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RESEARCH ARTICLE OPEN ACCESS

Decoding recurrent pregnancy loss: an integrated infectious-immune and multisystem biomarker signature associated with risk stratification

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Abstract
Objective: To assess associations between a panel of immunological (antinuclear antibodies [ANA], cytomegalovirus [CMV] IgM/IgG), thyroid (thyroid-stimulating hormone [TSH], T3, T4, free T3, free T4), coagulation (D-dimer, prothrombin time [PT], partial thromboplastin time [PTT]), haematological (haemoglobin, platelets) and metabolic (HbA1c) markers and the number of previous spontaneous abortions in women with recurrent pregnancy loss (RPL).
Methods: An analytical cross-sectional study was conducted among 100 women aged 26–39 years (mean 31.8 ± 3.7 years) with a history of 1–5 spontaneous abortions. Blood samples were collected during the follicular phase. Statistical analyses included the Kruskal–Wallis test, Spearman correlation, and Fisher's exact test.
Results: All continuous markers showed highly significant differences across abortion-frequency groups ($p < 0.001$). Median TSH, D-dimer, PT, PTT and HbA1c increased progressively with more abortions, whereas haemoglobin and platelets decreased. TSH showed the strongest positive correlation with abortion number ($\rho = +0.896$). ANA positivity rose from 2.9% (two abortions) to 100% (four to five abortions). CMV IgM positivity increased from 5.7% to 95.8–100%. All categorical markers demonstrated highly significant associations with abortion-frequency groups ($p < 0.001$).
Conclusions: This multi-system biomarker panel exhibits strong, dose-dependent associations with RPL severity. Prospective validation with healthy control groups is needed before clinical application.

Keywords: Recurrent Pregnancy Loss, Biomarkers, Thyroid Function, Autoimmunity, Thrombophilia

Plain English Summary

Recurrent pregnancy loss (RPL) strikes 1-5% of couples who are looking to conceive, but up to 50% are without a known cause. This study aimed to determine if blood tests of the immune system, thyroid gland, blood clotting, blood counts and blood sugar change based on the number of previous miscarriages. In this study, we studied 100 women with 1-5 pregnancy losses. Women who had more miscarriages showed higher levels of thyroid-stimulating hormone (TSH), clotting markers (D-dimer, PT, PTT) and a long-term blood sugar marker (HbA1c). They also had lower haemoglobin and platelet counts. Markers of immune activation (ANA and antibodies against cytomegalovirus) were almost always positive in women with four or five miscarriages but rare in those with only two miscarriages. These findings suggest that a panel of simple blood tests might help identify women at higher risk of repeated pregnancy loss, but further research is needed to confirm this.

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Introduction

Recurrent pregnancy loss (RPL), defined by the European Society of Human Reproduction and Embryology (ESHRE) as two or more consecutive pregnancy losses before 24 weeks' gestation, occurs in 1–5% of couples trying to conceive (1, 2). Even with modern diagnostic tools, up to 50% of cases remain unexplained (3). The pathophysiology of RPL is multifactorial, involving endocrine disorders, uterine anomalies, chromosomal defects, thrombophilia, infections and immune dysregulation (4, 5).

Among endocrine factors, overt thyroid disease is known to increase miscarriage risk. In the 17 studies included in the meta-analysis, women with thyroid autoimmunity had a significant increase in the chances of having RPL (odds ratio 1.94; 95% confidence interval 1.43–2.64) (6). Subclinical hypothyroidism has been reported in 12.9% of RPL patients [6]. Immunologically, antinuclear antibodies (ANA) are detected three times more frequently in women with RPL than in fertile controls, suggesting a role for autoimmunity in implantation failure and pregnancy loss (7). Human cytomegalovirus (CMV) has also been implicated: seropositivity for CMV IgM and IgG antibodies is associated with a miscarriage history, possibly by disrupting immune tolerance at the maternal–foetal interface (8).

Coagulation disturbances, particularly elevated D-dimer and reduced antithrombin III, may indicate a pre-thrombotic state leading to placental thrombosis and reduced perfusion in unexplained RPL (9). Metabolic dysregulation, reflected by elevated glycated haemoglobin (HbA1c), has been shown to impair endometrial receptivity and embryo development (10).

Despite extensive research on each of these systems, few studies have investigated them concurrently in the same population and stratified by abortion frequency. An integrated approach is essential to gaining insight into the interactions and possible interactions of these biological systems and their contribution towards each other in the context of progressive miscarriage. In the present study, however, we evaluated a multi-system biomarker panel in 100 women with RPL, and we sought to characterise the gradient of biological abnormalities between the women with increasing numbers of previous abortions.

Materials and Methods

Study design and population

This cross-sectional study was an analytical study conducted at Al Kadhimiya Teaching Hospital, Baghdad, Iraq, from March 2024 to March 2025. One hundred women with a history of recurrent pregnancy loss (RPL) were consecutively recruited. Inclusion criteria were a diagnosis of RPL (spontaneous pregnancy loss of 2 or more losses before 24 weeks), age of 26–39 years, and willingness to sign written informed consent. Exclusion criteria were current use of immunosuppressive drugs (such as for systemic lupus erythematosus or rheumatoid arthritis), anticoagulation therapy, or thyroxine treatment at the time of recruitment. One participant who had undergone only one abortion was selected for exploratory comparison because her data were a baseline reference.

Data collection and laboratory investigations

Demographic and clinical data, including age, marriage duration, number of previous spontaneous abortions, gestational age at abortion, number of previous full-term pregnancies and comorbidities, were collected through structured interviews and medical record reviews.

Venous blood samples were drawn during the follicular phase (days 2–5 of the menstrual cycle) using standardised venepuncture. All laboratory analyses were performed blinded to the participant's abortion history.

Immunological markers: Antinuclear antibodies (ANA) were measured using a MAGLUMI X3 automated chemiluminescence immunoassay analyser (Snibe Co., Ltd., Shenzhen, China). Cytomegalovirus (CMV) IgM and IgG were detected by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Dia.Pro Diagnostic Bioprobes, Italy).

Thyroid markers: Thyroid-stimulating hormone (TSH), free T3 (FT3), free T4 (FT4), total T3 and total T4 were determined on the MAGLUMI X3 platform according to the manufacturer's protocols.

Coagulation markers: D-dimer was measured using the MAGLUMI X3 analyser. Prothrombin time (PT) and activated partial thromboplastin time (PTT) were measured with reagents from Agappe Diagnostics Ltd. (India).

Haematological markers: Haemoglobin and platelet counts were obtained using an automated blood analyser (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Metabolic marker: Glycated haemoglobin (HbA1c) was measured by high-performance liquid chromatography using a GH-900 plus analyser (Lifotronic Technology Co., Ltd., Shenzhen, China).

Statistical analysis

Data were analysed using SPSS version 26. Continuous variables were summarised as mean ± standard deviation (SD) and median with interquartile range (IQR). Categorical variables were presented as frequencies and percentages. Normality was assessed with the Kolmogorov–

Smirnov test; all continuous variables showed a non-normal distribution ($p < 0.05$), so non-parametric methods were used throughout. The Kruskal–Wallis H test was applied to compare continuous laboratory markers across abortion-frequency groups (1, 2, 3, 4 and 5 previous abortions). Spearman's rank correlation coefficient (ρ) was used to evaluate associations between continuous markers and abortion number, as well as inter-marker relationships. Associations between categorical markers and abortion-frequency groups were assessed with Fisher's exact test. A p-value <0.05 was considered statistically significant. Given the exploratory nature of the study, no adjustment for multiple comparisons was made, but the reader is cautioned that the risk of type I error is increased.

Results

Baseline characteristics

The study included 100 women with a mean age of 31.80 ± 3.66 years (range 26–39). The median number of previous spontaneous abortions was 3 (range 1–5), and the median gestational age at miscarriage was 9 weeks (range 2–14 weeks). Baseline laboratory values are shown in Table 1.

Table 1: Baseline clinical and laboratory characteristics of the study population (N = 100)

Variable	Minimum	Maximum	Mean ± SD	Median
Age (years)	26	39	31.80 ± 3.66	31
Years of marriage	3	16	8.18 ± 3.40	7.5
Number of abortions	1	5	2.93 ± 0.87	3
Gestational age (weeks)	2	14	8.02 ± 3.49	9
Haemoglobin (g/dL)	10.5	13.2	11.81 ± 0.77	11.8
Platelets (×10³/μL)	150	345	217.2 ± 40.4	215
Prothrombin time (sec)	12.5	15.7	14.08 ± 0.94	14.1
PTT (sec)	29	43	36.14 ± 3.92	36
D-dimer (μg/mL)	0.3	1.5	0.75 ± 0.34	0.7
HbA1c (%)	5	6.5	5.69 ± 0.41	5.65

Abbreviations: PTT, partial thromboplastin time; HbA1c, glycated haemoglobin

Frequency distribution of categorical markers

Table 2 summarises the prevalence of categorical immunological and thyroid-related markers. ANA positivity was observed in 39% of participants. CMV IgM and CMV IgG were positive in 56% and 49%, respectively. Low levels of T3/FT3 were found in 56% and low T4/FT4 in 55% of women.

Table 2: Frequency distribution of categorical markers among women with recurrent pregnancy loss

Marker	Category	Frequency (n)	Percentage (%)
ANA	Negative	61	61
	Positive	39	39
CMV-IgG	Negative	51	51
	Positive	49	49
CMV-IgM	Negative	44	44
	Positive	56	56
FT3/T3	Low	56	56
	Normal	44	44
FT4/T4	Low	55	55
	Normal	45	45

Abbreviations: ANA, antinuclear antibody; CMV, cytomegalovirus; FT3, free triiodothyronine; FT4, free thyroxine

Differences across abortion-frequency groups

Kruskal–Wallis analysis revealed highly significant differences across the five groups (1, 2, 3, 4 and 5 abortions) for all continuous laboratory markers ($p < 0.001$). As shown in Table 3, median haemoglobin and platelet levels decreased progressively with increasing abortion number, whereas median PT, PTT, D-dimer, HbA1c and TSH increased progressively. The largest H-statistic was observed for TSH ($H = 80.5$), followed by PT (79.3), PTT (78.9) and D-dimer (77.9).

Table 3: Median (IQR) of continuous markers by number of abortions

Marker	1 abortion (n=1)	2 abortions (n=35)	3 abortions (n=37)	4 abortions (n=24)	5 abortions (n=3)	H (df=4)	p-value
Hb (g/dL)	13.2 (13.2–13.2)	12.6 (12.5–12.8)	11.8 (11.6–11.9)	10.9 (10.7–11.1)	10.5 (10.5–10.7)	70.5	<0.001
Platelets (×10³/µL)	230 (230–230)	255 (248–260)	210 (200–220)	168 (163–175)	155 (153–155)	67.1	<0.001
PT (sec)	12.5 (12.5–12.5)	13.0 (12.9–13.3)	14.2 (14.0–14.4)	15.3 (15.1–15.5)	15.6 (15.6–15.7)	79.3	<0.001
PTT (sec)	29.0 (29.0–29.0)	32.0 (31.0–33.0)	36.0 (36.0–37.0)	41.0 (40.5–42.0)	43.0 (42.5–43.0)	78.9	<0.001
D-dimer (µg/mL)	0.33 (0.33–0.33)	0.40 (0.35–0.46)	0.71 (0.70–0.80)	1.20 (1.10–1.30)	1.40 (1.35–1.45)	77.9	<0.001
HbA1c (%)	5.10 (5.10–5.10)	5.30 (5.20–5.35)	5.70 (5.60–5.80)	6.25 (6.00–6.40)	6.20 (6.15–6.25)	70.9	<0.001
TSH (µIU/mL)	2.10 (2.10–2.10)	2.00 (1.90–2.20)	4.40 (4.10–4.60)	5.75 (5.40–6.10)	6.20 (6.05–6.25)	80.5	<0.001

IQR, interquartile range; df, degrees of freedom; Hb, haemoglobin; PT, prothrombin time; PTT, partial thromboplastin time; TSH, thyroid-stimulating hormone

Correlations with abortion number

Spearman correlation analysis (Table 4) showed that all continuous markers were significantly correlated with the number of previous abortions ($p < 0.001$). TSH had the strongest positive correlation ($\rho = +0.896$), followed by PT ($\rho = +0.892$), PTT ($\rho = +0.891$) and D-dimer ($\rho = +0.884$). Haemoglobin and platelet counts showed strong negative correlations ($\rho = –0.836$ and –0.813, respectively).

Table 4: Spearman correlations between continuous markers and abortion number

Marker	ρ (rho)	p-value	Interpretation
Haemoglobin	–0.836	<0.001	Strong negative
Platelets	–0.813	<0.001	Strong negative
PT	0.892	<0.001	Strong positive
PTT	0.891	<0.001	Strong positive
D-dimer	0.884	<0.001	Strong positive
HbA1c	0.843	<0.001	Strong positive
TSH	0.896	<0.001	Strongest positive

Inter-marker correlations

The Spearman correlation matrix (Table 5) revealed very strong positive correlations among TSH, D-dimer, PT, PTT and HbA1c (ρ ranging from +0.84 to +0.99, all $p < 0.001$). Haemoglobin and platelets showed strong negative correlations with all other markers, suggesting that haematological decline is part of a broader multi-system dysregulation.

Table 5: Spearman correlation matrix between laboratory markers (all p < 0.001)

Marker	TSH	D-dimer	PT	PTT	Hb	PLT	HbA1c
TSH	—	0.966	0.965	0.953	–0.906	–0.881	0.941
D-dimer	—	—	0.987	0.987	–0.931	–0.907	0.945
PT	—	—	—	0.987	–0.933	–0.903	0.948
PTT	—	—	—	—	–0.922	–0.923	0.937
Hb	—	—	—	—	—	0.838	–0.935
PLT	—	—	—	—	—	—	–0.846

Abbreviations: Hb, haemoglobin; PLT, platelets; PT, prothrombin time; PTT, partial thromboplastin time; TSH, thyroid-stimulating hormone

Categorical markers across abortion-frequency groups

Fisher's exact tests (Table 6) demonstrated highly significant associations for all categorical markers ($p < 0.001$). ANA positivity increased from 2.9% in women with two abortions to 29.7% with three abortions, and reached 100% in those with four or five abortions. CMV IgM positivity rose from 5.7% (two abortions) to 73.0% (three abortions) and 95.8–100% (four to five abortions). Low thyroid hormone levels (T3/T4/FT3/FT4) were observed in only 2.9% of women with two abortions, but in 73–75.7% of those with three abortions and 100% of those with four or five abortions.

Table 6: Fisher's exact test for categorical markers across abortion-frequency groups

Marker	Category	1 ab. (n=1)	2 ab. (n=35)	3 ab. (n=37)	4 ab. (n=24)	5 ab. (n=3)	p-value (exact)
ANA	Positive	0 (0%)	1 (2.9%)	11 (29.7%)	24 (100%)	3 (100%)	<0.001

CMV-IgG	Positive	1 (100%)	4 (11.4%)	17 (45.9%)	24 (100%)	3 (100%)	<0.001
CMV-IgM	Positive	1 (100%)	2 (5.7%)	27 (73.0%)	23 (95.8%)	3 (100%)	<0.001
Low FT3/T3	Yes	0 (0%)	1 (2.9%)	28 (75.7%)	24 (100%)	3 (100%)	<0.001
Low FT4/T4	Yes	0 (0%)	1 (2.9%)	27 (73.0%)	24 (100%)	3 (100%)	<0.001

Abbreviations: ab., abortions

Discussion

This study evaluated a multi-system biomarker panel in 100 women with RPL and demonstrated a systematic, dose-dependent pattern of biological abnormalities that became more pronounced with an increasing number of previous abortions. The findings are notable for the strong correlations observed between immunological, thyroid, coagulation, haematological and metabolic markers and abortion frequency.

Thyroid markers

TSH showed the strongest correlation with abortion number (ρ = +0.896). Median TSH rose from 2.0 μIU/mL in women with two abortions to 6.2 μIU/mL in those with five abortions. Concomitantly, the proportion of women with low T3/T4 levels increased from near-zero to 100%. These observations are consistent with prior reports linking subclinical hypothyroidism and thyroid autoimmunity to RPL (6, 11). While a meta-analysis by Dong et al. did not find clear evidence for subclinical hypothyroidism alone as a cause of RPL, it did confirm a significant association with thyroid autoimmunity [6]. Our data extend these findings by showing a striking gradient of thyroid hormone suppression with increasing RPL severity. The mechanism may involve autoimmune thyroiditis leading to relative tissue-level hormone deficiency and immune dysregulation (12).

Immunological markers: ANA and CMV

The increase in ANA positivity from 2.9% in mild RPL (two abortions) to 100% in severe RPL (four or five abortions) represents the most pronounced gradient among the categorical markers. This aligns with a recent meta-analysis reporting an odds ratio of 3.22 for ANA positivity in RPL cases versus controls (7). Proposed mechanisms include complement activation, immune complex deposition at the maternal-foetal interface, and direct adverse effects on embryonic development (13).

CMV IgM positivity became nearly universal in women with four or five abortions (95.8–100%). This finding is consistent with a previous Iraqi study that reported a significant correlation between CMV seropositivity and RPL (8). In the general Iraqi population, CMV seroprevalence is high (e.g., 24.4% for active markers in healthy adults (14)), and viral reactivation under immunological stress may contribute to pregnancy loss. The progressive increase in CMV IgM positivity with abortion frequency raises the hypothesis that repeated pregnancy losses may be associated with recurrent or persistent viral reactivation, though causality cannot be inferred from this cross-sectional design.

Coagulation markers

PT, PTT and D-dimer all rose synchronously across abortion-frequency groups ($p < 0.001$). The paradoxical finding of prolonged PT/PTT (indicating in vitro clotting factor deficiency) together with elevated D-dimer (indicating in vivo fibrin formation and breakdown) is well recognised in antiphospholipid syndrome (APS), where lupus anticoagulant prolongs phospholipid-dependent clotting times while promoting thrombosis (15). This pattern suggests that a subset of our RPL patients may have undiagnosed APS or another thrombophilic disorder. Ye et al. previously reported that D-dimer levels increase with the number of miscarriages in unexplained RPL, with an area under the curve of 0.8776 for predicting the pre-thrombotic state (9). Our findings reinforce the potential value of coagulation markers in RPL risk assessment.

Haematological decline

The number of abortions correlated with decreasing haemoglobin and platelet values (ρ = –0.836 and –0.813, respectively). The blood loss from repeated miscarriages probably contributes to this drop, and this may be exacerbated by a consumptive process due to hypercoagulability. The most common cause for low haemoglobin in this context is IDA, which can also exacerbate immune function and pregnancy outcomes (16).

Metabolic marker

HbA1c, a marker of long-term glycaemic control, increased progressively from a median of 5.3% (two abortions) to 6.2% (five abortions), values within the prediabetes range. Liao et al. (2024) found that each 1% increase in HbA1c was associated with 40% higher odds of female infertility, with the strongest effect at HbA1c ≥6.5% (10). Our data suggest that even modest elevations in HbA1c are associated with more severe RPL, possibly through impaired endometrial receptivity and embryo development.

Integrated interpretation

The Spearman correlation matrix showed very strong inter-marker correlations (many $\rho > 0.9$), indicating that these biological systems do not operate in isolation. For example, TSH correlated strongly with D-dimer ($\rho = 0.966$) and HbA1c ($\rho = 0.941$), suggesting a functional link between thyroid dysfunction, coagulation abnormalities and metabolic dysregulation. Haemoglobin and platelets correlated negatively with all other markers, reinforcing the idea that haematological depletion is a downstream consequence of multi-system failure.

Taken together, our findings paint a picture of progressive biological deterioration across repeated pregnancy losses. Women with only two abortions generally had normal or near-normal biomarker profiles, whereas those with four or five abortions showed almost universal abnormalities in ANA, CMV IgM and thyroid hormones. This pattern suggests that RPL is not a binary condition but a spectrum of increasing systemic dysregulation.

Study limitations and future directions

There are several important caveats. One, it's a cross-sectional design; no causal inferences can be drawn. Whether the biomarker abnormalities observed stem from the pregnancy loss itself or are caused by repeated losses is unclear. Longitudinal studies tracking biomarker trajectories from before conception through subsequent pregnancies are urgently needed. Second, the absence of a healthy, fertile control group limits our ability to determine whether the biomarker levels in even the mild RPL group are abnormal relative to the general population. Third, the small sample sizes in the 1-abortion ($n = 1$) and 5-abortion ($n = 3$) subgroups mean that estimates for these groups are unstable and should be interpreted with caution. Fourth, the single-centre design in Baghdad, Iraq, may limit generalisability to other populations with different genetic, environmental and infectious disease backgrounds. Fifth, multiple statistical comparisons were performed without adjustment, raising the possibility of type I errors; confirmatory studies are required. Sixth, we did not measure other potentially relevant confounders such as body mass index, smoking, socioeconomic status, or paternal factors. Seventh, the study did not characterise the gestational age of each loss, which might influence biomarker profiles.

Future directions should include prospective cohort studies with preconception enrolment, inclusion of fertile control groups, multi-centre validation in diverse populations, and investigation of whether targeted interventions (e.g., thyroxine for subclinical hypothyroidism, antiviral therapy for CMV reactivation, or anticoagulation for thrombophilic abnormalities) can improve pregnancy outcomes in women with high-risk biomarker profiles.

Conclusion

The multi-system panel of immunological (ANA, CMV IgM/IgG), thyroid (TSH, T3/T4), coagulation (D dimer, PT, PTT), haematological (haemoglobin, platelets) and metabolic (HbA1c) markers is found to be strongly associated with the number of previous abortions in women with RPL in a dose-dependent manner. As the level of ANAs and CMV-IgM antibodies, as well as thyroid hormone suppression and coagulation disorders, progressively worsened with increasing severity of RPL, these markers could potentially be used for risk stratification. But, before clinical use, prospective validation through longitudinal follow-up with healthy control groups is necessary. This new biomarker panel could serve as an integrated tool to diagnose women with the highest risk for recurrent pregnancy loss and thereby provide the opportunity for personalised prevention.

List of Abbreviations

ANA: Antinuclear antibody
APS: Antiphospholipid syndrome
CBC: Complete blood count
CMV: Cytomegalovirus
ESHRE: European Society of Human Reproduction and Embryology
FT3: Free triiodothyronine
FT4: Free thyroxine
Hb: Haemoglobin
HbA1c: Glycated haemoglobin
IQR: Interquartile range
PLT: Platelets
PT: Prothrombin time
PTT: Partial thromboplastin time
RPL: Recurrent pregnancy loss
TSH: Thyroid-stimulating hormone

Declarations

Ethics Approval and Consent to Participate

The study protocol was approved by the Iraqi Ministry of Health and by the local ethics committee, College of Medicine, Karbala University. (Approval number: No:-24-15. 14/3/2024). All participants provided written informed consent before enrolment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent for Publication

All authors gave consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests
The authors declare that they have no competing interests.

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Author Contributions
YZAA worked on the conceptualisation, methodology, investigation, formal analysis, writing the original draft, review & editing and Supervision.
AFA: worked on the methodology, investigation, data curation, formal analysis, writing the original draft, review & editing.

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