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ORIGINAL RESEARCH

# An Analysis of Predictive Factors for Severe Neonatal Infection and the Construction of a Prediction Model

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**Objective:** To investigate the primary predictive factors for the occurrence of severe neonatal infection, construct a prediction model and assess its effectiveness.

**Methods:** A total of 160 neonates hospitalised in the Department of Neonatology at Suixi County Hospital from January 2019 to June 2022 were retrospectively analysed. Clinical data was analyzed to determine the primary predictive factors for the occurrence of severe neonatal infection. Predictive efficacy was evaluated using a receiver operating characteristic curve, and a nomogram model was constructed according to the predictors. A bootstrap technique was used to verify the accuracy of the model.

**Results:** The neonates were divided, based on the degree of infection, into a mild infection group (n = 80) and a severe infection group (n = 80) according to a 1:1 ratio. Multivariate logistic regression analysis showed that compared with the recovery stage, white blood cell count (WBC) and platelet count (PLT) in the two groups were significantly decreased in the early stage of infection, and the ratio of mean platelet volume to PLT, as well as C-reactive protein (CRP) and procalcitonin levels, was elevated ($P < 0.05$). The area under the curves (AUCs) of decreased WBC, decreased PLT and elevated CRP levels, and the combination of these three indicators, were 0.881, 0.798, 0.523 and 0.914, respectively. According to the filtered indicators, two models (a dichotomous variable equation model and a nomogram model) of continuous numerical variables were constructed, and their AUCs were 0.958 and 0.914, respectively. The calibration curve of the nomogram model was validated with a consistency index of 0.908 (95% confidence interval [0.862, 0.954]).

**Conclusion:** Decreased WBC and PLT levels and an elevated CRP level were the primary independent predictors of severe neonatal infection.

**Keywords:** neonatal infection, prediction, severe infection, risk factor, white blood cell count

## Introduction

Neonatal infection is one of the most common causes of morbidity and mortality among newborns, who are especially susceptible to infection due to the immaturity of their immune systems. Neonates most likely acquire infections before, during or after delivery. The symptoms of neonatal infection are elusive and often difficult to detect. Many severely infected neonates present only non-specific manifestations, including fever, hypothermia, depression, decreased responsiveness, insufficient milk intake and lethargy. This makes the diagnosis of neonatal infection extremely difficult, and a missed or delayed diagnosis can lead to rapid progression of the illness and even death.[1] At present, methods of diagnosing neonatal infection include growing pathogenic microorganism cultures, chest radiography, peripheral blood and umbilical cord blood tests and monitoring C-reactive protein (CRP) and procalcitonin (PCT) levels. Pathogenic microorganism culturing is the gold standard for diagnosing neonatal infection, but its low positivity rate and time-consuming process do not meet the current demand for clinical prediction and early diagnosis of infection.[2] Thus, identifying clinical indicators that can predict severe neonatal infection early and effectively and establishing predictive

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models based on these indicators is of great clinical significance for the early diagnosis, control and improved prognosis of neonatal infection.[3–5]

White blood cell count (WBC), CRP and PCT levels are currently used to diagnose and monitor neonatal infection,[6] white blood cells are essential components of the human immune system, responsible for identifying and eliminating pathogens such as bacteria, viruses, and parasites, when an infection occurs, white blood cells increase to counter the infection, platelets play a key role in coagulation and hemostasis while also participating in inflammatory responses. During an infection, the inflammatory response can lead to platelet activation and consumption, potentially resulting in a decreased platelet count, additionally, some infections (particularly bacterial infections) can cause a reduction in platelets as toxins or immune mediators produced by the pathogens may directly damage platelets or interfere with their production, but the sensitivity and specificity of any single peripheral blood index need to be studied further. Neonatal leukocyte levels fluctuate by the day, and although leukocyte detection is convenient, it is greatly affected by non-infectious factors, such as emotions. Studies have shown that the sensitivity and specificity of leukocyte and neutrophil counts are low. PCT is a calcitonin precursor and an important indicator of bacterial infection with high sensitivity and specificity. In addition, its concentration in adults is positively correlated with the degree of bacterial infection; however, PCT levels fluctuate greatly in the first 48 h after birth, so their value for the diagnosis of early neonatal infection needs to be investigated. A meta-analysis, performed by Vouloumanou et al,[7] suggests that PCT has great diagnostic value after the first 48 h of life. Meanwhile, Zhao et al[8] found that PCT was useful for the early diagnosis of severe infection but had limited value in the diagnosis of mild infection. CRP is an acute, time-phase protein that begins to rise 4–6 h after bacterial infection and usually reaches an effective threshold after 24–48 h. However, CRP fluctuates greatly in the first 3 days after birth, so errors arise in the diagnosis of neonatal infection based on CRP alone. Nevertheless, the combination of CRP and other peripheral blood indicators helps to improve diagnostic accuracy. Wu et al[9] found that the detection of PCT and high-sensitivity CRP in combination could promote diagnostic specificity. Previous studies have focused on diagnosis in the early stage of infection, but few studies have reported on changes in infection indicators during severe infection or in the progression from mild to severe infection. Therefore, this study retrospectively analyses clinical data related to the diagnosis and treatment of neonatal infection to explore changes in infection-related indicators over the course of a severe infection, find primary independent predictors of neonatal infection and establish predictive models based on these predictors. It will serve as a reference for clinicians concerning the timely identification and intervention of severe neonatal infections.

# Methods

## Study Subjects

Neonates hospitalised in the Department of Neonatology at Suixi County Hospital between January 2019 and June 2022 were retrospectively analysed and divided into two groups, based on the degree of infection as follows: a mild infection group and a severe infection group, using a 1:1 ratio. Samples were randomly selected using computer-generated random numbers. The minimum sample requirement was at least 40 neonates per group. The neonatologists in charge of the cases diagnosed cases of severe infections. The hospital has 1200 beds, 10 neonatologists on staff, and is a teaching and referral hospital. The inclusion criteria were as follows:

1. Diagnosis of neonatal infection according to Practical Neonatology,[10] with two or more of the following clinical signs:
   (a) Body temperature >37.5°C
   (b) Increased heart rate
   (c) Unstable or decreased percutaneous oxygen saturation ($SPO_2$)
   (d) Decreased milk intake
   (e) Abnormalities in one or more of the following: blood pressure; respiration; partial pressure of oxygen in arterial blood; pH, serum sodium, serum potassium, creatinine, blood urea nitrogen, haematocrit levels (all neonates included in the study underwent pathogenic microorganism culture analysis and were diagnosed with a neonatal infection);

2. Gestational age of <42 weeks and ≥24 weeks;
3. Admission age of 1–28 days;
4. Newborns who need to be treated with special grade antibiotics are considered severe infections, otherwise they are considered mild infections. Mild infections are treated with non-special grade antibiotics.

The purpose of this study was to explore the predictors of different severities of neonatal infection. Thus, study participants were divided into two groups: a severe infection group and a mild infection group. In the severe infection group, neonates were diagnosed with conditions such as sepsis, pneumonia, meningitis, and necrotising enterocolitis. In contrast, neonates in the mild infection group were diagnosed with less severe infections, including upper respiratory tract infections, urinary tract infections, and localised skin infections. Severe infections were defined as infections that are difficult to control with "restricted use"-grade antibiotics (ie, signs and symptoms of infection and/or abnormal indicators of infection were aggravated during the treatment) and require treatment with "special use" antibiotics. Restricted use antibiotics are defined as antibiotics that are reserved for specific situations or severe infections to prevent the development of antibiotic resistance. The World Health Organization's AWARE ("access, watch, and reserve") classification categorises antibiotics into three groups, with Reserve antibiotics being those that should be used as a last resort for treating multi-drug resistant infections. The remainder of the study population was treated as the mild-infection group. Non-infectious diseases, such as neonatal asphyxia, haematological diseases and immune abnormalities, were excluded. The exclusion criteria were (1) congenital abnormalities of platelets or coagulation factors; (2) the mother's uterine or placental dysfunction (eg, severe malnutrition, gestational hypertension, preeclampsia, placenta previa or placental abruption); and (3) instances where the mother suffered from known thrombotic tendencies or autoimmune diseases and used anticoagulants, antiplatelet drugs, steroidal anti-inflammatory drugs and/or blood products in the third trimester of pregnancy or during delivery.

Relevant indicators and possible predictors of neonatal infection during analysis included fever, increased heart rate, unstable percutaneous $SPO_2$ (often <90%), decreased milk intake, increased WBC, decreased WBC, increased neutrophils%, decreased neutrophils%, decreased platelet count (PLT), increased mean platelet volume (MPV), an increased MPV/PLT ratio and elevated CRP and PCT levels.[11] The WBC, CRP and PCT levels often increase within the first 72 h after birth due to non-infectious factors, such as labour pressure, foetal distress, perinatal asphyxia and ischemic tissue injury, which affect the determination of infection. The upper bounds of the WBC, CRP and PCT levels should be specified; namely, the normal reference ranges within 72 h of birth are[12,13] as follows: WBC: (5.0–25.0) × $10^9$/L; PLT: (100–300) × $10^9$/L; CRP: (0–20) mg/L; and PCT: (0–10) ng/mL. The study was approved by the medical ethics committee of the study hospital (ethics no.: KY-2019002), and informed consent was obtained from the families of all neonatal study participants. Although data collection was based on already completed medical records, we still made efforts to ensure informed consent was provided by the patients' families. To do this, we contacted patient families either by phone or in writing, informing them of the purpose, methods, and potential risks of the study, and sought their consent to use patient data for research purposes. We respected the wishes of the families, and if they did not agree to participate, we refrained from using their related data. During data analysis, we ensured that all data were anonymised to protect patient privacy.

## Data Collection

General data (eg, gender, age and body weight), clinical manifestations (eg, body temperature, heart rate, percutaneous $SPO_2$ and milk intake volume), laboratory results (eg, WBC, N%, PLT, MPV, MPV/PLT, CRP and PCT levels) and clinical severity of infection were collected by two research assistants.

## Specimen Collection and Detection

Two millilitres of peripheral venous blood were drawn from each neonatal study participant and placed in a heparin lithium anticoagulant tube and an EDTA2K anticoagulant tube. A Mindray BC-5390 Auto Haematology Analyzer and a Roche Cobas® 8000 biochemical immune-detection system were used to measure WBC, N%, PLT, MPV, CRP and PCT levels. All blood specimens were tested by the professionals of the laboratory department of the study hospital

according to strict operating instructions. The data collection and laboratory results were obtained within 72 hours after birth. For serial data, the highest values within the 72-hour time frame were used in the analysis.

The normal reference ranges 72 h after birth[10] were as follows: WBC (5.0–14.5) × $10^9$/L; PLT (100–300) × $10^9$/L; CRP (0–10) mg/L; and PCT (0–0. 5) ng/mL.

## Statistical Methods

Statistical analysis of the data was performed using SPSS v. 21.0 statistical software and the R3.6.1 software and extension package. Measurement data that did not conform to a Gaussian distribution were expressed by M (P25, P75), and a Mann–Whitney *U*-test was used. Categorical variable data are expressed as n (%), and a chi-squared ($\chi^2$) test or Fisher exact probability method was adopted. Considering both the statistical significance and clinical relevance of the variables to identify the primary predictive factors for the occurrence of severe neonatal infection, multivariate analysis was performed using logistic regression (progressive forward, α in: 0.05, α out: 0.10). A receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) in the analysis of predictive efficacy. Logistic regression was used to predict the probability of the occurrence of an event by fitting data to a logit function.[14] R3.6.1 software was used to draw a nomogram model, and the calibration curve was drawn with a bootstrap method (1000 sampling repeats) for the internal verification of the established model and to test the accuracy of the prediction model. The results were considered statistically significant at $P < 0.05$.

# Results

From January 2019 to June 2022, consecutive neonates with sepsis hospitalised at Suixi County Hospital were enrolled in this retrospective study. According to the inclusion criteria, out of the initial 200 neonates, 40 were excluded for reasons such as a lack of complete clinical and laboratory data; 80 newborns diagnosed with severe infection and admitted to the neonatal intensive care unit (NICU) were placed in the severe infection group, and 80 neonates diagnosed with mild infection during the same period (none of whom were admitted to the NICU) were randomly selected at a ratio of 1:1 and placed in the mild infection group (Figure 1). Microbiological culture results in the severe infection group revealed that most infections were due to gram-negative rods. Positive and negative likelihood ratios were 5.67 and 0.42, respectively.

## The Comparison of General Clinical Characteristics Between the Two Groups in the Early Stage of Infection

Eighty neonates with severe infection and 80 with mild infection were included in this study. There was no statistical difference in gender, age or weight distribution between the two groups. The male-to-female ratios of the severe infection group and the mild infection group were 54:26 and 51:29, respectively, and the average body weight was 2.3 kg and 3 kg, respectively. The average age of neonates in both groups was 1 day. Newborns with severe infections were more likely to have an increased heart rate and decreased milk intake. Routine analysis of blood data showed that, compared with the mild infection group, neonates in the severe infection group were more likely to have decreased WBC and N% levels and a significantly increased MPV/PLT ratio. Compared with the recovery stage, the CRP and PCT levels in both groups were increased in the early stage of infection and the difference was statistically significant ($P < 0.05$), as shown in Table 1 and Table 2. There was no significant change in MPV in the early stage of infection in either group.

## The Comparison of Infection-Related Indicators in the Severe Infection Group Between the Early and Recovery Stages of Infection

The mean routine blood data of the severe infection group in the early stage of infection showed a significant decrease in WBC and PLT levels, while the MPV/PLT ratio, as well as CRP and PCT levels, was elevated. The above indicators tended to be within the normal range during the recovery stage. The MPV level during the early stage of infection was normal, but it increased during the recovery period, as shown in Table 1.

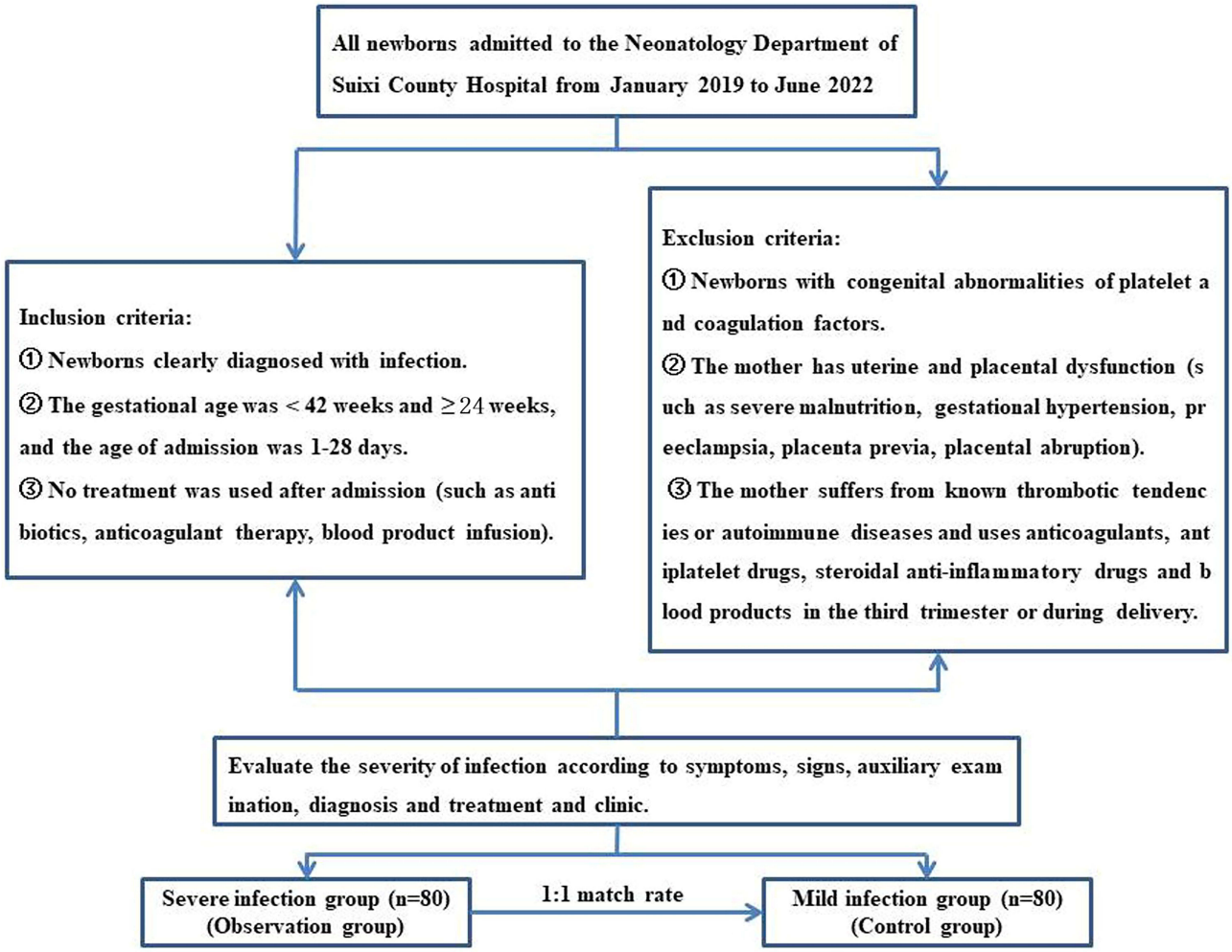


**Figure 1** Flow chart of patient recruitment.

## The Comparison of Infection-Related Indicators in the Mild Infection Group Between the Early and Recovery Stages of Infection

Similar to severely infected patients, the WBC, CRP and PCT levels in the mild infection group increased in the early stage of infection and tended to be normal in the recovery stage. The MPV level was normal in the early stage of infection but increased significantly in the recovery stage. Moreover, the MPV/PLT ratio in the early stage of infection did not differ from that of the recovery stage (see Table 1).

## The Univariate Analysis of Possible Predictors of Severe Neonatal Infection

As shown in Table 2, there was no difference in sex, weight or day age between the two groups ($P > 0.05$). Possible predictors of severe neonatal infection were determined, based on clinical symptoms, as well as a comparison of baseline levels of routine bloodwork in patients with severe and mild infections. The proportion of neonates with increased heart rate, decreased milk intake, decreased WBC, N% and PLT levels and an increased MPV/PLT ratio in the severe infection group was found to be higher than in the mild infection group. However, the proportion of neonates with increased WBC and N% levels (with correction) was lower in the severe infection group than in the mild infection group ($P < 0.05$) (see Table 2).

**Table 1** Comparison of Relevant Indicators Between the Early Stage and the Recovery Stage of Neonatal Infection in the Two Groups $M(P_{25},P_{75})$

| Group | Time | WBC($\times10^9$/L) | N(%) | PLT($\times10^9$/L) | MPV(fL) | MPV/PLT | CRP(mg/L) | PCT(ng/mL) |
|---|---|---|---|---|---|---|---|---|
| The severe infection group n=80 | The early stage | 3.9(3.5,8.0) | 46.3(32.3,59.6) | 177.0(92.3,211.8) | 9.5(8.7,10.0) | 0.05(0.04,0.10) | 2.6(0.8,18.3) | 0.5(0.2,2.2) |
| | The recovery stage | 8.3(7.0,9.9) | 39.9(32.6,47.6) | 254.0(172.5,308.5) | 10.3(9.7,11.1) | 0.04(0.03,0.06) | 1.0(0.4,3.1) | 0.2(0.1,0.4) |
| The mild infection group n=80 | The early stage | 15.6(10.6,23.3) | 73.4(61.8,80.6) | 251.5(203.8,293.6) | 9.4(8.9,9.9) | 0.04(0.03,0.05) | 3.4(0.5,11.7) | 1.2(0.5,3.1) |
| | The recovery stage | 9.9(7.2,12.1) | 42.6(34.5,54.1) | 291.0(229.0,346.0) | 10.1(9.3,10.8) | 0.04(0.03,0.05) | 1.0(0.4,1.9) | 0.2(0.1,0.5) |
| *Z/P* The early stage between the two groups | – | −8.312/<0.001 | −6.897/<0.001 | −6.506/<0.001 | −0.401/0.688 | −6.008/<0.001 | −0.109/0.913 | −2.055/0.040 |
| *Z/P* The recovery stage between the two groups | – | −3.568/<0.001 | −3.112/0.002 | −3.111/0.002 | −0.555/0.579 | −2.784/0.005 | −0.129/0.897 | 0.000/1.000 |
| *Z/P* The severe infection group | – | −5.610/<0.001* | −2.737/<0.001* | −5.010/<0.001* | −3.507/<0.001* | −3.679/<0.001* | −3.065/0.002* | −3.912/<0.001* |
| The early stage - The recovery stage | | | | | | | | |
| *Z/P* The mild infection group | – | −5.493/<0.001* | −8.474/<0.001* | −2.800/0.005* | −4.317/<0.001* | −0.927/0.354 | −3.758/<0.001* | −5.189/<0.001* |
| The early stage - The recovery stage | | | | | | | | |

**Note**: *With statistically significant difference.

**Abbreviations**: SpO2, peripheral oxygen saturation; WBC, white blood cell; N, neutrophils; PLT, platelet; MPV, mean platelet volume; CRP, C-reactive protein; PCT, procalcitonin; *Z/P*, Z-score/ P-value.



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**Table 2** Comparison of Clinical Indicators in the Early Stage of Neonatal Infection Between the Two Groups

| Index | The Severe Infection Group (n=80) | The Mild Infection GROUP (n=80) | $Z/\chi^2$ value | *P* value |
|---|---|---|---|---|
| Gender(number, male/female) | 54/26 | 51/29 | 0.249 | 0.618 |
| Day age[$M(P_{25},P_{75})$,d] | 1.0(1.0,1.8) | 1.0(1.0,1.8) | −0.256 | 0.798 |
| Weight[$M(P_{25},P_{75})$,kg] | 2.3(1.6,3.4) | 3.0(2.4,3.3) | −1.822 | 0.068 |
| Fever[n(%)] | 8(10.0) | 3(3.8) | 2.441 | 0.118 |
| Increased heart rate [n (%)] | 10(12.5) | 3(3.8) | 5.010 | 0.025* |
| Decreased milk intake [n (%)] | 5(6.3) | 0 | 5.161 | 0.023* |
| Unstable or decreased $SPO_2$ [n(%)] | 8(10.0) | 5(6.3) | 0.754 | 0.385 |
| (with correction) Increased WBC [n(%)] | 7(8.8) | 19(23.8) | 6.613 | 0.010* |
| Decreased WBC[n(%)] | 52(65.0) | 1(1.3) | 73.384 | <0.001* |
| Increased N%[n(%)] | 6(7.5) | 29(36.3) | 19.346 | <0.001* |
| Decreased N%[n(%)] | 19(23.8) | 2(2.5) | 15.841 | <0.001* |
| Decreased PLT[n(%)] | 29(36.3) | 2(2.5) | 29.167 | <0.001* |
| Increased MPV[n(%)] | 21(26.3) | 14(17.5) | 1.792 | 0.181 |
| Increased MPV/PLT[n(%)] | 49(61.3) | 17(21.3) | 26.409 | <0.001* |
| (with correction) Increased CRP[n(%)] | 20(25.0) | 11(13.8) | 3.241 | 0.072 |
| (with correction) Increased PCT[n(%)] | 7(8.8) | 6(7.5) | 0.084 | 0.772 |

**Note**: *With statistically significant difference.
**Abbreviations**: SpO2, peripheral oxygen saturation; WBC, white blood cell; N, neutrophils; PLT, platelet; MPV, mean platelet volume; CRP, C-reactive protein; PCT, procalcitonin.

## Independent Predictors of Severe Neonatal Infection and Associated Dichotomous Variable Model Equation

Crucial indicators from the univariate analysis were subjected to multivariate logistic regression analysis to identify independent predictors of severe infection. Decreased WBC, decreased PLT and increased CRP levels were found to be the three major independent predictors of severe neonatal infection, as shown in Table 3. Model equation (dichotomous variable): $\text{logit}P = 7.402 \times$ decreased WBC (no = 0, yes = 1) + 5.134 × decreased PLT (no = 0, yes = 1) + 3.148 × increased CRP (no = 0, yes = 1) − 3.787.

As $\text{logit}P = \ln(P / [1 - P])$, the predicted probability is $P = 1/(1 + 2.718^{-\text{logit}P})$. The corresponding values of WBC, PLT and CRP were inserted into the equation to obtain a logit*P* score for each patient. Each score was then inserted into $P = 1/[1 + 2.718^{-\text{logit}P}]$ to calculate the predicted probability of severe infection for each neonate. An ROC analysis of logit*P* scores yielded an AUC of 0.958, a sensitivity of 85.0% and a specificity of 96.2%, which showed that the model equation has good predictive value for severe neonatal infection (see Figure 2).

## An Analysis of the Predictive Value of Independent Indicators and Their Combined Prediction of Severe Neonatal Infection

ROC curves were drawn for the three independent predictors (WBC, PLT and CRP levels), as well as the three in combination, to analyse their predictive value for severe neonatal infection (see Figure 2). The AUCs of decreased WBC

**Table 3** Multivariate Logistic Regression Analysis of Predictors of Severe Neonatal Infection

| Items | Value | $\beta$ | *SE* | Wald$\chi^2$ | *P* | *OR* | 95% CI |
|---|---|---|---|---|---|---|---|
| Increased White Blood Cells | No=0, Yes=1 | 1.529 | 0.791 | 3.735 | 0.053 | 4.616 | 0.979~21.770 |
| Decreased White Blood Cells | No=0, Yes=1 | 7.402 | 1.265 | 34.263 | <0.001* | 1639.611 | 137.507~19,550.483 |
| Decreased Platelets | No=0, Yes=1 | 5.134 | 1.061 | 23.434 | <0.001* | 169.774 | 21.234~1357.378 |
| Increased C-reactive Protein | No=0, Yes=1 | 3.148 | 0.791 | 15.850 | <0.001* | 23.289 | 4.944~109.700 |
| Constant | – | −3.787 | 0.759 | 24.868 | <0.001* | 0.023 | – |

**Note**: *With statistically significant difference.

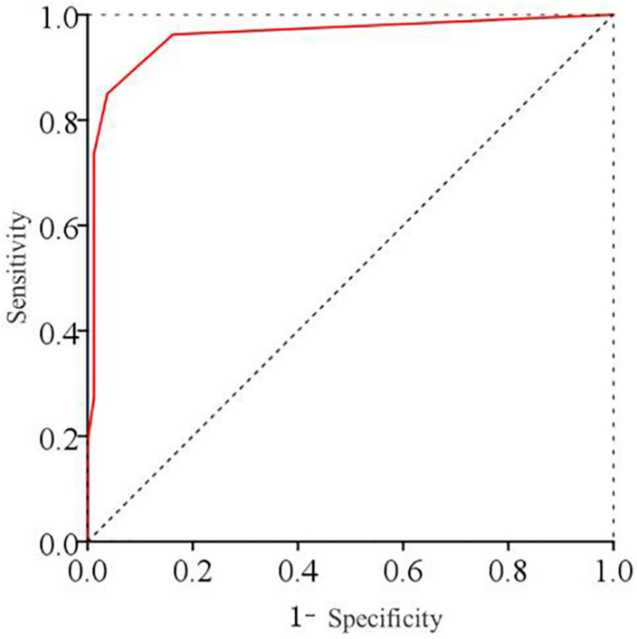


**Figure 2** ROC curves of the model equation for dichotomy variables (decreased WBC, decreased PLT, increased CRP) for risk of severe neonatal infection The AUC was 0.958.

and PLT levels, increased CRP levels and the combination of these three indicators were 0.881, 0.798, 0.523 and 0.914, respectively, among of which that decreased WBC, decreased PLT and the three indicators together show the predictive value with statistical significance for severe neonatal infection ($P < 0.05$). The combination of the three indexes had the highest predictive value for severe neonatal infection, and its sensitivity and specificity were 91.3% and 77.5%, respectively (see Table 4 and Figure 3).

## The Establishment and Validation of a Nomogram Model for the Prediction of Severe Neonatal Infection

All independent risk predictors (continuous numerical variables) were used to establish a nomogram for the risk estimation of severe neonatal infection, as shown in Figure 4. The accuracy of the model was validated using the bootstrap technique (see Figure 5). After obtaining WBC, PLT and CRP values for each newborn, corresponding nomogram segments were checked. The corresponding scores were obtained, and a total score was calculated. After that, the total score and predicted probability segments of the nomogram were checked to obtain the predicted probability of severe infection for each newborn (Figure 4). The internal verification results of the nomogram model show the

**Table 4** ROC Analysis of Predictive Efficacy for Predictors of Severe Neonatal Infection

| Predictors | AUC | 95% CI | Youden Index | The Best Cut-Off Value | Sensitivity (%) | Specificity (%) | *P* value |
|---|---|---|---|---|---|---|---|
| Decreased White Blood Cells | 0.881 | 0.826~0.935 | 0.675 | 4.08 | 98.8 | 68.7 | <0.001 |
| Decreased Platelets | 0.798 | 0.728~0.868 | 0.538 | 191.50 | 83.8 | 70.0 | <0.001 |
| Increased C-reactive Protein | 0.523 | 0.432~0.614 | 0.125 | 6.62 | 33.8 | 78.7 | 0.618 |
| Combination of three indicators | 0.914 | 0.872~0.956 | 0.688 | – | 91.3 | 77.5 | <0.001 |

**Abbreviations**: ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

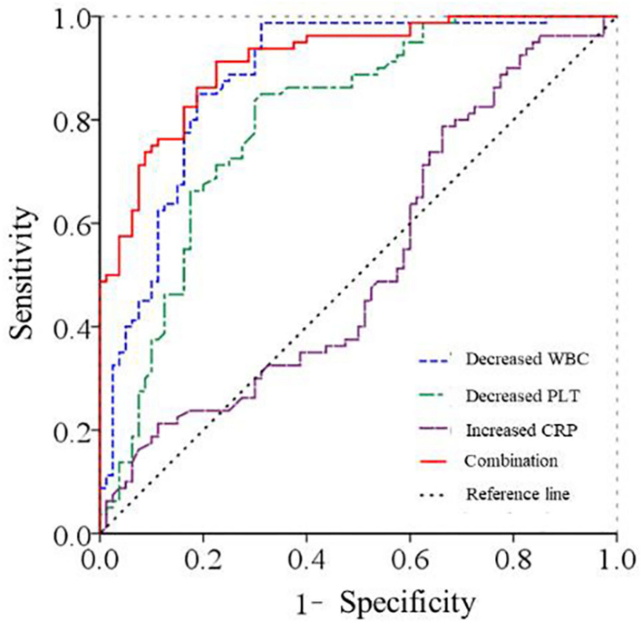


**Figure 3** ROC curves of continuous numerical variables (decreased WBC, decreased PLT, increased CRP) and their combination for risk of severe neonatal infection. The AUC was 0.881, 0.798, 0.523, and 0.914, respectively.

consistency index of the calibration curve to be 0.908 (95% confidence interval [CI] [0.862, 0.954]), which indicates that the predictions of the nomogram model (AUC = 0.914; 95% CI [0.872, 0.956]) were highly consistent with the results, thus demonstrating the model's excellent predictive performance and high accuracy (see Figure 5).

## Discussion

By comparing the routine blood results and clinical symptoms of severe and mild infection in neonates, this study found that decreased WBC and PLT levels and elevated CRP levels were the main independent predictors of severe neonatal infection. Two prediction models (a dichotomous variable model equation and a nomogram model) were established to predict the incidence of severe neonatal infection. While both models were found to be highly accurate, the visualisation of the nomogram was more intuitive and convenient to use than the dichotomous variable model.

Newborns are particularly susceptible to pathogenic infections, and significant rates of morbidity have been observed worldwide. This may be due to an immature immune system. The pathogen spectrum varies with the healthcare setting and pathogen complexity, and the early symptoms of neonatal infection are insidious and atypical. Some newborns exhibit only non-specific manifestations, such as decreased milk intake. The presence of infection in newborns with low body weight is particularly difficult to detect and diagnose. Missed diagnoses and delays in treatment can result in serious infections and have a great impact on the health of the child. Therefore, early detection of severe neonatal infection is particularly important for early infection control and improved prognosis. WBC, CRP and PCT levels have some value in the diagnosis of neonatal infection,[6] but there are few studies on the trends of infection indicators during severe infection or during the progression of infection from mild to severe.

As reported in the literature, the AUC of increased peripheral WBC during neonatal infection was 0.833, with a sensitivity of 64.0% and a specificity of 94.0%.[6] This study found that the proportion of newborns with an increased WBC level in the severe neonatal infection group (8.8%) was lower than that in the mild neonatal infection group (23.8%); however, the proportion of newborns with a decreased WBC level in the severe neonatal infection group was higher (65.0%) than that in the mild neonatal infection group (1.3%). The AUC of decreased WBC levels predicting severe neonatal infection was 0.881 with a sensitivity of 98.8% and a specificity of 68.7%. This study achieved good diagnostic efficacy using decreased WBC levels as an early independent predictor of severe neonatal infection or progression from mild to severe infection. The results of this study show that WBC levels decreased in a majority of

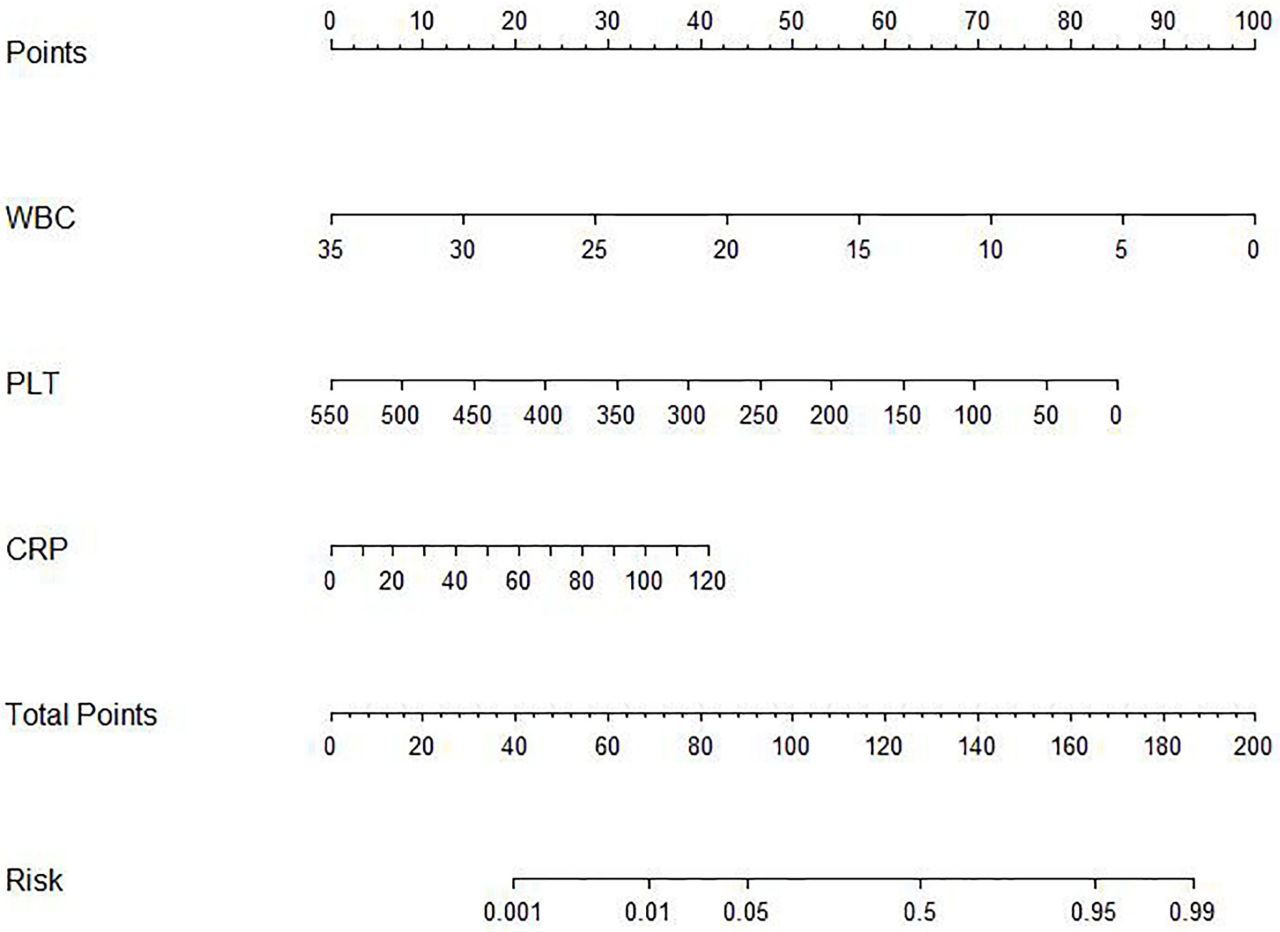


**Figure 4** Nomogram model for predicting severe neonatal infection Check the corresponding nomograms of WBC, PLT and CRP values respectively to get the corresponding scores, and add the scores to calculate the total score. Then check the total score and predicted probability segments of the nomogram to obtain the predicted probability of severe infection of each newborn.

cases of severe neonatal infection, which differs from the trend of increasing WBC levels that were previously reported concerning neonatal infection. The reasons for this may be as follows: (1) the increase in neonatal WBC levels (up to 25,000–30,000/mm$^3$) in the first 72 h after birth which was reported in the literature was due to perinatal risk factors (such as neonatal asphyxia), and they gradually recovered to the normal range after the 72-h period; the increase was not associated with an increased risk of infection; [13] (2) increased WBC levels 72 h after birth indicate mild infection, this finding is consistent with existing literature, which points out that an early increase in WBC levels may be related to infection; [15] and (3) in severe infection, a decrease in WBC levels is due to low immunity and bone marrow depletion. As reported in Austria, the low WBC levels associated with neonatal infection have high probability, high specificity and a negative predictive value (73.3–99.9% and >99.8%, respectively).[16] A decreased WBC level is reported in approximately 35% of neonatal patients with sepsis. In contrast, the probability of an increased WBC level in neonatal patients with sepsis is only 17.7% or even as low as 4%.[17] A decreased WBC level occurs in more than 40% of neonatal patients with sepsis caused by *Escherichia coli, Klebsiella pneumoniae (K. pneumoniae)* or *Candida spp*. and in approximately 30% of neonatal patients with septicaemia caused by *Streptococcus agalactiae* or *Enterococcus spp*. An increased WBC level was observed in only 22% of neonatal patients with coagulase-negative staphylococcal sepsis.[18] The results of this study show that the probability of a decreased WBC level in severe neonatal infection is higher than the probability of an increased WBC level, which is consistent with the results of studies from other countries.[19]

As about 75% of neonatal sepsis is caused by a gram-negative bacterial infection, and reduced PLT levels are associated with gram-negative sepsis, thrombocytopenia is a common problem in neonatal sepsis.[17] Approximately 75%

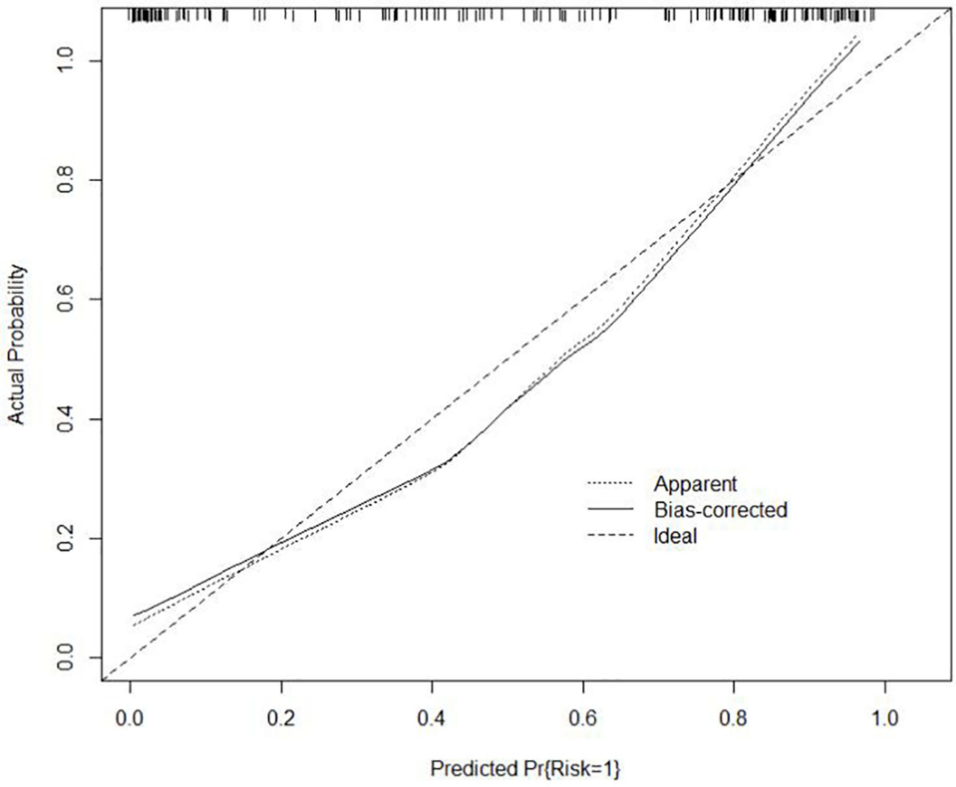


**Figure 5** Internal validation calibration curve of the Nomograph model The validation result showed that the consistency index (C index) of the calibration curve is 0.908 (95% CI: 0.862~0.954).

of neonatal sepsis cases present with reduced PLT levels, which increase mortality almost fourfold.[17,20,21] Moreover, the MPV/PLT ratio in adult patients with infection is higher than that in neonates, and it is a predictor of the clinical severity and mortality of sepsis;[21] however, this is rarely mentioned in current domestic reports of neonatal infection. This study shows that the proportion of patients with a decreased PLT level in the severe neonatal infection group in the early stage of infection was higher (36.3%) than the proportion in the mild neonatal infection group (2.5%). After logistic multivariate regression and ROC analysis, a decreased PLT level was an early independent predictor of severe neonatal infection, with an AUC of 0.798, 83.8% sensitivity, 70.0% specificity and good diagnostic efficacy. The reasons for reduced PLT levels may be as follows: (1) the direct pathophysiological role of endotoxins produced by gram-negative bacteria is to decrease PLT levels;[17] (2) inflammatory mediators in neonatal infection lead to platelet activation and aggregation and induce platelet adhesion to the endothelial cells of blood vessels. Some platelets can cross endothelial cells and fail to return to the blood, while others are destroyed by megakaryocytes. Moreover, some platelets bind to inflammatory mediators and lipopolysaccharides, which accelerates their destruction. Decreased PLT levels were reported in 75% of neonatal patients with sepsis, and 72.0% of neonatal patients with sepsis exhibiting severe thrombocytopenia.[17] In cases of neonatal sepsis caused by *Candida* species or *K. pneumoniae*, PLT levels were decreased in 53.0% and 65.0% of cases, respectively. PLT levels decreased by 17.0% in neonates with gram-positive septicaemia.[19] In addition, the results of this study show that there was no difference in MPV levels between the two groups in the early stage of infection; MPV levels increased in both groups during the recovery stage (compared with the early stage) and MPV/PLT ratios increased in the early stage. However, since MPV in the early stages of infection did not differ, multivariate logistic regression analysis showed that the MVP/PLT ratio is not an early predictor of severe neonatal infection; this result is inconsistent with the findings of related studies.[22,23] This change may be due to the following reasons: (1) PLT levels increase in the early stage of infection, while the number of immature platelets in the blood increases in the recovery stage; an increase in MPV levels compensates for the adverse consequences of decreased PLT;[24] (2) relevant studies do not indicate clearly whether data were drawn from the early or the recovery stage of infection.

Therefore, the increase in MPV levels may derive from the recovery stage of infection rather than the early stage, which necessitates further research and discussion.

Studies have reported that CRP levels increased in 38.5% and PCT levels increased in 98.8% of neonatal sepsis patients. This study found that CRP and PCT levels rose in 25.0% and 8.8% of severe neonatal infection cases, respectively. Logistic multivariate regression and ROC analyses found decreased CRP to be an early independent predictor of severe neonatal infection with an AUC of 0.523, 33.8% sensitivity, 78.7% specificity and low diagnostic efficacy. CRP is an acute-phase protein, and its concentration rapidly increases in response to infection and inflammation. However, in certain cases, a decrease in CRP levels may indicate that the infection is under control or that the inflammatory response within the body has subsided. Therefore, based on the trend of CRP changes, it can serve as an early independent predictor of neonatal infection.[25] However, this study found that a PCT level is not an early predictor of severe neonatal infection, which is inconsistent with the related studies;[26–28] this may be related to the characteristics of neonatal physiology and immune response, as well as the sample size and types of infections included in the study.[29] The low sensitivity of CRP and PCT in predicting severe neonatal infection may be due to their physiological increase in the first three days of life,[12,30] which is a topic that requires further exploration.

As a single-centre, retrospective study with a limited sample size, this study has certain limitations. First, as this study was a retrospective investigation conducted at a single hospital, its results may be influenced by sample selection bias and data completeness. Second, since our research was conducted at a regional hospital, the study findings might not be directly generalisable to different regions and types of healthcare institutions. Furthermore, we did not perform separate analyses for each type of infection, which may limit our understanding of the impact of various infection types on WBC, PLT, CRP, and PCT. Lastly, although we utilised multivariable regression analysis to identify independent predictors of severe infection, there may still be other unconsidered confounding factors. Therefore, future studies should be conducted in larger patient cohorts and different types of healthcare institutions to further validate and expand our findings.

## Conclusion

Decreased WBC and PLT levels and elevated CRP levels are the primary independent predictors of severe neonatal infection. The two models (the dichotomous variable model equation and the nomogram model) that were constructed, based on the major independent predictors, performed comparably in predicting severe neonatal infection with good differentiation and high accuracy. However, the nomogram visualisation is more intuitive and convenient to use than the dichotomous variable model. Both models can effectively predict the occurrence of severe neonatal infections and provide a scientific basis for clinicians to identify such infections early and formulate treatment strategies.

## Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

## Ethics Approval and Consent to Participate

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of The Hospital of Suixi County. Written informed consent was obtained from all parents/local guardians.

## Consent for Publication

The manuscript is not submitted for publication or consideration elsewhere.

## Consent Statement

Written informed consent was obtained from all parents/local guardians.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare that they have no competing interests.

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