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Predictive Value of Maternal Parvovirus B19 IgG and Hematologic Profiles in An Exploratory Miscarriage Risk Model

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ABSTRACT

Background: Parvovirus B19 (B19V) is a known cause of first-trimester miscarriages. Although diagnosis using serology has been extensively documented in the literature, no hematology-based risk scoring parameters for the evaluation of pregnant women exposed to (B19V) infection based on their risk of developing complications.

Objectives: To evaluate maternal parvovirus B19 IgG antibodies and hematological profiles as potential component of an exploratory miscarriage risk model.

Methods: The case-control design included 40 women diagnosed with miscarriage (cases) and 40 women during their full-term pregnancy (controls). Anti-B19 IgG was assayed using Enzyme-Linked Immunosorbent Assay (ELISA). The following complete blood count values (CBC) were determined: hemoglobin (Hb), white blood cell count (WBC),red blood cell count (RBC), platelet count (PLT), absolute neutrophils, and absolute lymphocytes count. Associations were evaluated using binary logistic regression, Pearson correlation analysis, analysis of variance across gestational-age categories, and receiver operating characteristic (ROC) curve analysis.

Results: B19 IgG seropositivity (OR= 7.154; p< 0.001), RBC count (OR = 4.349; p = 0.016), and gestational age (OR = 1.686; p = 0.046) were found to be independent predictors of risk in logistic regression analysis. The prevalence of B19 IgG positivity was significantly higher in miscarriage cases than in controls (67.5% vs. 22.5%; p< 0.001). Significantly lower RBC counts were observed in miscarriages (4.07 ± 0.07 vs. 4.51 ± 0.20 $x10^6/\mu L$; p = 0.017). Miscarriage cases in the first trimester constituted 60% of all cases. The cut-off points calculated via ROC analysis were: 1.85 U/ml (AUC = 0.664; 72.5% specificity) for B19 IgG, 4.21 $x10^6/\mu L$ (AUC = 0.654) for RBC, and 1.25 $x10^3/\mu L$ (AUC = 0.635) for WBC. The levels of B19 IgG and lymphocyte count varied significantly in gestational windows (p=.031 and p=.015, respectively).

Conclusions: A three-factor model comprising B19 IgG status, RBC count, and gestational age showed moderate discriminatory ability for miscarriage in the present study. external validation in a larger independent sample is required before its clinical application.

KEYWORDS: *Parvovirus B19 IgG; hematological profile; risk score; miscarriage; gestational age*

1. INTRODUCTION

Miscarriage, clinically, is defined as the spontaneous loss of a pregnancy before 20 weeks of gestational age (GA). One of the worst possible complications of pregnancy that is routinely encountered in clinical practice. It is estimated that 10-15% of clinically recognized pregnancies are affected by this condition (1). This condition is a significant health problem worldwide, with estimated pregnancy losses of almost 23 million per year in the world. It also has a psychological and social effect, as there can be serious psychological and social consequences for women affected and their families (2). Miscarriage may be due to several factors that may interact including chromosomal abnormalities, uterine abnormalities, hormonal imbalance, immunological disorder, thrombophilic issues, and maternal infections (3). Viral pathogens that can cross the placenta have been highlighted amongst infectious factors as they not only disrupt fetal development, but are also able to cause damage to the placenta during critical time points of pregnancy (4).

Human parvovirus B19 (B19V) is a small, non-enveloped, single-stranded DNA virus in the family Parvoviridae, genus Erythroparvovirus, and the only virus of this family known to be able to infect humans at present (5). The virus is mainly spread by respiratory secretions, direct contact or, as is so important in the obstetric context, through the placental route (6). B19V has a limited cellular tropism and is known to replicate primarily in erythroid progenitor cells in the bone marrow. This infection is usually lytic and can lead to a reversible or irreversible inhibition of the erythropoiesis (7). Specific interactions between B19V and receptors on erythroid lineage cells are the major explanation for the cellular tropism. The VP1 unique region (VP1u) is important for viral entry and globoside (blood group P antigen) is an important receptor for endosomal uptake. Furthermore, efficient viral replication in erythroid progenitor cells (EpoR) is dependent on signalling from EpoR (8).

Recent research has suggested that VP1u receptor and globoside are expressed by cytotrophoblasts and syncytiotrophoblasts of the human placenta. These results indicate that placental tissue in early pregnancy may be susceptible to B19V infection via EpoR independent pathways (9). These observations may offer a potential mechanism for the vulnerability of the first trimester fetoplacental unit to B19V induced injury. During pregnancy, the B19V infection is associated with known pregnancy complications, including fetal anaemia, non-immune hydrops fetalis, intrauterine fetal death and pregnancy loss (10). Vertical transmission occurs in almost a third to half of cases when maternal primary infection occurs during pregnancy. This risk is more clinically significant if the infection occurs prior to 20 weeks of gestation, as fetal death has been reported in about 9-17% of these cases (11).

Serological assessment of B19V infection depends mainly on the detection of virus-specific antibodies. The presence of IgG reflects previous exposure, as it usually develops within 7-14 days after primary infection and persists lifelong (12). Meanwhile, IgM is considered a marker of recent or acute infection and generally remains detectable for 3-6 months following infection (13). Available seroprevalence data suggest considerable variation in B19V IgG positivity among women of reproductive age. This variation appears to be influenced by geographic location, socioeconomic background, and age, with reported global rates ranging from approximately 40% to 80% (14). In the Middle East and Arab population, regional seroprevalence rates between 53.3% and 74% have been documented (15). Although B19V IgG seropositivity is commonly interpreted as evidence of past infection and acquired immunity, its clinical relevance during pregnancy remains unclear. Pregnancy-related immune modulation, together with the possibility of asymptomatic viral reactivation or infection occurring around conception, may complicate the interpretation of IgG positivity in pregnant women (16). Earlier seroprevalence studies have shown that B19V IgG seroprevalence may be higher among women with adverse pregnancy outcomes, particularly those with a history of miscarriage (17, 18).

The methodological limitations in the existing literature, including reliance on serology alone, failure to adjust for gestational age as a confounder, and the lack of formal predictive modeling, have precluded definitive conclusions regarding the independent contribution of B19V IgG seropositivity to pregnancy loss. Furthermore, the relationship between B19V-mediated erythroid suppression and peripheral hematological parameters, specifically the red blood cell (RBC) count, in the context of miscarriage has not been adequately characterized. Neither the utility of B19V IgG as an independent predictor of miscarriage nor the complementary diagnostic value of a combined serological-hematological biomarker panel has been systematically evaluated. The biological argument for RBC counts as B19V-specific hematological risk marker supported by the well-mechanically consistent body of evidence at several levels of investigation. The virus B19V uses the P antigen (globoside) as a receptor and it is abundantly expressed on erythroid progenitor cells and since B19V cannot replicate independently, it is only able to exert cytotoxic effect on rapidly dividing erythroid precursor cells (19). The in vitro experiments involving bone marrow cultures demonstrated that B19V infection results in a profound decline in CFU-E and BFU-E number without affecting the granulocyte and megakaryocyte progenitors — the mechanism responsible for the fact that RBC count is the CBC parameter mostly sensitive to the B19V-induced disruption of haematopoiesis (20). On molecular level, the nonstructural protein NS1 of B19V is responsible for the inhibition of erythroid differentiation and maturation through induction of viral DNA damage responses and cell cycle arrest while the binding of B19V to Gb4 rich erythrocyte membrane results in morphological changes leading to shortened RBC half-life (21). The clinical manifestation of this condition is a reduced level of reticulocytes of 0-1%, where the reduction of RBCs precedes the reduction of haemoglobin, and hence there is an important pre-anaemic detection period which plays an important role in risk stratification (22). Importantly, in the obstetric setting, the presence of P antigen is found

not only in RBCs and RBC precursors, but also in the fetal liver cells, where the haematopoiesis process occurs in the first trimester of pregnancy (23). Thus, this study aims to evaluate maternal parvovirus B19 IgG antibodies and hematological profiles as potential predictive biomarkers for the B19-induced miscarriage risk model.

2-MATERIALS AND METHODS

Study Design and Setting

The study was a prospective case-control study, which was conducted at Al-Elwyia Maternity Hospital, Baghdad, Iraq for three months from 28 November 2025 – 28 February 2026. This study aimed to assess the predictive role of maternal serum Parvovirus B19 IgG antibody level, peripheral red blood cell count (RBC) which were grouped according to the gestational age among the women who presented with miscarriage. The Institutional Ethics Committee (Reference No. FM.SA.22, dated 04/02/2026) approved the study, and all participants gave written informed consent before being included in the study, which was conducted in accordance with the principles of the Declaration of Helsinki.

Sample size calculation

The sample size was calculated in advance using the two-proportion z test equation, assuming a seroprevalence of 60% in the cases and 20% in the controls ($\alpha = 0.05$, two-tailed; power = 80%). A minimum of 37 subjects in each group was computed, increased to 40 in each group (n = 80 in total) allowing for possible data loss. The 40 cases recruited satisfy the requirement of EPV ≥ 10 needed for a binary logistic regression model (24).

Study Population and Eligibility Criteria

Eighty pregnancies were registered and split into two 40-pregnancy groups. The case group which is women who had experienced clinically confirmed and ultrasonographically verified spontaneous abortion occurring before 20 weeks of gestation. The control group were healthy pregnant women who had normal pregnancies and were attending normal prenatal care. Gestational age (GA) was estimated from the last period and confirmed by first trimester ultrasonographic biometry, and the subjects were divided into three gestations: less than 12 weeks, 12-20 weeks and greater than 20 weeks.

The exclusion criteria were the same for both groups and included: known autoimmune or connective tissue disease; known preexisting hematological disease; multiple gestation; current or recent immunosuppressive therapy; known hepatitis B or C infection; documented TORCH infection other than B19V; and incomplete clinical or laboratory data. These were to assure that the differences observed in hematological and immunological variables were due to B19V exposure, and not to other confounding diseases.

Data Collection and Hematological Assessment

Demographic information, such as maternal age, and gestational age at presentation, was systematically collected for each participant. Blood samples of 5 mL volume were collected from all enrolled women under standardized conditions. A complete blood count (CBC) was performed on EDTA-anticoagulated whole blood using a calibrated, automated hematology analyzer. The following parameters were measured: hemoglobin concentration (Hb, g/dL), white blood cell count (WBC, $\times 10^3/\mu L$), red blood cell count (RBC, $\times 10^6/\mu L$), platelet count (PLT, $\times 10^3/\mu L$), absolute neutrophil count ($\times 10^3/\mu L$), and absolute lymphocyte count ($\times 10^3/\mu L$).

Detection of Parvovirus B19-Specific Antibodies

For serological analysis, serum was separated from clotted blood after using gel tube by centrifugation at 3,000 rpm for 10 minutes and subsequently stored at −20°C until the time of assay. Parvovirus B19-specific IgG antibodies were quantified using an automated enzyme-linked immunosorbent assay (ELISA: the Alegria® Anti-Parvovirus B19 IgG kit (Catalog No. ORG 912G; ORGENTEC Diagnostika GmbH, Mainz, Germany), performed on the Alegria®fully automated immunoassay analyzer. This commercially available system complies with the requirements of CE marking and is widely employed in clinical diagnostic laboratories for quantitative B19V serology.

Assay Principle and Antigen Configuration

The assay employs recombinant Parvovirus B19 major capsid protein VP2, expressed in the form of virus-like particles (VLPs) as the solid-phase capture antigen. The use of VP2-based VLPs ensures faithful presentation of the three-dimensional native conformational epitopes of the viral capsid, thereby enabling highly sensitive

detection of both conformational and linear antibody specificities relevant to both acute-phase and post-exposure immunity [19].

Assay Procedure

All testing was performed in strict accordance with the manufacturer's validated instructions on the Alegria® automated immunoassay platform, using the following sequential procedural steps:

(1) Sample incubation: Patient serum samples, diluted according to the manufacturer's protocol, were dispensed onto microtiter strip wells pre-coated with recombinant B19V VP2 VLPs and incubated to allow antibody-antigen binding.

(2) Washing: Automated multi-cycle washing removed unbound serum constituents and non-specifically adsorbed proteins to minimize background signal and optimize assay specificity.

(3) Conjugate incubation: Each well was incubated with an enzyme-labeled anti-human IgG conjugate for the detection of the bound B19-specific IgG antibodies.

(4) Substrate incubation: After another wash cycle, a chromogenic substrate solution was applied to start a colorimetric reaction that is proportional to the amount of bound conjugate.

(5) Signal termination and reading: The enzymatic reaction was stopped by addition of Stop Solution and the optical density (OD) of each well was read spectrophotometrically at 450nm using the Alegria® integrated reader.

The Alegria® system software automatically calculated antibody concentration, by application of pre-programmed multi-point calibration curve.

Calibration and Interpretation of Results

The quantitative B19 IgG results were calibrated against WHO International Standard for Human Parvovirus B19 antibodies (NIBSC reference code 01/602) and expressed as International Units per milliliter (IU/mL). The manufacturer recommended seropositivity cut-off value as 3 IU/mL and a reportable quantitative range of 0-50 IU/mL. Those specimens that gave values greater than this were considered seropositive and those less than this were considered seronegative. Quantitative IgG levels were divided into three categories: low (less than 3 IU/mL), medium (3–49 IU/mL), and high (50 IU/mL or higher), for exploratory categorical analysis.

Laboratory Quality Control

For the Alegria assay, test results were accepted only when the assay-specific internal control fulfilled the manufacture's validity criteria. The instrument automatically verified the test-strip barcode, lot-specific calibration data, and the expiration date before processing. Reagent were stored at 2-8°C according to the instruction of the manufacturer's , also the repeated freezing and thawing of serum sample was avoided.

The CBC measurement were performed by using a calibrated automated hematology analyzer under the hospital laboratory's routine internal quality control procedure. Patient result was accept only when the control measurement within the laboratory's predefined acceptable ranges. If the samples showing clotting, insufficient volume, or analyzer waning flags were rechecked or repeated before inclusion in the statistical analysis.

Statistical Analysis

IBM SPSS Statistics software (version 27.0, IBM Corp., Armonk, NY, USA) was used for all the statistical calculations. Data for continuous variables are expressed as mean ± standard deviation (SD) or mean ± standard error (SE) as appropriate. Independent samples t-test was used for between group comparisons of continuous variables. The Pearson Chi-Square test was used to compare categorical variables. Pearson correlation coefficients were used to study bivariate associations between continuous variables. Analysis of covariance (ANCOVA) was used to test group differences in immunological and hematological parameters controlling for the large and inherent difference in gestational age between groups. ABO blood groups was initially evaluated as an exploratory candidate variable because it was routinely available and to determine whether differences in blood groups distribution could represent a potential confounding factor between the study groups. As no significant association was observed ($p=0.528$), ABO blood group not included in the final risk score model. To generate risk-scores, a binary logistic regression model was used with an adverse pregnancy outcome as dependent variable. A combination of different co-variates namely B19 IgG, RBC, WBC, GA, neutrophils were simultaneously entered into the model. OR (95% CI) refers to odds ratio (95% confidence interval) which indicates the weight of each covariate in the development of the risk score. For each of the selected covariates, Receiver operating characteristic (ROC) curves were

constructed, and discriminatory performance was assessed using the area under the curve (AUC), sensitivity and specificity. A p-value of < 0.05 (two sided) was considered statistically significant.

3-RESULTS

Table 1 presents the baseline demographic and hematological characteristics of each group. Maternal age was similar for both groups (cases 27.6 ± 7.2 vs controls 25.2 ± 6.9 yr, p=.138), providing further evidence that maternal age was not a confounding factor. Gestational age was significantly lower in the miscarriage group than in the healthy control group (10.59 ± 4.8 vs. 37.4 ± 3.2 weeks, p < 0.001). This marked difference primarily reflects the case-control design, because cases were enrolled at the time of pregnancy loss, whereas controls were enrolled during ongoing pregnancies near term. Therefore, gestational age was considered a potential confounding factor when interpreting differences in serological and hematological parameters, rather than solely as an independent biological risk factor. From the hematological variables included in the CBC analysis, only RBCs were found to be statistically significant (cases 4.07 ± 0.07 vs control 4.51 ± 0.20 x10^6/µL, p=0.017). The specific reduction in RBCs in the absence of any changes in other hematological indicators such as Hb, WBC, PLT, or white cell differential counts represents the hematological risk factor that most closely matches the predicted effects of infection on erythroid precursors and forms part of the proposed risk score. Antigen-specific IgG levels were significantly increased in case samples (18.25 ± 3.8 vs controls 9.53 ± 2.9 U/ml, p<0.001).

Table 1. Baseline demographic and hematological risk profile in study groups

Hematological / Clinical Parameter	Miscarriage Group (Mean ± SD)	Healthy Controls (Mean ± SD)	95% CI Lower	95% CI Upper	p-value
Maternal Age (yr)	27.6 ± 7.2	25.2 ± 6.9	−0.777	5.527	.138
Gestational Age (wk) †	10.59 ± 4.8	37.4 ± 3.2	−28.243	−24.607	< 0.001*
Hemoglobin — Hb (g/dl)	11.4 ± 1.5	11.2 ± 1.3	−0.479	0.744	0.668
White Blood Cells — WBC (×10^3/µL)	10.01 ± 0.59	11.69 ± 0.66	0.062	−3.448	0.084
Red Blood Cells — RBC (×10^6/µL)	4.07 ± 0.07	4.51 ± 0.20	0.042	−0.859	0.017*
Platelets — PLT (×10^3/µL)	263.68 ± 9.13	282.55 ± 10.23	0.173	−46.173	0.423
Neutrophils (×10^3/µL)	7.47 ± 0.61	8.83 ± 0.57	0.862	−2.180	0.084
Lymphocytes (×10^3/µL)	2.07 ± 0.15	2.09 ± 0.11	0.919	−0.404	0.364
B19 IgG Antibody Titer (U/ml)	18.25 ± 3.8	9.53 ± 2.9	0.070	0.127	< 0.001*

** p < 0.05, statistically significant. † Gestational age difference reflects case-control design. Hb = haemoglobin; WBC = white blood cell count; RBC = red blood cell count; PLT = platelet count; B19 IgG = anti-Parvovirus B19 immunoglobulin G; CI = confidence interval.*

Table 2. Distribution of participants according to gestational-age categories

Group	<12 weeks	12–20 weeks	>20 weeks	Total	Asymptotic Sig. (2-sided)
Miscarriage	24 (60.0%)	13 (32.5%)	3 (7.5%)	40	< 0.001*
Healthy Controls	0 (0%)	0 (0%)	40 (100%)	40	—

** p < 0.001.*

As shown in Table 2, gestational age distribution differed significantly between the two study groups ($p < 0.001$). Among miscarriage cases, 60.0% occurred before 12 weeks, 32.5% occurred between 12 and 20 weeks, and 7.5% occurred after 20 weeks, whereas all healthy controls were recruited after 20 weeks. This difference is largely related to the study design and the different timing of participant recruitment. Therefore, gestational age should be considered a potential confounding factor, and the observed association should not be interpreted as evidence that gestational age independently predicts miscarriage in the present study.

Table 3. ABO blood group distribution among the study groups

Group	O	A	B	AB	Total	Approx. Sig.
Miscarriage	15 (37.5%)	10 (25.0%)	12 (30.0%)	3 (7.5%)	40	0.528 (NS)
Healthy Controls	20 (50.0%)	9 (22.5%)	5 (12.5%)	6 (15.0%)	40	—

NS = not statistically significant (p = 0.528). ABO blood type is excluded from the risk-scoring model on this basis.

The results of the ABO blood groups are shown in Table 3 below. There was no statistically significant association between ABO blood groups and disease status (P=0.528). The group with the highest incidence in both groups was group O (37.5% in the case group and 50% in the control group). This finding plays a particular role in our study because it ensures that ABO phenotype cannot be a confounding factor in the model of risk of infection with B19V.

Table 4. B19 IgG serostatus among the study groups

B19 IgG Serostatus	Miscarriage Group n (%)	Healthy Controls n (%)	Chi-Square p-value
Seropositive	27 (67.50%)	9 (22.50%)	< 0.001*
Seronegative	13 (32.50%)	31 (77.50%)	—
Total	**40**	**40**	—

** p < 0.001, statistically significant. Seropositivity was classified as high-risk; seronegativity as low-risk for the composite score.*

B19 IgG serostatus is categorized as a dichotomous risk predictor in Table 4. Of all the cases, 67.5% (n = 27) were seropositive or considered high-risk, as opposed to just 22.5% (n = 9) of the control samples. The significant prevalence of seropositivity among miscarriage samples ($p < 0.001$) is evidence that IgG serostatus is the strongest independent variable in the risk model. It is also worth noting that the vast majority of control samples (77.5%) were seronegative.

Table 5 and Figure 1 highlight changes that occur within the risk-relevant hematological parameters and IgG titer values between the three gestational windows. There were two parameters that changed significantly with respect to gestational windows, both being directly related to the risk score computation.

B19V Anti-IgG titer was significantly lower during later pregnancies ($p = .031$). The average anti-IgG titer was higher in the high-risk group (< 12 weeks: 18.99 ± 24.44 U/ml) compared to the mid-risk group (12-20 weeks: 16.58 ± 22.94 U/ml). The lowest titer was seen in the low-risk group (> 20 weeks: 10.23 ± 19.14 U/ml). The correlation between gestational age and IgG titer is the manifestation of the increased prevalence of B19V in early pregnancy.

Variations were also observed in the lymphocytes ($p = .015$), where the minimum mean values were recorded in the < 12 weeks group (1.96 ± 0.64 $\times 10^3/\mu L$), while maximum values were seen in the 12-20 weeks group (2.42 ± 1.46 $\times 10^3/\mu L$). While the number of lymphocytes did not meet the criteria to become an independent component in the risk score calculation by logistic regression, the significant variation in this variable among different windows may indicate an underlying biological immune response which could be used in further analyses. There was no significant change in the total white blood cells during pregnancy ($p = .063$).

Table 5. Hematological parameters and B19 IgG titer stratified by gestational age (one-way ANOVA)

Parameter	Gestational Age	n	Mean	SD	p (ANOVA)	95% CI Lower	95% CI Upper
IgG Titer (U/ml)	< 12 wk	24	18.99	24.44	.031*	8.67	29.31
	12–20 wk	13	16.58	22.94		2.72	30.45
	> 20 wk	43	10.23	19.14		4.34	16.12
Hemoglobin (g/dl)	< 12 wk	24	11.68	1.43	.408	11.07	12.28
	12–20 wk	13	10.90	1.55		9.96	11.84
	> 20 wk	43	11.23	1.26		10.84	11.62
WBC (×10³/µL)	< 12 wk	24	9.11	2.66	.063	7.98	10.23
	12–20 wk	13	10.25	2.54		8.71	11.79
	> 20 wk	43	11.99	4.65		10.57	13.43
RBC (×10⁶/µL)	< 12 wk	24	4.16	0.38	.616	3.99	4.32
	12–20 wk	13	3.92	0.55		3.59	4.25
	> 20 wk	43	4.48	1.22		4.11	4.86
Platelets (×10³/µL)	< 12 wk	24	263.88	62.43	.453	237.51	290.24
	12–20 wk	13	266.38	44.79		239.32	293.45
	> 20 wk	43	280.30	65.80		260.05	300.55
Neutrophils (×10³/µL)	< 12 wk	24	3.69	1.78	.141	2.94	4.44
	12–20 wk	13	3.63	1.45		2.75	4.51
	> 20 wk	43	5.51	5.81		3.72	7.30
Lymphocytes (×10³/µL)	< 12 wk	24	1.96	0.64	.015*	1.69	2.23
	12–20 wk	13	2.42	1.46		1.54	3.31
	> 20 wk	43	2.04	0.71		1.82	2.26

** p < 0.05, statistically significant. GA = gestational age; Hb = haemoglobin; WBC = white blood cell count; RBC = red blood cell count; PLT = platelets.*

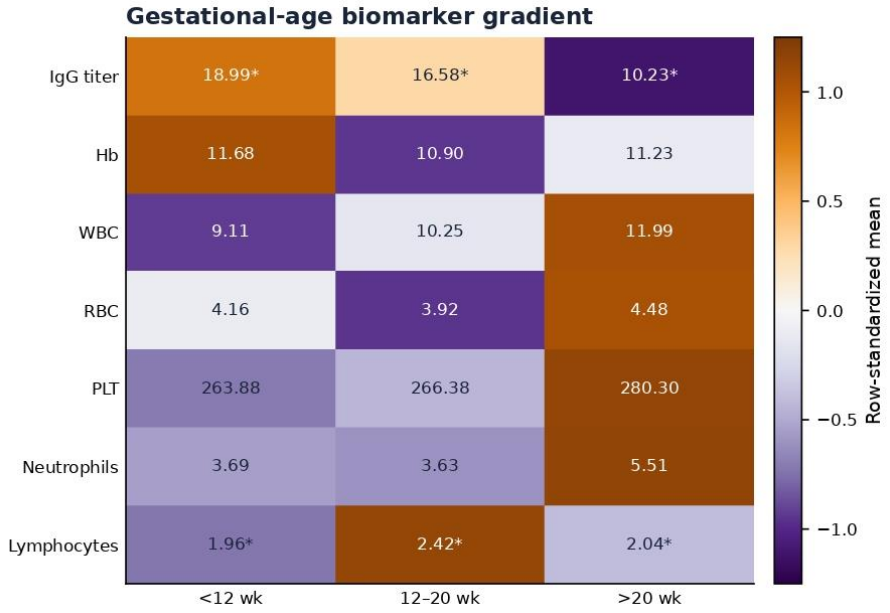

Figure 1. Hematological parameters and B19 IgG titer stratified by gestational age (one-way ANOVA)

Table 6. Pearson correlation analysis: gestational age as a continuous risk modifier

Parameter	Pearson r	Significance (2-tailed)
Study Group (Miscarriage vs. Control)	.956**	< 0.001*
ABO Blood Group	−.049	.665
B19 IgG Seropositivity (binary)	.394**	< 0.001*
B19 IgG Titer Level	−.233*	.038*
Hemoglobin — Hb (g/dl)	−.076	.500
White Blood Cells — WBC (×10³/µL)	.248*	.027*
Red Blood Cells — RBC (×10⁶/µL)	.081	.473
Platelets — PLT (×10³/µL)	.163	.105
Neutrophils (×10³/µL)	.093	.413
Lymphocytes (×10³/µL)	.010	.933
Neutrophil-to-Lymphocyte Ratio (NLR)	.036	.752

*** p < 0.01; * p < 0.05. Significant correlates are candidates for risk-score inclusion. NLR = neutrophil-to-lymphocyte ratio.*

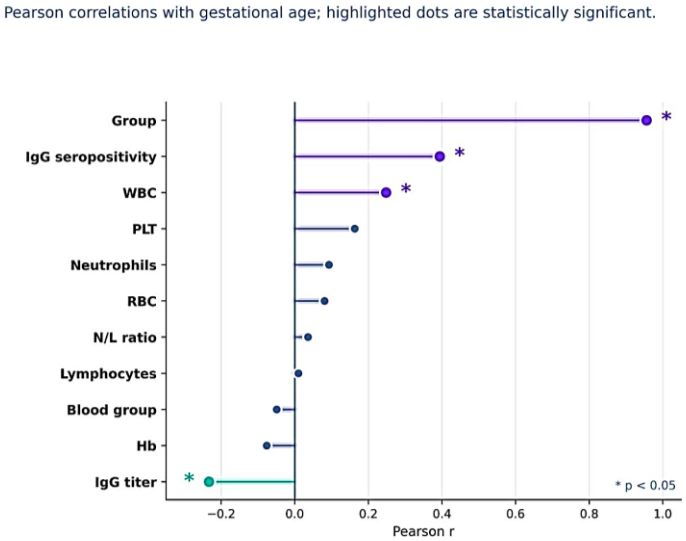

Figure 2: Pearson correlation analysis: gestational age as a continuous risk modifier

In Table 6 and Figure 2, gestational age is tested as a continuous modifier of each of the study variables, thereby determining those variables that vary with the timing of gestation and hence contain intrinsic risk temporal information. Group membership was unsurprisingly found to be highly correlated ($r = .956$, $p < 0.001$). There were three other significant correlations: IgG seropositivity for B19 ($r = .394$, $p < 0.001$) — which is consistent with the observation that seropositive cases occur at earlier gestation periods; IgG titer ($r = -.233$, $p = .038$) — consistent with the negative correlation between titer values and gestational age; and WBC count ($r = .248$, $p = .027$) — indicative of physiologic, and not B19V-induced, leukocytosis.

Notably, parameters showing no correlation with gestational age, such as Hb, RBC, PLT, neutrophils, lymphocytes, and NLR, provide information independent of gestational age to the risk score. This is consistent with the lack of correlation of ABO blood group ($r = -.049$, $p = .665$).

Table 7. Binary logistic regression: derivation of risk-score component weights

Risk Predictor	B	SE	Wald χ^2	df	p-value	Odds Ratio	95% CI Lower	95% CI Upper
B19 IgG Seropositivity	1.968	.507	15.045	1	< 0.001*	7.154	2.647	19.335
RBC Count (×10⁶/µL)	1.470	.610	5.806	1	.016*	4.349	1.316	14.379
Gestational Age (wk)	.522	.261	3.994	1	.046*	1.686	1.010	2.814
WBC Count (×10³/µL)	.115	.064	3.241	1	.072	1.122	.990	1.271
Neutrophils (×10³/µL)	.107	.069	2.407	1	.121	1.113	.972	1.273

** p < 0.05, statistically significant. B = regression coefficient; SE = standard error; Wald χ^2 = Wald chi-square statistic; df = degrees of freedom; OR = odds ratio (Exp[B]); CI = confidence interval.*

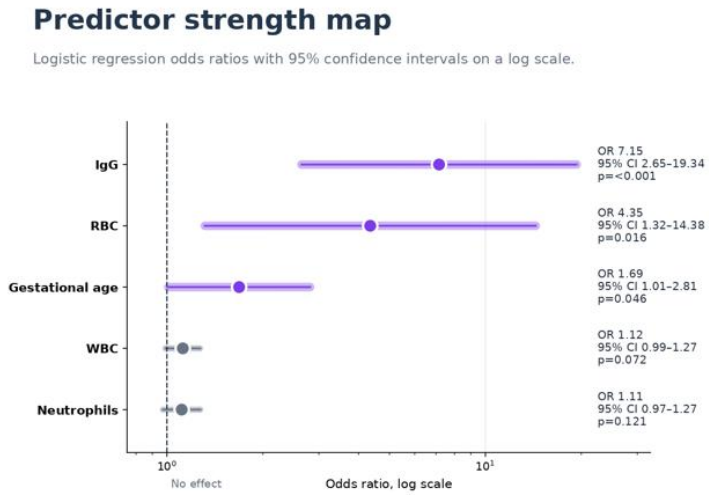

Figure 3. Binary logistic regression: derivation of risk-score component weights

Table 7 and Figure 3 display the results of the regression analysis, in which three independent variables were included in the derivation of the three-factor risk score. There were three independent variables that fulfilled the p<0.05 criterion: Component 1 – B19 IgG Seropositivity (OR=7.154, 95% CI: 2.647–19.335, p<0.001): This was the most important predictor in the risk-score equation. Being seropositive increased the odds of having an adverse outcome 7.15 times after controlling for all other covariates. The Wald value (15.045) was the highest of the three, suggesting that B19 IgG positivity is the main driver of the score. Component 2 – RBC Count (OR=4.349, 95% CI: 1.316–14.379, p=0.016): This represents the erythroid component of the risk score. Every unit decrease in RBC increases the odds of an adverse outcome by 4.35 times, showing the effect on erythropoiesis caused by B19V as the measurable outcome of virus infection. Component 3 - Gestational Age (OR = 1.686; 95% CI: 1.010-2.814; p = .046): This refers to the temporal component in the scoring system. One week earlier gestational age is associated with an increased risk of having an adverse event by 1.69 times, reflecting the biological risk gradient within each of the gestational windows shown in Table 5. The white blood cell count (OR = 1.122; p = .072) and neutrophil count (OR = 1.113; p = .121) were close to being significant predictors but were not, implying a possible contribution from inflammation as a secondary factor.

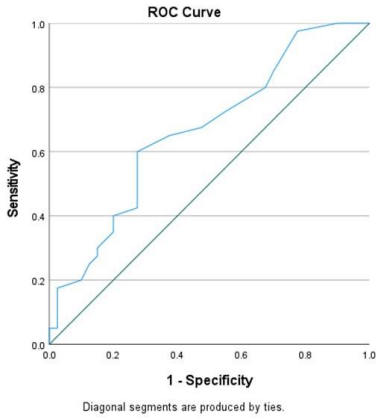

Figure 4. ROC analysis: Discriminative performance B19V IgG titer in the study Groups

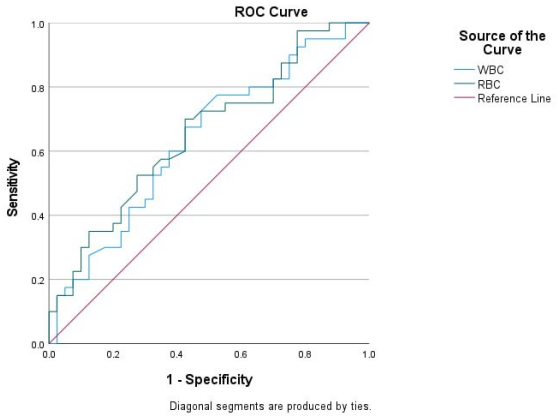

Figure 5. ROC Analysis: Discriminative performance of Hematological Parameters in the study groups.

Table 8. ROC curve analysis: optimal threshold values for composite risk-score components

Biomarker	AUC	p-value	Optimal Cut-Off	Sensitivity	Specificity	95% CI Lower	95% CI Upper
B19 IgG Titer	.664	0.012*	1.85 U/ml	60.0%	72.5%	.545	.782
RBC (×10⁶/µL)	.654	.017*	4.21 ×10⁶/µL	70.0%	57.0%	.535	.774
WBC (×10³/µL)	.635	.038*	1.25 ×10³/µL	67.5%	57.5%	.513	.757

** p < 0.05, statistically significant. AUC = area under the ROC curve; CI = confidence interval. Cut-off values represent Youden Index-optimized thresholds for clinical score application.*

For the assessed markers, ROC analysis shown statistically significant but moderate discrimination. B19 IgG had an AUC of 0.664 (95% CI: 0.545–0.782), with 60.0% sensitivity and 72.5% specificity at a cut-off value of 1.85 U/mL. RBC count had an AUC of 0.654 (95% CI: 0.535–0.774), with 70.0% sensitivity and 57.0% specificity at a cut-off value of 4.21 × 10⁶/µL. WBC count had an AUC of 0.635 (95% CI: 0.513–0.757), with 67.5% sensitivity and 57.5% specificity at a cut-off value of 1.25 × 10³/µL. The suggested cut-off values should be regarded as exploratory until they are confirmed in a larger independent sample, as these results show moderate discriminatory performance,

4-DISCUSSION

In this study, B19 IgG status reflects the patient's immune history of prior infections, with an odds ratio of 7.154, the largest contributing factor. RBC count represents the erythroid response to B19V infection — the consequence of viral replication in progenitor cells. Gestational age represents the increased susceptibility of pregnant women to B19V infection. Whereas the parameter most frequently linked to anaemia in clinical practice – hemoglobin – showed no difference between the two groups ($p = 0.668$), the RBC count showed a significant decrease ($p = 0.017$). This Hb-RBC discrepancy is not counterintuitive: since the B19V-caused lysis of Colony-Forming Unit-Erythroid (CFU-E) results in a halt to RBC production while sparing the pre-existing circulating RBC in the maternal bloodstream (25), there is a decrease in RBC numbers accompanied by an increase in hemoglobin per RBC (increased mean corpuscular hemoglobin) (26). This is a unique pattern reflecting the inhibition of erythropoiesis – which confers the predictive power of the RBC count over Hb (25). The combination of B19 IgG titer gradient in different gestational windows ($p = .031$; Table 5) and its negative correlation with gestational age ($r = -.233$) indicates one more key characteristic of the risk profile: the maximum serological risk signal corresponds to the period of lowest gestational age, which coincides with the maximum presence and vulnerability of fetal erythroid precursors (5,16,27). Consequently, the combination of the gestational risk window and high serological signal intensifies the predictive capacity of the risk score in the first-trimester window group (5). However, the lymphocyte count (p-value from ANOVA = .015 in various gestation windows) is worthy of mention as another possibility for inclusion in future test scores. Even though the absolute lymphocyte count failed to attain significance in logistic regression (p-value not provided for the model), the varying lymphocyte counts in relation to the various gestation windows imply that changes in the kinetics of the lymphocytes might provide clues regarding the changing host reaction against the B19 virus infection at different gestation periods. The reduced lymphocyte counts during gestations less than 12 weeks likely reflect an active immune response and can be explained by active migration into the uterus placental compartment or a peripheral depletion by the B19 virus (28).

The thresholds generated from ROC analysis for B19 IgG (1.85 U/mL; specificity of 72.5%) serve as the triage gate — any level below this significantly lowers the post-test probability of a B19V-related adverse event, thus allowing reduction in surveillance. The threshold for RBCs (4.21 x 106/μL; sensitivity of 70%) is used as a safety catch — any level lower than this, regardless of B19 IgG test results, will elevate the score. Finally, the threshold for WBC (1.25×10^3/μL; sensitivity of 67.5%) ranks below that of RBCs in terms of prognostic significance, but can serve as an inflammatory index when B19 IgG and RBC levels border on the positive side. These hierarchical thresholds, led by specificity for B19 IgG and sensitivity for RBC, supplemented by WBC, fit logically within the biologic progression of B19V infections: exposure, erythroid inhibition, then inflammatory activation (19,29). The lack of association between any ABO blood group and B19V infection ($p = 0.528$) allows blood group to be excluded from consideration when developing the risk score, thereby simplifying the use of the score in clinical settings. This result agrees with what is known about the independent expression of P antigen (globoside) from the ABO locus and from B19V receptor genetics in general (30). The suggested model potential clinical is found in its application as preliminary risk-stratification strategy based on commonly accessible metrics. Together, B19V IgG status, RBC count, and gestational age could be used to determine which women might need more laboratory testing or a more thorough clinical evaluation. However, this model should not be used as a diagnostic tool in a place of routine obstetric evaluation, PCR, or as a substitute for B19V IgM testing. Before it can be used to direct the treatment of individual patient managements, its clinical application is still in its early stages and needs to be validated in larger, gestational-age-matched populations . The limitations of this study include a small sample size, with only 40 patients per group. By considering only IgG and not IgM or B19V DNA load by PCR, this test fails to differentiate acute, recent, and remote infection, a consideration that is pertinent since the fetus is most at risk during the acute phase of primary infection. The variations in gestational age between the research groups was another drawback. Healthy controls were enrolled after 20 weeks of gestation, while miscarriage cases were primarily recruited during the early stages of pregnancy. When interpreting the observed associations with miscarriage, this imbalance should be taken into account as it may have introduced confounding. To lesson this possible source of bias, gestational-age-matched controls should be used in future research.

CONCLUSION

The present study found that (B19V) IgG seropositivity, lower RBC count, and gestational age were associated with miscarriage and could be combined in an exploratory three-factor model. However, the model showed only moderate discriminatory performance, and the proposed ROC-derived cut-off values should be interpreted with caution. Since B19V IgG mainly indicates previous exposure rather than acute infection, and because gestational age differed considerably between the study groups, these findings do not confirm a causal relationship between active B19V infection and miscarriage. Larger studies using gestational-age-matched controls and additional confirmation by IgM and/or PCR are required before this model can be considered for clinical application.

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