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Complement levels and their diagnostic utility in neonatal sepsis

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Abstract

Background This study evaluated the diagnostic value of complement levels in neonatal sepsis and their roles in disease progression.

Methods This diagnostic accuracy study, conducted in Guangdong Province, China (January 2021-February 2022), involved 41 neonates with sepsis and 41 controls matched by sex and gestational age. Cases included neonates with culture-positive or clinically diagnosed sepsis, while controls consisted of neonates hospitalized for non-septic conditions, confirmed by negative blood cultures. Ten complement components (C1q, C3, C3c, C3b, C4, C5, C5a, H, B, mannose-binding lectin [MBL]) were quantified using residual specimens from routine clinical tests. Descriptive statistics, logistic regression, ROC curve analysis, and correlation assessments were introduced in this study.

Results The neonatal sepsis group had significantly higher levels of C3c (0.68 vs. 0.49 ng/mL, $P=0.007$) and MBL (518.81 vs. 397.06 pg/mL, $P<0.001$) compared to controls. In contrast, C5a levels were significantly lower in neonates with sepsis (51.18 vs. 57.25 ng/mL, $P=0.042$). C5a demonstrated limited individual performance (AUC = 0.63, 95% CI: 0.51–0.75; sensitivity = 65.9%, specificity = 65.9% at 55.63 ng/mL), while MBL showed moderate accuracy (AUC = 0.75, 95% CI: 0.64–0.85; specificity = 90.2%, sensitivity = 53.7% at 512.86 pg/mL). Notably, their combined C5a + MBL indicator achieved exceptional discrimination (AUC = 0.92, 95% CI: 0.85–0.99) with 85.4% sensitivity and 97.6% specificity, yielding 97.2% positive predictive value (PPV) and 87.0% negative predictive value (NPV).Positive correlations were found between C4 levels and C-reactive protein (CRP), and C3 levels and neutrophil percentage (Neut%), while negative correlations were observed between C5 and MBL levels and total cholesterol (TCH).

Conclusions This study highlights the diagnostic significance of combined C5a + MBL indicator in neonatal sepsis and underscores the association between complement levels and disease progression.

Keywords Complement, Neonatal sepsis, Diagnostic value, Mannose-binding lectin

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Background

Neonatal sepsis, a life-threatening systemic infection driven by immature immunity, causes significant global morbidity and mortality, affecting about 1.3 million infants annually with 17.6% mortality [1]. Severe complications like meningitis (23% of cases) exacerbate neurological and multi-organ damage [2, 3]. While blood culture remains the diagnostic gold standard, its limitations, including low sensitivity, high false positives, and delayed results, hinder timely intervention [4]. In recent years, biological factors, such as C-reactive protein (CRP), leukocytes, and procalcitonin (PCT), have been commonly used as diagnostic markers; however, these markers have low sensitivity and poor specificity [5].

Studies indicate the complement system's crucial role in sepsis via diverse immune mechanisms, with complement levels closely correlated to sepsis patient conditions [6]. Key components like C1q and C3 correlate strongly with inflammation intensity and sepsis progression, offering diagnostic advantages over traditional markers. For instance, serum C1q levels aid in initial neonatal sepsis diagnosis and severity classification, as blood C1q levels are a good indicator of its severity [7]. Also, C3 and C5 levels in neonatal sepsis patients correlate positively with inflammation severity, suggesting their use as markers for sepsis onset and severity [8]. Other research highlights the associations between complement, lipid, and vitamin levels in sepsis development [9, 10]. In neonatal sepsis diagnosis, complement levels such as C3 and C1q offer advantages over common biomarkers like CRP and PCT. They can detect sepsis earlier, responding more rapidly to infection onset [11, 12]. Due to direct involvement in immune responses to bacterial infections, they show greater early-stage specificity [12], enabling more precise diagnosis and better early detection and treatment outcomes. However, few studies have explored the connection between various complements and biochemical indicators in neonatal sepsis patients.

This study addresses these gaps through a comprehensive evaluation of 10 complement components spanning classical, lectin, and alternative pathways in neonatal sepsis. We characterized complement level distributions, assessed their diagnostic accuracy for neonatal sepsis against conventional biomarkers. Furthermore, the associations among complement levels, inflammatory factors, lipids, and vitamin levels were explored to uncover holistic pathophysiological insights.

Methods

Study population

This diagnostic accuracy study was conducted between January 2021 and February 2022 at the Guangdong Women and Children Hospital, a large teaching tertiary public hospital with three hospital districts located in Guangzhou, Guangdong Province, China. The participants were enrolled in neonatal wards and intensive care units based on the 2019 Chinese Neonatal Sepsis Expert Consensus [13], the present study included both culture-positive and clinically proven neonatal sepsis: (1) Culture-positive Neonatal Sepsis: A neonate with clinical signs of sepsis (e.g., lethargy, hypothermia, hyperpyrexia, poor feeding, apnea, tachypnea, bradycardia, tachycardia, cyanosis, and poor perfusion) and a positive blood culture. (2) Clinically Proven Neonatal Sepsis: A neonate with negative blood culture but showing clinical signs of sepsis, along with at least one of the following: (1) ≥ 2 positive non-specific blood tests (e.g., leukocyte count, platelet count, C-reactive protein, procalcitonin), or (2) purulent meningitis changes in cerebrospinal fluid.

The inclusion criteria for the control group were: (1) neonates admitted during the same period; (2) matched by sex and gestational age; and (3) without clinical signs of sepsis, with negative blood cultures, and not meeting diagnostic criteria for neonatal sepsis. Blood cultures were obtained as part of routine clinical care, in accordance with national expert consensus [13–14], which recommends blood culture sampling in asymptomatic neonates with perinatal risk factors (e.g., maternal chorioamnionitis, intrapartum fever, premature rupture of membranes ≥ 18 h, or preterm birth). The exclusion criteria for this study were: (1) immune system abnormalities, including immunodeficiency diseases and autoimmune diseases; (2) severe neurological disorders, such as severe brain injury, hydrocephalus, or other neurological abnormalities (except for mild conditions such as meningocele); (3) genetic metabolic diseases or severe congenital abnormalities that could complicate the comparison or diagnosis of neonatal sepsis. (4) incomplete clinical medical records; (5) insufficient samples available for testing. Finally, a total of 41 neonates with sepsis and 41 controls were enrolled. Informed consent was obtained from the guardians of the enrolled children, and the study was approved by the Medical Ethics Committee of Guangdong Women and Children's Hospital (No.202001102).

Sample collection and information extraction

Blood samples from the neonates were collected using sterile techniques by a trained healthcare professional, after obtaining informed consent from the parents or legal guardians. The blood samples were taken from the appropriate site depending on the neonate's condition. The samples were drawn from residual blood collected during routine clinical tests, ensuring that no additional invasive procedures were performed. The collected blood was transferred into vacuum blood collection tubes, which were appropriately labeled. After collection, the samples were anonymized, centrifuged at 3000 r/min to separate the serum within 30 min, and immediately

stored at − 80 ℃ for later analysis. Samples exhibiting visible hemolysis were excluded in our study. Basic information including sex, age, birth weight, and gestational age at birth, and medical records including admission diagnosis, discharge diagnosis, and category of infectious pathogens were obtained from the hospital's medical record information system.

Complement and other biochemical tests

The sample was removed from the − 80 °C refrigerator and thawed to 25 °C at least 30 min. Quantitative analysis of complement in serum from children was performed following the manufacturer's instructions and protocols. Immunoturbidimetric method was used to determine complement component levels including C3c, C1q, factor H and B, using detection kits (Shanghai Beijia Biochemistry Reagents Co., Ltd., China). OD values at a 340 nm wavelength were measured at a Beckman Coulter AU5800 automatic analyzer (Beckman Coulter, USA), and then were converted to the concentration values based on the standard curve. For C3, C4, C3b, C5a, C5, and mannose-binding lectin (MBL), enzyme-linked immunosorbent assay (ELISA) was applied, and serum samples were processed using reagents from Beijing Enzyme Immuno Bio-Technology Co (Beijing, China). The pretreated samples were detected in an automated enzyme immunoassay workstation (ADC ELISA 1800, Adikai Biotechnology Co., Ltd, China) at a wavelength of 450 nm. Two levels of quality control samples were conducted for each assay to monitor the precision and performance of the instrument following the calibration range. In addition, a blank sample was included in each experimental procedure to avoid potential contamination.

Inflammatory factors, including white blood cell count (WBC), neutrophils (Neut), neutrophil percentage (Neut%), and CRP, were measured using the Mindray BC-7500 hematology analyzer (Mindray Bio-Medical Electronics Co., Ltd., China). PCT levels were measured using the Roche cobas e411 electrochemiluminescence immunoassay analyzer (Roche Diagnostics, Germany). Triglycerides (TG), total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using the Beckman AU5800 automated biochemical analyzer (Beckman Coulter, USA). Vitamin A and Vitamin E levels were measured using the Waters TQS micro liquid chromatography-tandem mass spectrometry system (Waters Corporation, USA).

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics of the study population. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range), depending on their distribution. Categorical variables were expressed as counts and percentages. Group comparisons of complement levels were conducted using the Mann–Whitney U test. Logistic regression analysis was performed to examine the association between complement levels and neonatal sepsis risk. Model 1 was unadjusted, while Model 2 was adjusted for neonatal sex and age at sampling. Complement concentrations were standardized using Z-scores before inclusion in regression models, and odds ratios (ORs) were reported per 1-SD increase in complement level.

In accordance with the STARD (Standards for Reporting Diagnostic Accuracy Studies) 2015 guidelines [15], diagnostic performance was assessed for C5a, MBL, and their combination using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was calculated along with 95% confidence intervals. Optimal cut-off values were determined using the Youden index. For each biomarker and the combined model, we calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), each with 95% confidence intervals derived using the Wilson score method. A joint diagnostic model combining C5a + MBL was developed using a logistic regression–based predictive probability approach. AUC values were compared among individual and combined models to evaluate improvement in diagnostic performance. To investigate the association between complement levels and progression of neonatal sepsis, the data was normalized and subjected to Pearson correlation analysis to correlate complement levels with lipid and inflammatory factor concentrations in children with neonatal sepsis. Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). A two-tailed P value < 0.05 was considered statistically significant.

Results

Basic characteristics of the study population

A total of 41 neonates with sepsis and 41 controls were included in this study. The basic characteristics of the study population and the distribution of types of infectious pathogens are shown in Table 1. The mean age of the study participants was 7.7 ± 8.4 days in the neonatal sepsis group and 4.0 ± 7.7 days for the control group, which was not significantly different (P = 0.772). Each group comprised 31 male subjects. The mean gestational age at birth for neonates with sepsis was 33.1 ± 6.7 weeks, with a mean birth weight of 2134.2 ± 935.5 g. In contrast, the mean gestational age at birth for neonates in the control group was 33.0 ± 3.1 weeks, with a mean birth weight of 2639.5 ± 837.1 g. The types of pathogenic bacteria and rates of infection with a particular type of pathogen among the study participants are listed in Table 1. A total

Table 1 Basic characteristics of the study population

Characteristics (mean ± SD or *n*%)	Neonatal sepsis group(*n* = 41)	Control group(*n* = 41)	*P*-value[a]
Age (days)	7.7 ± 8.4	4.0 ± 7.7	0.772
Male infant (n%)	31 (75.6%)	31 (75.6%)	1.000
Gestational age at birth (weeks)	33.1 ± 6.7	33.0 ± 3.1	0.904
Birth weight (g)	2134.2 ± 935.5	2639.5 ± 837.1	**0.014**
Types of infectious agents (n%)[b]			
Escherichia coli	7 (17.1%)	NA	NA
Streptococcus agalactiae	2 (4.9%)	NA	NA
Enterococcus faecalis	2 (4.9%)	NA	NA
Staphylococcus aureus	3 (7.3%)	NA	NA
Klebsiella pnenmoniae	3 (7.3%)	NA	NA
Enterococcus faecium	1 (2.4%)	NA	NA
Streptococcus pyogenes	2 (4.9%)	NA	NA
Streptococcus agalactiae	2 (4.9%)	NA	NA
Staphylococcus haemolyticus	1 (2.4%)	NA	NA
Corynebacterium philippinum	1 (2.4%)	NA	NA
No bacteria detected	18 (43.9%)	NA	NA

[a]Differences between the two groups were calculated using t-test or Mann-Whitney testo; [b]One of the patients was co-infected with *Staphylococcus aureus* and *Klebsiella pneumoniae*; NA: not available

Table 2 Distributions of complement levels in neonatal sepsis and control groups

Complement	Neonatal sepsis group(*n* = 41)	Control group(*n* = 41)	*P*-value
C1q (ng/ml)	70.70 (44.05 – 92.48)	57.70 (42.00 – 68.55)	0.057
C3 (ng/ml)	0.80 (0.60 – 1.04)	0.76 (0.67 – 0.85)	0.639
C3c (ng/ml)	0.68 (0.49 – 1.01)	0.49 (0.39 – 0.71)	**0.007**
C3b (ng/ml)	8.87 (7.70 – 9.92)	9.29 (6.89 – 11.26)	0.376
C4 (ng/ml)	0.20 (0.11 – 0.26)	0.16 (0.14 – 0.21)	0.461
C5 (pg/ml)	12.69 (7.67 – 15.90)	13.27 (6.78 – 21.42)	0.519
C5a (ng/ml)	51.18 (43.55 – 59.47)	57.25 (40.63 – 69.85)	**0.042**
H (ng/ml)	432.40 (347.45 – 518.45)	397.50 (332.30 – 458.60)	0.128
B (ng/ml)	358.70 (277.65 – 427.60)	331.10 (294.35 – 373.50)	0.276
MBL (pg/ml)	518.81 (355.80 – 587.97)	397.06 (221.90 – 479.35)	**< 0.001**

Note: Data was presented as median (25th–75th percentile). *P*-values were calculated using Mann-Whitney tests, and *P*-values in bold were < 0.05

of 23 patients in the neonatal sepsis group had pathogenic bacteria in their blood samples, whereas no bacteria were detected in the remaining 18 subjects. One of the patients was co-infected with *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Distribution of ten complement levels in the neonatal sepsis group and the control group

The distribution of the ten complements in the neonatal sepsis and control groups is shown in Table 2. In the control group, factor H had the highest median level (397.50 ng/mL), while C5 had the lowest median level (13.27 pg/mL). The results demonstrated that C3c in the neonatal sepsis group were significantly higher than those in the control group (0.68 vs. 0.49 ng/mL, $P = 0.007$). In addition, neonatal sepsis subjects tended to have higher MBL levels than the control neonates (518.81 vs. 397.06 pg/mL, $P < 0.001$). In contrast, the median C5a concentration was 51.18 ng/ml in the neonatal sepsis group, which was significantly lower than the concentration in the control group (57.25 ng/ml), with the $P = 0.042$.

Regression models of the ten complement levels in relation to neonatal sepsis

The measured complement data were Z-score-standardized to ensure the reliability and accuracy of the results, and the associations between complement levels and the odds of sepsis in neonates were analyzed using regression models (Table 3). The crude models used univariate regression models for each complement, whereas the adjusted models were adjusted for age and sex. The results of the crude model showed a significant association between complement C5a and MBL levels and the odds of neonatal sepsis ($P < 0.05$). These relationships remained statistically significant in adjusted models. For each 1-SD increase in complement C5a levels, the odds of neonatal sepsis was reduced by 53% (odds ratio [OR] = 0.47, 95% confidence interval [CI]: 0.24–0.90,

Table 3 Associations between complement levels and the risk of neonatal sepsis

complement (per 1-SD)	Crude model		Adjusted model	
	OR(95% CI)	*P*-value	OR(95% CI)	*P*-value
C1q (per 67.29 ng/ml)	1.00 (0.99 – 1.01)	0.810	1.04 (0.65 – 1.66)	0.867
C3 (per 0.38 ng/ml)	1.08 (0.67 – 1.60)	0.892	1.01 (0.64 – 1.60)	0.986
C3c (per 0.49 ng/ml)	1.41 (0.86 – 2.42)	0.166	1.49 (0.85 – 2.63)	0.167
C3b (per 3.81 ng/ml)	0.74 (0.46 – 1.19)	0.214	0.72 (0.44 – 1.16)	0.172
C4 (per 0.11 ng/ml)	1.16 (0.75 – 1.81)	0.505	1.15 (0.73 – 1.81)	0.536
C5 (per 6.87 pg/ml)	0.85 (0.54 – 1.33)	0.463	0.87 (0.55 – 1.4)	0.545
C5a (per 26.36 ng/ml)	0.52 (0.27 – 0.97)	**0.040**	0.47 (0.24 – 0.90)	**0.023**
H (per 132.89 ng/ml)	1.40 (0.88 – 2.23)	0.161	1.45 (0.86 – 2.45)	0.160
B (per 101.28 ng/ml)	1.17 (0.75 – 1.82)	0.480	1.17 (0.73 – 1.85)	0.516
MBL (per 168.97 pg/ml)	2.97 (1.63 – 5.45)	**< 0.001**	2.96 (1.61 – 5.44)	**< 0.001**

Note: Adjusted models were adjusted for neonatal age and sex. ORs and 95% CIs were calculated as changes in the effect estimates per 1-SD increase

Table 4 Diagnostic values of complements in neonatal sepsis

Variable	C5a (ng/ml)	MBL (pg/ml)	C5a + MBL
AUC (95% CI)	0.63 (0.51 – 0.75)	0.75 (0.64 – 0.85)	0.92 (0.85 – 0.99)
Sensitivity (95% CI)	65.9% (50.5–78.4%)	53.7% (38.7%, 67.9%)	85.4% (71.6%, 93.1%)
Specificity (95% CI)	65.9% (50.5–78.4%)	90.2% (77.5%, 96.1%)	97.6% (87.4%, 99.6%)
PPV (95% CI)	65.9% (50.5–78.4%)	84.6% (66.5%, 93.8%)	97.2% (85.8%, 99.5%)
NPV (95% CI)	65.9% (50.5–78.4%)	66.1% (53.0%, 77.1%)	87.0% (74.3%, 93.9%)
Cut-off value	55.63	512.86	NA
True positive	27	22	35
False positive	14	4	1
False negative	14	19	6
True negative	27	37	40

Note: C5a + MBL, combination indicator of C5a and MBL through the calculation of a joint predictive probability; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; NA, non-available

$P = 0.023$). In contrast, for each 1-SD increase in complement MBL levels, the odds of neonatal sepsis increased by 2.96-fold (OR = 2.96, 95% CI: 1.61–5.44, $P < 0.001$).

Clinical diagnostic values of C5a, MBL, and C5a + MBL indicator in neonatal sepsis

Based on the results of regression models, C5a and MBL were selected as potential diagnostic biomarkers for neonatal sepsis, and their clinical diagnostic values were evaluated in accordance with STARD guidelines. As summarized in Table 4, C5a demonstrated an AUC of 0.63 (95% CI: 0.51–0.75, $P = 0.042$). Using a cut-off value of 55.63 ng/mL determined by the Youden index, C5a achieved a sensitivity of 65.9% (95% CI: 50.5–78.4%), specificity of 65.9% (95% CI: 50.5–78.4%), PPV of 65.9% (95% CI: 50.5–78.4%), and NPV of 65.9% (95% CI: 50.5–78.4%). In contrast, MBL showed improved diagnostic accuracy, with an AUC of 0.75 (95% CI: 0.64–0.85, $P < 0.001$). At a cut-off value of 512.86 pg/mL, the sensitivity was 53.7% (95% CI: 38.7–67.9%), specificity 90.2% (95% CI: 77.5–96.1%), PPV 84.6% (95% CI: 66.5–93.8%), and NPV 66.1% (95% CI: 53.0–77.1%). Notably, the combination of C5a + MBL, derived from a joint predictive probability model, achieved substantially better diagnostic performance. True positives, 4 false positives, 19 false negatives, and 37 true negatives. As shown in Fig. 1, the combined C5a + MBL indicator yielded an AUC of 0.92 (95% CI: 0.85–0.99, $P < 0.001$), with a sensitivity of 85.4% (95% CI: 71.6–93.1%), specificity of 97.6% (95% CI: 87.4–99.6%), PPV of 97.2% (95% CI: 85.8–99.5%), and NPV of 87.0% (95% CI: 74.3–93.9%).

Correlation analysis of complement levels in relation to inflammatory factors, lipid, and vitamins

The levels of complement, inflammatory factors, lipid, and vitamins were logarithmically transformed, and the correlations were assessed by Pearson's correlation coefficients (r) in the neonatal sepsis group. As shown in Fig. 2, there were moderate to high correlations between the ten complements, with Pearson's r ranging from 0.34 (C1q and C3) to 0.90 (C3b and C5a). A strong correlation was observed between C3b, C5, C5a, and MBL ($r > 0.70$).

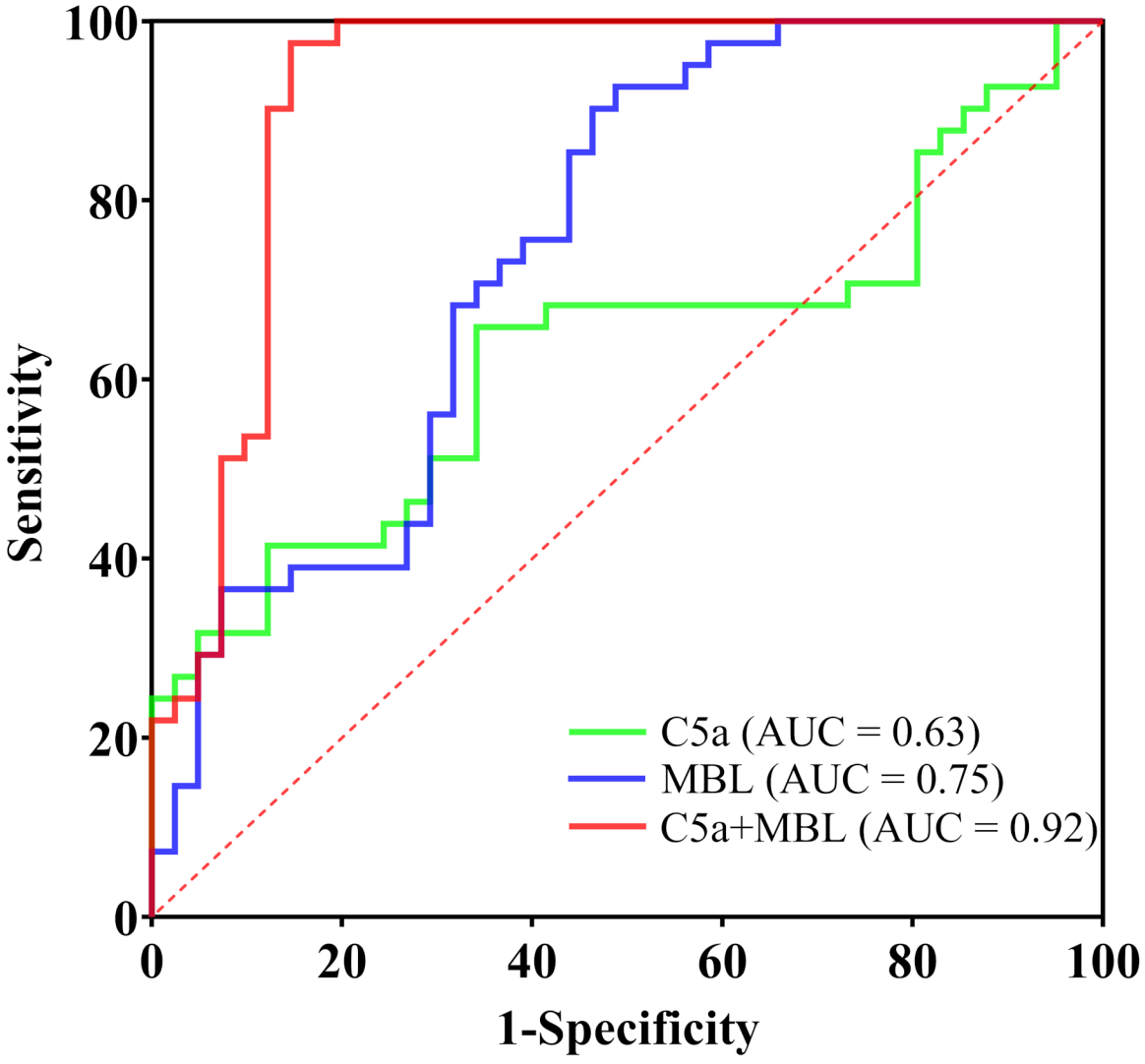

Fig. 1 ROC curve for the diagnostic values of C5a, MBL, and the combined C5a + MBL indicator in neonatal sepsis. Green line = C5a, blue line = MBL, and red line = C5a + MBL

C3c was not correlated with any of the other complementary components. The correlations between complement levels and inflammatory factors were assessed. Positive trends of C4 levels in relation to CRP ($r = 0.33$, $P = 0.047$) and C3 levels in relation to Neut% ($r = 0.34$, $P = 0.036$) were suggested. Regarding the correlations between complement levels and lipid levels, TG exhibited a moderately positive correlation with complement C3, C4, factor H, and B (r ranging from 0.34 to 0.64). Increasing C5 ($r = -0.40$, $P = 0.014$) and MBL ($r = -0.46$, $P = 0.004$) levels could decrease TCH levels at a statistically levels. In addition, positive correlations of factor H levels in relation to HDL-C ($r = 0.39$, $P = 0.015$) and LDL-C levels ($r = 0.62$, $P < 0.001$), and factor B levels in relation to LDL-C levels ($r = 0.63$, $P < 0.001$) were observed. No significant correlation was suggested between the ten complements, vitamin A, and E (all $P > 0.05$).

Discussion

This study investigated complement levels in neonates with sepsis and matched controls, revealing significant differences in C3c, C5a, and MBL. C5a levels were inversely associated with sepsis odds, while MBL showed a positive correlation. The combination of C5a + MBL demonstrated high diagnostic utility, suggesting their potential as biomarkers for neonatal sepsis.

The complement system, a key immune effector, comprises classical, lectin, and alternative pathways, mediating inflammation and pathogen clearance [16, 17]. Dysregulation of this system contributes to sepsis

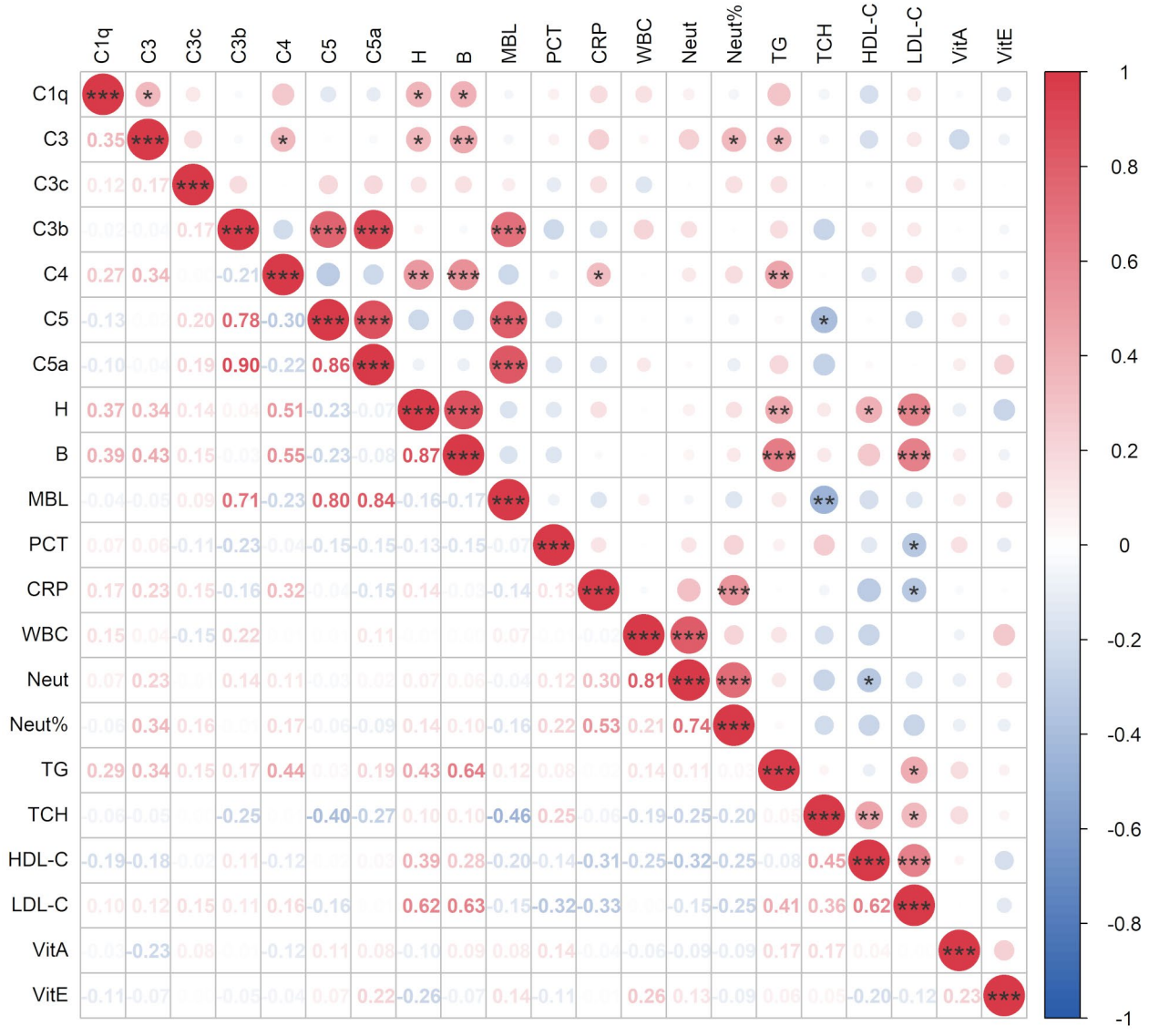

Fig. 2 Pearson correlation matrix of complement, inflammation factors, lipids, and vitamins among neonates with sepsis. The red color represented a positive correlation, while blue color represented a negative correlation. The darker the color, the greater the correlation coefficient. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

pathogenesis, making it a promising target for diagnostic exploration. In neonatal sepsis, we observed distinct activation patterns across complement components, with C3c (a C3 activation product) and MBL showing significant intergroup differences, while C5a exhibited paradoxical anti-inflammatory associations. C3c emerged as a dynamic marker of disease progression, with sensitivity superior to native C3. While prior studies linked low C3 levels to poor outcomes in septic meningitis [18], our findings suggest C3c reflects ongoing complement activation rather than static depletion. Conversely, C5a is a potent inflammatory mediator, which displayed a protective role, with higher levels reducing sepsis odds by 53% in our study. This aligns with mechanistic studies where C5a enhances leukocyte recruitment and activation. He et al. demonstrated that C5a plays a crucial role in activating the inflammatory response and that inhibiting its production effectively reduces inflammation in patients [19].

MBL plays a critical role in the inflammatory response and influences the regulation of inflammatory processes by triggering the complement cascade. MBL binds to pathogen-associated molecular patterns to activate the lectin complement pathway, facilitating pathogen clearance [20]. In our study, there was a significant disparity in the distribution of MBL between the two groups (median: 518.81 pg/ml for case vs. 397.06 pg/ml for control), and our results were consistent with those of Özkan

et al. [21]. While MBL classically enhances pathogen clearance via complement activation, our study revealed elevated serum MBL levels in septic neonates. This may reflect a compensatory host response to acute infection, wherein MBL production is upregulated to neutralize pathogens [22]. However, excessive MBL could paradoxically exacerbate inflammation through uncontrolled complement activation, contributing to tissue damage and septic shock. The opposing roles of C5a and MBL suggest a complex interplay between pro- and anti-inflammatory complement mediators in neonatal sepsis. Excessive MBL activation may overstimulate the alternative complement pathway, leading to increased C3 cleavage and production of pro-inflammatory mediators (e.g., C3a). While C3a amplifies inflammation, C5a was downregulated as part of a feedback mechanism to limit excessive inflammation [23]. In our study, elevated MBL levels may drive complement activation beyond regulatory thresholds, suppressing C5a generation and limiting inflammation. This aligns with Neth et al.'s findings, where MBL enhances pathogen clearance but can also trigger excessive complement activation [24].

The C5a + MBL combination exhibits high diagnostic accuracy in this study, highlighting its potential as a supplementary tool for neonatal sepsis suspicion. Compared to the weak to moderate diagnostic value of C5a and MBL independently, the combined marker C5a + MBL (AUC = 0.92) has superior diagnostic accuracy to distinguish between septic and non-septic neonates. In addition, the sensitivity and specificity of C5a + MBL are significantly higher (85.4% and 97.6%, respectively), reducing the likelihood of both false negatives and false positives. The predictive values further support the superior performance of the combined marker. Furthermore, the PPV of 97.2% and the NPV of 87.0% indicated the reliability in accurately identifying both true-positive and true-negative neonatal sepsis cases. Among the current diagnostic markers for neonatal sepsis, microRNAs are emerging with high diagnostic potential. MiR-141 was shown by Jouza et al. to have a high diagnostic efficacy for neonatal sepsis, with an AUC of 0.87 [25]. In addition, a study by Fouda et al. also yielded high diagnostic efficacy for miR-15b and miR-378a (AUC 0.97, 0.95), with excellent diagnostic efficacy [26]. Elevated miR-15b levels suggest further exacerbation of neonatal sepsis. Conversely, a reduction in miR-378a levels in children with neonatal sepsis indicated a less severe condition. The diagnostic efficacy of these miRNA markers was comparable with that of the C5a + MBL combination used in this study. However, compared with the measurement of complement levels using serum samples, these markers were commonly complex, poorly localized, and time-consuming, which were significant limitations in early diagnosis for neonatal sepsis. Further prospective studies are warranted to validate the clinical applicability of C5a + MBL across diverse populations and integrate it with existing diagnostic protocols.

Lipid profiles, particularly TG, HDL-C, and LDL-C, showed significant alterations in sepsis, consistent with previous findings [10]. We observed a moderate positive correlation between C3, C4, factor H, and B with TG, HDL-C, and LDL-C levels, while C5 and MBL showed significant negative correlations with TCH, supporting the link between complement activation and metabolic dysregulation [27]. Regarding inflammation, elevated levels of inflammatory factors in the cerebrospinal fluid indicate potential inflammation, blood-brain barrier disruption, or CNS infection [28]. While no significant association was found between complement levels and PCT, WBC, or Neut%, we identified positive correlations between C4 and CRP, and between C3 and Neut%. Mechanistically, CRP activates the classical complement pathway by binding pathogens, triggering C4 cleavage [29], while C3 cleavage likely promotes neutrophil activation and inflammatory amplification, as C3 products enhance neutrophil responses and mediator release from macrophages/mast cells [30, 31].

This study measured the distribution characteristics of 10 complement components within three activation pathways and explored their diagnostic values in relation to neonatal sepsis. To the best of our knowledge, this is the first study to indicate a high diagnostic value of the combination of C5a + MBL indicator. However, several limitations warrant consideration. First, the limited sample size may restrict statistical power and generalizability. Second, pathogens were undetected in 43% of sepsis cases, likely due to prior antibiotic exposure, which could confound inflammatory marker levels. Third, combining early- and late-onset sepsis cohorts may obscure infection timing-specific biomarker variations. Finally, the control group did not explicitly include neonates clinically suspected but ruled out for sepsis, restricting applicability to ambiguous clinical scenarios. Future studies should validate our findings in larger cohorts, stratify analyses by infection onset timing, and prospectively incorporate clinically ambiguous subgroups.

Conclusion

In conclusion, this study demonstrates significant dysregulation of complement components in neonatal sepsis, characterized by elevated C3c and MBL levels coupled with reduced C5a concentrations compared to controls. The clinical diagnostic values of C5a, MBL, and their combined indicator had been explored. Individually, C5a and MBL biomarkers showed low-to-moderate diagnostic accuracy. The combined C5a + MBL indicator substantially enhanced diagnostic performance, achieving an excellent AUC along with high sensitivity and specificity.

These findings advance our understanding of complement dysregulation in neonatal sepsis and provide novel insights into potential clinical applications. Identification of distinct complement signatures associated with sepsis could facilitate targeted diagnostic strategies. Future studies are warranted to validate these results in larger, prospective cohorts and to explore mechanistic pathways that participate in complement-mediated sepsis progression.

Abbreviations

AUC	Area Under The Curve
CI	Confidence Interval
CRP	C-Reactive Protein
ELISA	Enzyme-Linked Immunosorbent Assay
HDL-C	High-Density Lipoprotein Cholesterol
LDL-C	Low-Density Lipoprotein Cholesterol
MBL	Mannose-Binding Lectin
NPV	Negative Predictive Value
Neut	Neutrophils
Neut%	Neutrophil Percentage
OR	Odds Ratio
PPV	Positive Predictive Value
PCT	Procalcitonin
ROC	Receiver Operating Characteristic
SD	Standard Deviation
TCH	Total Cholesterol
TG	Triglycerides
WBC	White Blood Cell Count

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Author contributions
Fuqiang Diao: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft. Feng Zhong: Conceptualization, Data curation, Methodology, Formal analysis, Software. Lingling Tang: Methodology, Formal analysis, Visualization, Writing-review & editing. Chunming Gu: Investigation, Methodology. Junfei Guo: Methodology, Formal analysis. Lihong Wu: Investigation, Validation. Weixiang Wu: Conceptualization, Methodology, Formal analysis, Funding acquisition, Visualization, Writing-original draft. Mingyong Luo: Funding acquisition, Project administration, Supervision, Resources, Writing-review & editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Medical Ethical Committee of Guangdong Women and Children Hospital (No.202001102). All healthcare procedures were conducted in accordance with approved guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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