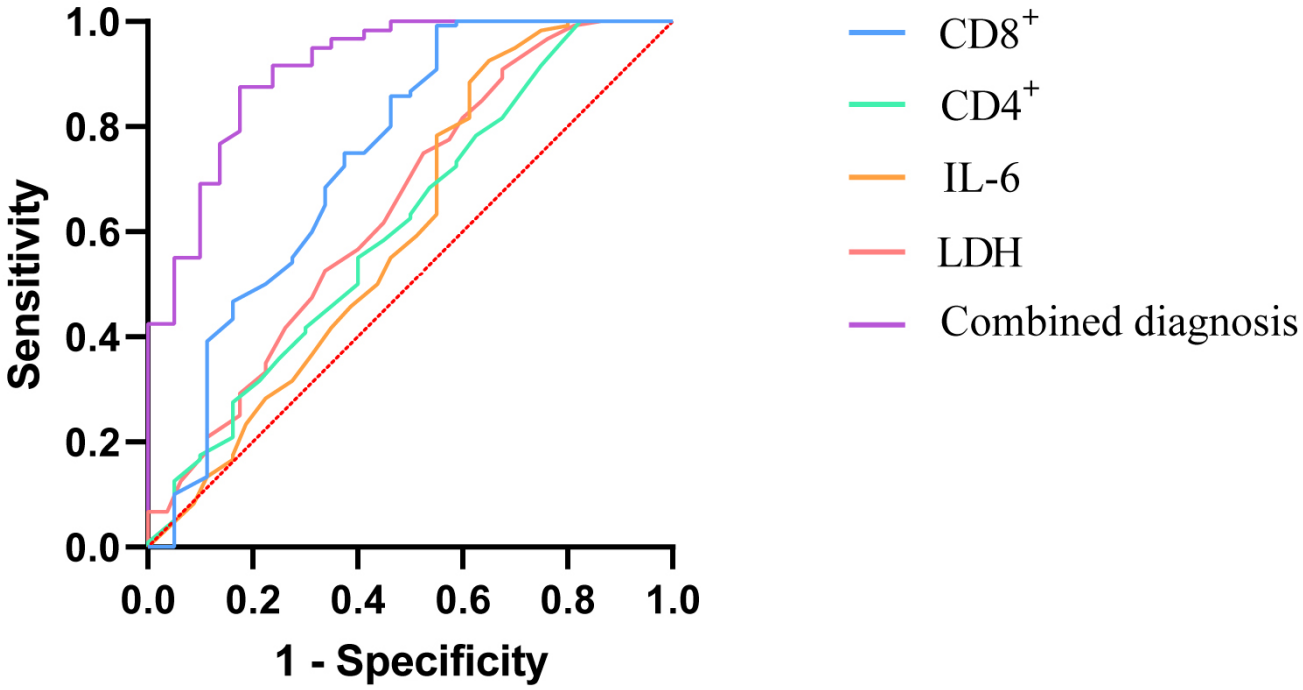

Fig. 2. Receiver operating characteristic (ROC) curves of serum LDH and immune-inflammatory markers for the diagnosis of recurrent spontaneous abortion.

Table 7. Diagnostic performance of serum LDH and immune-inflammatory markers for recurrent spontaneous abortion.

Variables	Cut-off	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	*p*-value
LDH	139.00	0.647 (0.567–0.726)	0.775 (0.692–0.841)	0.425 (0.323–0.534)	<0.001
IL-6	8.81	0.603 (0.519–0.688)	0.783 (0.702–0.848)	0.450 (0.346–0.559)	0.016
$CD4^+$	38.17	0.612 (0.531–0.693)	0.733 (0.648–0.804)	0.413 (0.311–0.522)	0.007
$CD8^+$	25.55	0.741 (0.666–0.816)	0.750 (0.666–0.819)	0.625 (0.516–0.723)	<0.001
Combined model	-	0.908 (0.859–0.945)	0.875 (0.802–0.928)	0.823 (0.721–0.900)	<0.001

AUC, area under the curve.

cell levels were significantly reduced. These findings may reflect enhanced cellular immune activity in RSA. Disruption of maternal immune tolerance may increase immune rejection responses, leading the maternal immune system to recognize the fetus as an allogeneic graft and subsequently trigger abortion [18].

IFN-γ is primarily produced by Th1 cells and can inhibit the proliferation of Th2 cells. The results of this study showed that the serum IFN-γ level in the RSA group was significantly higher than that in the control group. IFN-γ exerts cytotoxic effects on embryos, inhibiting cell growth and embryonic development, ultimately leading to embryo loss, consistent with the findings of Mousavi-Salehi et al.

[19]. In addition, reduced IL-6 was identified as an independent risk factor for RSA, and IL-6 levels in RSA patients were lower than those in normal pregnancy. This may be attributed to the role of IL-6 in stimulating immune cells to produce antibodies, suppressing maternal immune rejection, promoting the expression of adhesion molecules, and participating in the regulation of angiogenic factor synthesis, all of which are critical for maintaining pregnancy. Decreased serum IL-6 levels may therefore lead to a Th1/Th2 imbalance, consistent with the findings of Ma et al. [20].

To date, a series of factors have been identified in the plasma of patients with RSA and evaluated as predictors of RSA, including indoleamine 2,3-dioxygenase and human chorionic gonadotropin (hCG) [21,22]. In this study, variations in LDH levels and immune-inflammatory markers were used to evaluate recurrent abortion. The results demonstrated that the sensitivity, specificity, and area under the ROC curve of combined detection of LDH and immune-inflammatory markers were higher than those of individual indicators, suggesting that combined detection has meaningful diagnostic value for recurrent abortion in patients with RSA.

In addition to LDH and the aforementioned immune-inflammatory biomarkers, other novel peripheral inflammatory biomarkers have attracted increasing attention in recent years. Although no studies have yet evaluated the relationship between butyrylcholinesterase (BChE) and RSA, as an inflammation-related indicator, future research could investigate BChE level fluctuations in RSA patients and evaluate its potential to provide additional value to the combined diagnostic model proposed in this study.

We observed that the positivity rate of APA in the RSA group was significantly higher than in the control group, consistent with the established role of APA in placental thrombosis and hypoxia. However, due to the retrospective design of this study and the use of a single test at enrollment, APA positivity could not be confirmed according to standard diagnostic criteria. Therefore, the reported APA positivity may include transient positivity related to infection or other conditions, and these findings should be interpreted with caution. Future prospective studies with confirmatory assays are warranted to validate the association between persistent APA positivity and RSA in this population.

Notably, the RSA group in this study exhibited a higher proportion of noise exposure and irregular work and rest patterns, suggesting that these patients may originate from a specific occupational background. This phenomenon may be associated with the geographical location and socio-economic characteristics of the hospital. Yongkang City is a well-known hardware manufacturing center in China, where a large number of women are engaged in industrial processing, assembly line operations, or small family workshops. These occupations are often accompanied by prolonged exposure to mechanical noise and shift or overtime work, leading to circadian rhythm disruption. Increasing evidence indicates that chronic noise exposure and circadian rhythm disturbances can affect neuroendocrine function, interfere with the hypothalamic-pituitary-ovarian axis, and may induce immune-inflammatory imbalance, thereby increasing the risk of RSA [23,24,25]. Therefore, the occupational background of the sample population in this study may reflect the combined effects of environmental and behavioral factors on RSA, which warrants further investigation in future studies.

Several limitations of this study should be acknowledged. This was a single-center retrospective study with a relatively small sample size and a homogeneous occupational background, which may have introduced selection bias. APA was measured only once at enrollment, without confirmatory testing at 12 weeks; thus, some positive results may be transient, and the association between APA and RSA should be interpreted cautiously. Additionally, the lack of longitudinal monitoring of LDH and immune-inflammatory markers limits causal inference. Other potential confounding factors, such as genetic predisposition and psychological stress, were not comprehensively evaluated. Future multicenter prospective studies with larger sample sizes, standardized APA confirmation, dynamic biomarker monitoring, and investigation of environment-immune-metabolism interactions are required to address these limitations.

5. Conclusion

In summary, serum LDH, IL-6, $CD4^+$, and $CD8^+$ are influencing factors for recurrent spontaneous abortion in patients with RSA. The combined detection of LDH, IL-6, $CD4^+$, and $CD8^+$ demonstrates marked value for disease diagnosis.

Key Points

• Recurrent spontaneous abortion can lead to intrauterine adhesions and contribute to female infertility.

• Serum LDH levels in RSA patients are significantly elevated and are closely associated with adverse pregnancy outcomes.

• Lactate dehydrogenase promotes lactic acid accumulation under hypoxia-driven glycolysis and influences T cell subsets, resulting in immune-inflammatory dysregulation at the maternal-fetal interface and ultimately leading to abortion.

• Multivariate logistic regression analysis showed that elevated LDH levels, increased $CD4^+$ proportion, decreased IL-6, and reduced $CD8^+$ proportion are independent risk factors for RSA.

• ROC curve analysis indicated that the combined diagnostic model incorporating LDH, IL-6, $CD4^+$, and $CD8^+$ exhibits strong discriminative performance for RSA, providing robust support for the development of non-invasive and efficient auxiliary diagnostic tools in clinical practice.

Availability of Data and Materials

The analyzed data are available from the corresponding author upon reasonable request.

Author Contributions

JFW had the original conception of the work. WJC and SSH collected the clinical data. SNX and LMS performed the research. JFW drafted the manuscript. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Review Committee of Yongkang Women and Children's Health Hospital [Approval No. AF/SQ-01(03)/02.01.04], and informed consent was obtained from all participants. All procedures adhered to the Declaration of Helsinki.

Acknowledgment

The authors would like to acknowledge the support and assistance of Yongkang Women and Children's Health Hospital.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Deng T, Liao X, Zhu S. Recent Advances in Treatment of Recurrent Spontaneous Abortion. Obstetrical & Gynecological Survey. 2022; 77: 355–366. https://doi.org/10.1097/OGX.0000000000001033

[2] Li D, Zheng L, Zhao D, Xu Y, Wang Y. The Role of Immune Cells in Recurrent Spontaneous Abortion. Reproductive Sciences. 2021; 28: 3303–3315. https://doi.org/10.1007/s43032-021-00599-y

[3] Wu A, Zhao Y, Yu R, Zhou J, Tuo Y. Untargeted metabolomics analysis reveals the metabolic disturbances and exacerbation of oxidative stress in recurrent spontaneous abortion. PloS One. 2023; 18: e0296122. https://doi.org/10.1371/journal.pone.0296122

[4] Albright CM, Ali TN, Lopes V, Rouse DJ, Anderson BL. Lactic acid measurement to identify risk of morbidity from sepsis in pregnancy. American Journal of Perinatology. 2015; 32: 481–486. https://doi.org/10.1055/s-0034-1395477

[5] Tang C, Hu W. Non-coding RNA regulates the immune microenvironment in recurrent spontaneous abortion (RSA): new insights into immune mechanisms†. Biology of Reproduction. 2024; 110: 220–229. https://doi.org/10.1093/biolre/ioad157

[6] Talukdar A, Sharma KA, Rai R, Deka D, Rao DN. Effect of Coenzyme Q10 on Th1/Th2 Paradigm in Females with Idiopathic Recurrent Pregnancy Loss. American Journal of Reproductive Immunology. 2015; 74: 169–180. https://doi.org/10.1111/aji.12376

[7] Kanellopoulos-Langevin C, Caucheteux SM, Verbeke P, Ojcius DM. Tolerance of the fetus by the maternal immune system: role of inflammatory mediators at the feto-maternal interface. Reproductive Biology and Endocrinology. 2003; 1: 121. https://doi.org/10.1186/1477-7827-1-121

[8] Ye L, Jiang Y, Zhang M. Crosstalk between glucose metabolism, lactate production and immune response modulation. Cytokine & Growth Factor Reviews. 2022; 68: 81–92. https://doi.org/10.1016/j.cytogfr.2022.11.001

[9] Obstetrics Subgroup, Chinese Society of Obstetrics and Gynecology, Chinese Medical Association; Chinese Expert Consensus Group on Diagnosis and Management of Recurrent Spontaneous Abortion. Chinese expert consensus on diagnosis and management of recurrent spontaneous abortion (2022). Chinese Journal of Obstetrics and Gynecology. 2022; 57: 653–667. https://doi.org/10.3760/cma.j.cn112141-20220421-00259 (In Chinese)

[10] Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). Journal of Thrombosis and Haemostasis. 2006; 4: 295–306. https://doi.org/10.1111/j.1538-7836.2006.01753.x

[11] Jauniaux E, Poston L, Burton GJ. Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Human Reproduction Update. 2006; 12: 747–755. https://doi.org/10.1093/humupd/dml016

[12] Tian P, Xu Z, Guo J, Zhao J, Chen W, Huang W, et al. Hypoxia causes trophoblast cell ferroptosis to induce miscarriage through lnc-HZ06/HIF1α-SUMO/NCOA4 axis. Redox Biology. 2024; 70: 103073. https://doi.org/10.1016/j.redox.2024.103073

[13] Huang X, Lin Z, Zheng ZM, Shi JL, Lu KY, Wang JR, et al. A Hypoxia-Decidual Macrophage Regulatory Axis in Normal Pregnancy and Spontaneous Miscarriage. International Journal of Molecular Sciences. 2024; 25: 9710. https://doi.org/10.3390/ijms25179710

[14] Alijotas-Reig J, Esteve-Valverde E, Anunciación-Llunell A, Marques-Soares J, Pardos-Gea J, Miró-Mur F. Pathogenesis, Diagnosis and Management of Obstetric Antiphospholipid Syndrome: A Comprehensive Review. Journal of Clinical Medicine. 2022; 11: 675. https://doi.org/10.3390/jcm11030675

[15] Gao L, Xu QH, Ma LN, Luo J, Muyayalo KP, Wang LL, et al. Trophoblast-derived Lactic Acid Orchestrates Decidual Macrophage Differentiation via SRC/LDHA Signaling in Early Pregnancy. International Journal of Biological Sciences. 2022; 18: 599–616. https://doi.org/10.7150/ijbs.67816

[16] Chen X, Song QL, Ji R, Wang JY, Cao ML, Guo DY, et al. JPT2 Affects Trophoblast Functions and Macrophage Polarization and Metabolism, and Acts as a Potential Therapeutic Target for Recurrent Spontaneous Abortion. Advanced Science. 2024; 11: e2306359. https://doi.org/10.1002/advs.202306359

[17] Tang C, Hu W. The role of Th17 and Treg cells in normal pregnancy and unexplained recurrent spontaneous abortion (URSA): New insights into immune mechanisms. Placenta. 2023; 142: 18–26. https://doi.org/10.1016/j.placenta.2023.08.065

[18] Thellin O, Heinen E. Pregnancy and the immune system: between tolerance and rejection. Toxicology. 2003; 185: 179–184. https://doi.org/10.1016/s0300-483x(02)00607-8

[19] Mousavi-Salehi A, Ghafourian M, Amari A, Zargar M. Evaluation of NKT Cell Percentage and Function and Its Relationship with Serum IFN-γ and Vitamin D Levels in Women with Recurrent Spontaneous Abortion and Recurrent Implantation Failure. Journal of Obstetrics and Gynaecology of India. 2024; 74: 391–397. https://doi.org/10.1007/s13224-023-01894-1

[20] Ma W, Gu Z, Lin Q, Cao M, Zhong J, Li X, et al. Integrative genetic and multi-omics analysis reveals the interleukin-6 re-

ceptor's role in recurrent spontaneous abortion. Frontiers in Immunology. 2025; 16: 1659251. https://doi.org/10.3389/fimmu.2025.1659251

[21] Liu Y, Liu Y, Li X, Jiao X, Zhang R, Zhang J. Predictive value of serum β-hCG for early pregnancy outcomes among women with recurrent spontaneous abortion. International Journal of Gynaecology and Obstetrics. 2016; 135: 16–21. https://doi.org/10.1016/j.ijgo.2016.03.007

[22] Ban Y, Chang Y, Dong B, Kong B, Qu X. Indoleamine 2,3-dioxygenase levels at the normal and recurrent spontaneous abortion fetal-maternal interface. The Journal of International Medical Research. 2013; 41: 1135–1149. https://doi.org/10.1177/0300060513487642

[23] Cui L, Xu F, Xu C, Ding Y, Wang S, Du M. Circadian gene Rev-erbα influenced by sleep conduces to pregnancy by promoting endometrial decidualization via IL-6-PR-C/EBPβ axis. Journal of Biomedical Science. 2022; 29: 101. https://doi.org/10.1186/s12929-022-00884-1

[24] Baroeva MD, Datieva FS, Popova LS, Tsallagova LV. Characteristics of circadian regulation in patients with miscarriage. Obstetrics and Gynecology. 2024; 5–11. https://doi.org/10.18565/aig.2024.95

[25] Haldar C, Chowdhury JP. Chronodisruption and Loss of Female Reproductive Potential Due to Shift Work. In Wani KA, Naha N (eds.) Climate Change and Its Impact on Fertility and Life Forms (pp. 70–98). IGI Global Scientific Publishing: Hershey, PA. 2021.