

A Combined Diagnostic Model for Distinguishing Mycoplasma pneumoniae Infection from Carriers in Children

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Research Article

Keywords: Mycoplasma pneumoniae, pneumonia, carriers, children, combined diagnostic model

Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11199862/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background: *Mycoplasma pneumoniae* (*M.pneumoniae*) is a crucial pathogen responsible for an important causative pathogen of community-acquired pneumonia in children. Conventional upper respiratory infection symptoms Polymerase Chain Reaction (PCR) detection and serological assays fail to differentiate genuine *M.pneumoniae* infection from asymptomatic carriers, the diagnostic PCR or serology cannot discriminate between *M.pneumoniae* infection and carriers. This study aimed to establish a combined diagnostic model for distinguishing *M.pneumoniae* infection from carriers.

Methods: The study included 1320 children with upper respiratory infection symptoms. The clinical features, and laboratory examinations of blood, C-reactive protein (CRP), White Blood Cells (WBC) and MP-IgM, MP-DNA were collected and retrospectively analyzed.

Results: The WBC and CRP level of the patients' blood have a significant difference between the *M.pneumoniae* patients and carriers, as well as the other respiratory infected patients ($P < 0.001$), and the level of CRP and WBC was closely correlated with had a special relationship of the pediatric patients fever duration. We constructed a new combined diagnosis model by logistic regression analysis to improve the diagnostic efficiency.

Conclusion: The results show the WBC and CRP level of the *M.pneumoniae* patients and carriers had a significant difference. And the constructed combined diagnostic model helps the help for rapid clinical diagnosis.

Introduction

In recent years, the epidemiologic feature of upper respiratory infection among children was shown the high prevalence and frequently, and also easily develop into pneumonia[1, 2]. There are many kinds of pathogens that can cause upper respiratory infections. *Mycoplasma pneumoniae* (*M.pneumoniae*) is one of the most prevalent atypical respiratory pathogens causing Community Acquired Pneumonia (CAP) in preschool and school-age children worldwide[3–5], accounting for 10%–40% of all paediatric CAP hospitalizations[6]. Current mainstream diagnostic approaches include nucleic acid PCR and serological IgM/IgG antibody detection. However, we found the PCR and serological test was limited in our daily work that *M.pneumoniae* nucleic acid positive or serology positive hardly to distinguish pathogenic active infection from asymptomatic upper respiratory carriers[7–9]; The asymptomatic carriers carry the low-load *M.pneumoniae* in nasopharynx without any pneumonia-related clinical manifestations from several weeks to several months[10, 11]. Even several kids had serological IgM antibody weak positive could last 2–3 years. The clinical manifestations and chest radiography lack specific signs for *M.pneumoniae* infection, leading to frequent misdiagnosis between true pneumonia patients and asymptomatic carriers and inappropriate antibiotic abuse in primary medical institutions[12–15]. Routine blood test had some inflammatory biomarkers such as CRP or WBC have limited diagnostic accuracy when used alone. Therefore, it is clinically urgent to develop an easy-to-perform combined diagnostic model relying on conventional blood tests to distinguish active *M.pneumoniae* pneumonia from asymptomatic carriers[16–19].

Materials and methods

We enrolled pediatric patients defined as younger than 14-years-old from the Xiamen Hospital of Traditional Chinese Medicine from 2020 to 2022, including include outpatient department and hospitalized patients. All patients were enrolled initially according to clinical symptoms, Patients need to fulfill three criteria: (1) respiratory tract infection symptoms, (2) a positive finding of chest x-ray (CXR). Clinical symptoms include fever, cough and abnormal auscultation of the lungs. Exclusion criteria were severe concomitant disease (chronic pulmonary disease except asthma, cardiovascular disease, neoplasia, kidney or liver disease and immunodepression), nosocomial-acquired infection and recently being antibiotic therapy.

Throat swabs and blood samples were obtained from all children at the time of enrolment, all the sample were tested in routine clinical lab tests without extra specimen collection were detected after the normal clinical laboratory diagnosis projects. So we didn't take any additional blood samples or throat swabs. *M.pneumoniae*-specific IgM and IgG antibodies were determined using a commercially available chemiluminescence assay (YHLO, Shenzhen, China). Acute *M.pneumoniae* infection was diagnosed if the child had a significant antibody response (IgM antibodies detected > 1.1 in single serum or if at least 4-fold increase in the IgG antibody titre detected in the paired sera) and/or if throat swabs were RT-PCR-positive (SANSURE BIOTECH INC, Changsha, China).

Statistical analysis

Student's t-test and Mann–Whitney U-test were used to compare continuous variables between the group, and χ^2 test and Fisher's exact test were used for categorical variables. Statistical analysis and graphics were performed by SPSS 19.0 statistical software package (SPSS, Chicago, IL, USA) and GraphPad Prism (GraphPad Software, Inc., California, USA). P -values < 0.05 were considered statistically significant.

Result

Comparison of WBC and CRP between MP-IgM positive and negative cohorts

The level of WBC and CRP differed across was showed in difference group patients. The group of the MP-IgM positive patients had a lower WBC level (7.77 ± 1.79 , $P = 0.013$) and higher CRP (21.31 ± 16.38 , $P < 0.001$) compared with the MP-IgM negative patients (Fig. 1.).

Comparison of WBC and CRP between MPP patients and asymptomatic carriers

We divided the IgM-positive patient into MPP patient (MP-IgM antibody was negative then developed to positive in several day) and carrier (MP-IgM antibody was positive but low titer last few months to a year) for distinguishing *Mycoplasma pneumoniae* infection from carriers. The data shows WBC (8.21 ± 3.04 , $P < 0.001$) and CRP (18.49 ± 17.45 , $P < 0.001$) level of MPP patient group, which MP-IgM antibody was negative then developed to positive in several day, had greater difference than the MP carriers (Fig. 2.).

Correlation between the level of CRP and WBC and fever duration

The level of CRP and WBC had a special relationship of the pediatric patients fever duration. The correlation of the level of CRP with the fever duration before admission showed CRP peaked significantly at 2–5 days of fever As the Fig. 3. shows the level of CRP when the duration of fever was 2–5 days had a significant high level than other period ($P < 0.001$).

The clinical characteristics

We collected the clinical characteristics of patients, including WBC and WBC classification count, PLT (Platelet) and CRP are presented in Table 1. And divided the patients into six groups (Ful-A: Influenza A Virus, Ful-B: Influenza B Virus, RSV: Respiratory Syncytial Virus, ADV: Adenovirus, HRV: Human Rhinovirus, MP: *Mycoplasma pneumoniae*) by the pathogen nucleic acid detection result. It shows the level of WBC of MPP patients (7.77 ± 1.79) was normal and had no significant difference with the Flu-A (7.46 ± 3.43), Flu-B (6.75 ± 2.65), RSV (10.16 ± 4.41) patients ($P > 0.05$), meanwhile the ADV (16.27 ± 6.86 , $P < 0.001$), HRV (12.04 ± 5.22 , $P = 0.0065$) patients had high abnormally high level. The performance had a completely difference on the level of CRP among those groups; the Flu-A (6.57 ± 7.46 , $P < 0.001$), Flu-B (5.02 ± 6.16 , $P < 0.001$), RSV (9.62 ± 17.93 , $P < 0.001$) patients had lower level of CRP than MPP patients (21.31 ± 16.38).

Table 1
The clinical characteristics of the patients

Characteristic	Flu-A	Flu-B	RSV	ADV	HRV	MP
WBC (10 ⁹ /L)	7.46 ± 3.43	6.75 ± 2.65	10.16 ± 4.41	16.27 ± 6.86	12.04 ± 5.22	7.77 ± 1.79
Lymphocyte (%)	23.31 ± 13.68	30.91 ± 14.84	34.88 ± 16.55	17.26 ± 11.47	26.73 ± 14.18	26.08 ± 9.06
Monocyte (%)	9.00 ± 3.72	7.91 ± 2.93	8.18 ± 3.21	7.62 ± 3.38	7.84 ± 2.75	7.39 ± 1.92
Neutrophil (%)	66.33 ± 15.61	59.68 ± 15.99	55.60 ± 18.10	74.68 ± 13.27	63.28 ± 15.95	62.22 ± 14.64
Eosinophil (%)	1.17 ± 2.56	1.26 ± 1.88	1.19 ± 1.25	0.60 ± 0.89	1.96 ± 1.87	1.52 ± 1.63
Basophil (%)	0.16 ± 0.11	0.13 ± 0.16	0.15 ± 0.17	0.12 ± 0.12	0.16 ± 0.15	0.20 ± 0.13
PLT (mg/L)	223 ± 44	237 ± 83	290 ± 88	289 ± 63	319 ± 109	257 ± 67
CRP (mg/L)	6.57 ± 7.46	5.02 ± 6.16	9.62 ± 17.93	24.68 ± 23.85	21.90 ± 27.28	21.31 ± 16.38
+The mean ± SD of a representative result of experiments is shown.						
⊠ CRP:C-reactive protein, WBC:White Blood Cells, PLT:Platelet, Ful-A:Influenza A Virus, Ful-B: Influenza B Virus, RSV: Respiratory Syncytial Virus, ADV: Adenovirus, HRV: Human Rhinovirus, MP: <i>Mycoplasma pneumoniae</i> .						

The diagnostic performance of the WBC and CRP for the MPP patients. Additionally, an ROC curve analysis was carried out to assess the serum levels of CRP in children with MPP (Fig. 5). When the cut-off of CRP was 15.95 mg/mL, the ROC curve of CRP show the sensitivity and specificity was 86.96%, 64.17% respectively. As for WBC, when the cut-off was 10.45*10⁹/L, the sensitivity and specificity was 71.56%, 36.49%.

The ROC analysis of single index and combined logistic model.

In order to obtain higher diagnostic efficiency, the diagnostic efficiency of difference combinations of the CRP with WBC. The cut-off of the ROC for WBC, CRP were difference from clinical reference value. Then we use the clinical reference value of WBC, CRP, it was 11.9*10⁹/L and 10.0 mg/mL respectively, to evaluate diagnostic performance of that combination. The results show in the Table 2.

Table 2
The diagnostic performance of WBC combined with CRP in the MP patients

	IgM		Sensitive % (CI)	Specificity% (CI)	PPV % (CI)	NPV % (CI)	PLR	NLR	Accuracy (%)	<i>P</i> value
	Positive	Negative								
Cut-off value of diagnostic combination: WBC > 11.9*10^9/L, CRP > 10.0mg/L										
Positive	56	71	52.830	85.714	44.094	89.496	3.698	0.550	79.934	< 0.0001
Negative	50	426	(42.894–62.604)	(82.325–88.671)	(37.334–51.082)	(87.411–91.270)	(2.793–4.896)	(0.448–0.675)	(76.511–83.060)	
+CRP:C-reactive protein, WBC:White Blood Cells, PPV:Positive Predictive Value, NPV:Negative Predictive Value, PLR:Positive Likelihood Ratio, NLR:Negative Likelihood Ratio.										

This simple combined cannot significantly improve the diagnostic efficiency, so we set a new combined diagnosis model by logistic regression analysis. As was shown in Table 3, the diagnosis efficiency of combinations were all higher than that of each marker used alone and the simple combined. The regression coefficient of CRP and WBC was 0.061 and 0.178 in binary logistic regression (Forward) and set a mathematical diagnostic model through Logistics regression was obtained:Y = 0.061CRP + 0.178WBC-0.66. The ROC curve analysis showed that the AUC of the panel was 0.812, with sensitivity and specificity reaching 79.05% and 75.00%

Discussion

Mycoplasma pneumoniae is an important causative pathogen of CAP in children[20–22]. Currently the detection rates of polymerase chain reaction (PCR) or serology in children with CAP admitted to the hospital amount 14%-39%[23, 24]. At the same time, the

diagnostic PCR or serology cannot discriminate between MP infection and carriers[9, 19, 25]. Further, symptoms and Medical imaging features are not specific for *M.pneumoniae* infection[8]. The rapid and reliable laboratory diagnosis of *M.pneumoniae* infection facilitates targeted clinical medication convenient for the clinical specific medicine treatment[3, 26, 27]. And we found a part of the IgM positive patients had a long-term lower level IgM positive, without causing disease, for up to 3–6 months. And the detection rates of *M.pneumoniae* DNA in the respiratory tract of healthy children without respiratory symptoms were 21% in a Dutch study and 56% in a U.S. study. These data suggest that carriers rate of MP had a great interference in the diagnosis of disease[11, 16, 28, 29].

So, in this study, we focus on finding a method to distinguish between carrier and infection of *M.pneumoniae* in the children with a respiratory symptom. We collected and analysed the data of routine blood test of the patients and found the WBC and CRP level of the patients' blood have a significant difference between the *M.pneumoniae* patients and the other respiratory infected patients. The IgM positive patients have more lower WBC and higher CRP than other respiratory infected patients. In order to accurately identify the infected and carrier, we divide the MP-IgM positive patients into the two group, include the carrier, who had lower level IgM positive over 2 months, and the infected people, the MP-IgM performance negative translate to positive during this treatment. In this model we found the combined diagnosis of the WBC and CRP level could diagnose the carrier and the infected, but also distinguish the MPP from other respiratory pathogen infected patients. And the combined diagnosis model had significant difference during the 2th-5th fever days, so it could help to diagnosis at the early stage of MPP. Finally, We hope this simple and effective diagnosis method help the primary hospital and the community hospital without the PCR method to diagnose and thread the pediatric *Mycoplasma pneumoniae* patients as early as possible.

However, this study is still in its infancy, requiring more difference types of respiratory pathogen samples and more markers to ultimately optimize the detection system and method. Additionally, we will take follow-up studies to determine whether this combined model had more function of MPP. This single-center retrospective study has limitations, and we will carry out multi-center follow-up research to further optimize the model.

Declarations

Ethics approval and consent to participate

This study has been cleared by the Ethics Committee of Xiamen Hospital of Traditional Chinese medicine, and that participant have signed informed consent, the NO:2026-K058-01.

Consent for publication

All authors have read and approved the content of the manuscript.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by funding:

- 1.National Innovation Center for Advanced Medical Devices: NMED2024CX-04-007,
- 2.Xiamen STI Medical and Health Guidance Project:3502ZZ20254ZD1153,
- 3.Xiamen High Quality Development Youth Research Project:2024GZL-QN051

Authors' contributions

All authors confirmed they had contributed to the intellectual content of this paper, Zhiyuan Lin, and Ping Chen conceived experiments. Zhiyuan Lin and Minjing Cai wrote the manuscript. Hanyu You, Zhen Lin provided technical support.

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Table 3

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Figures

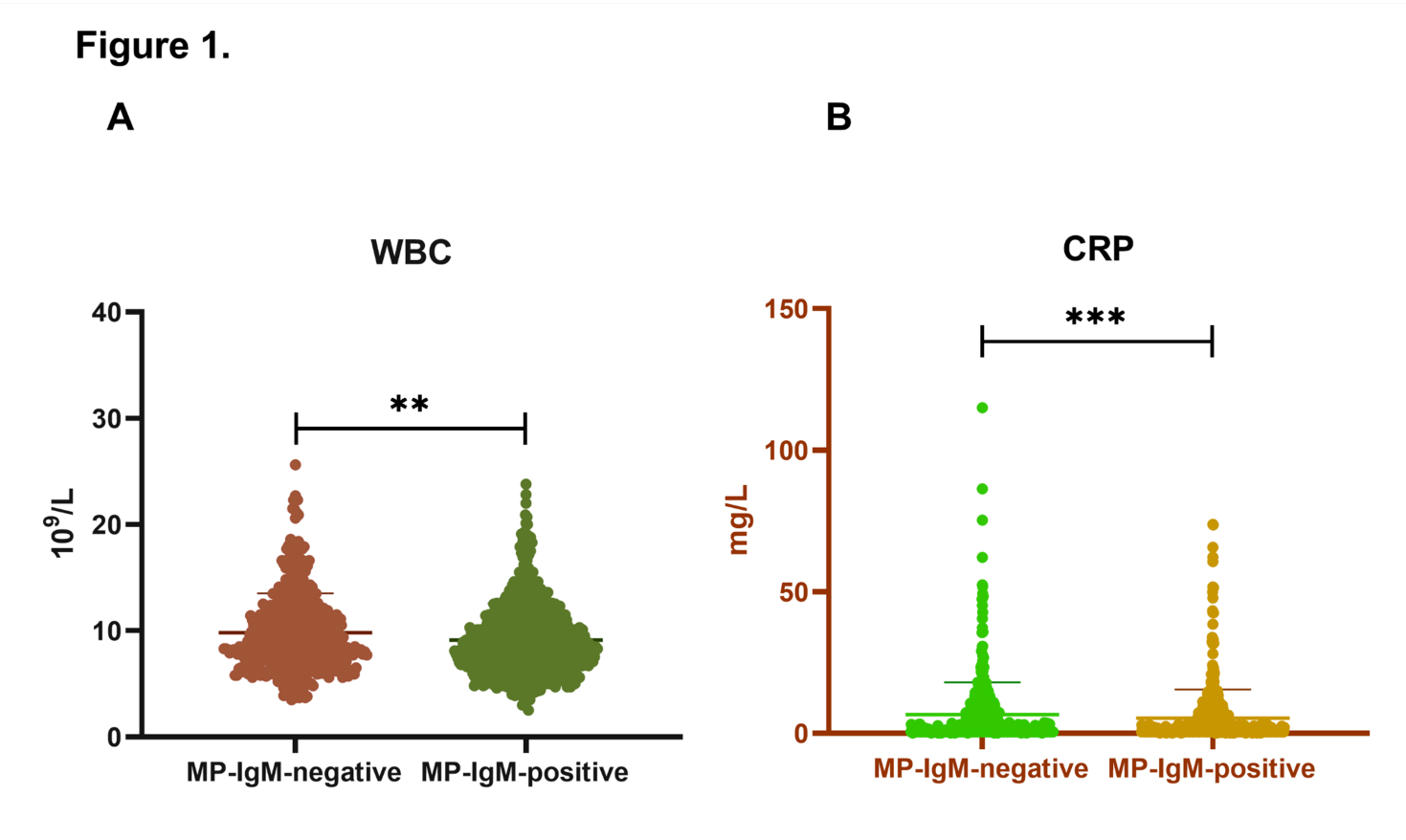


Figure 1

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Figure 2.

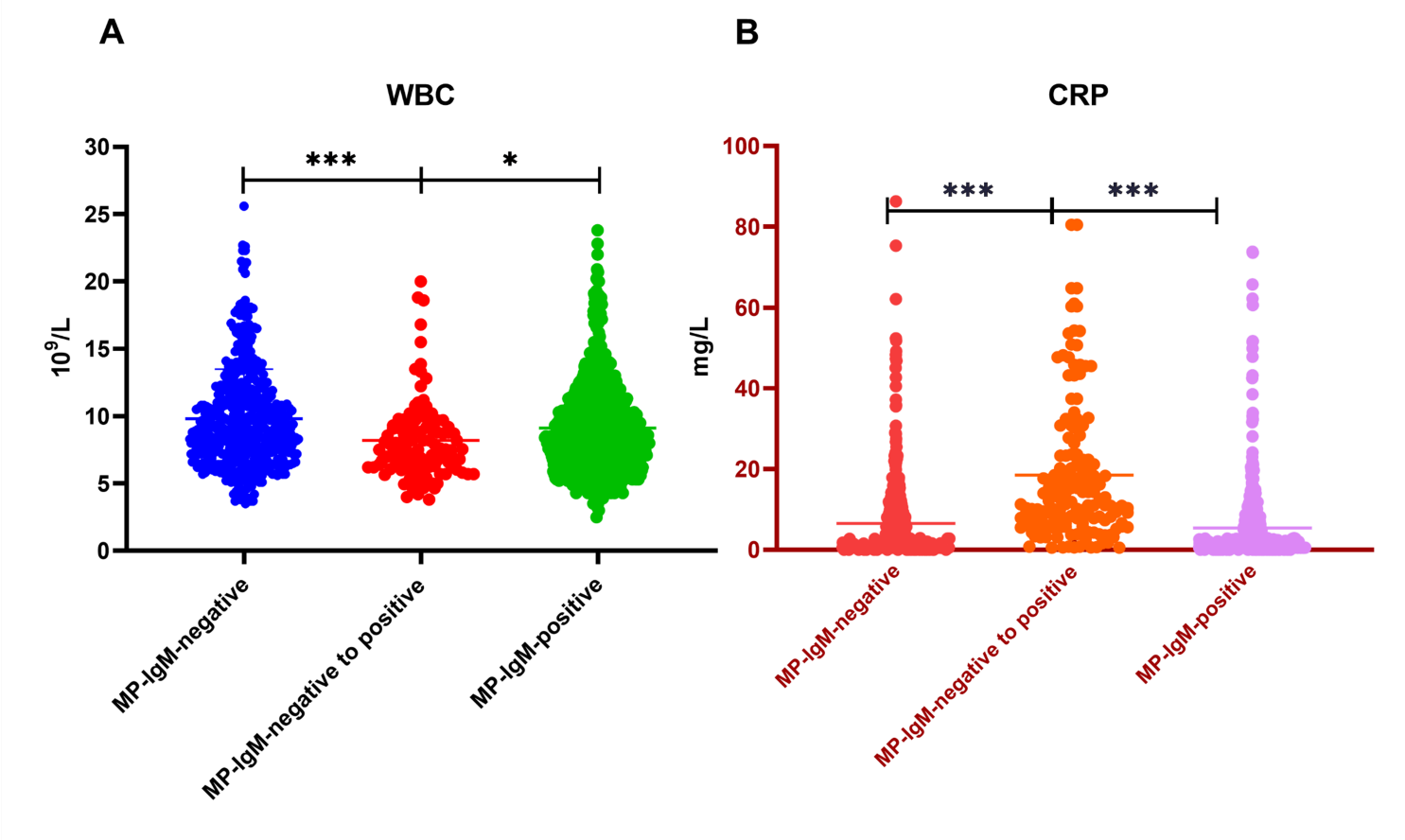


Figure 2

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Figure 3.

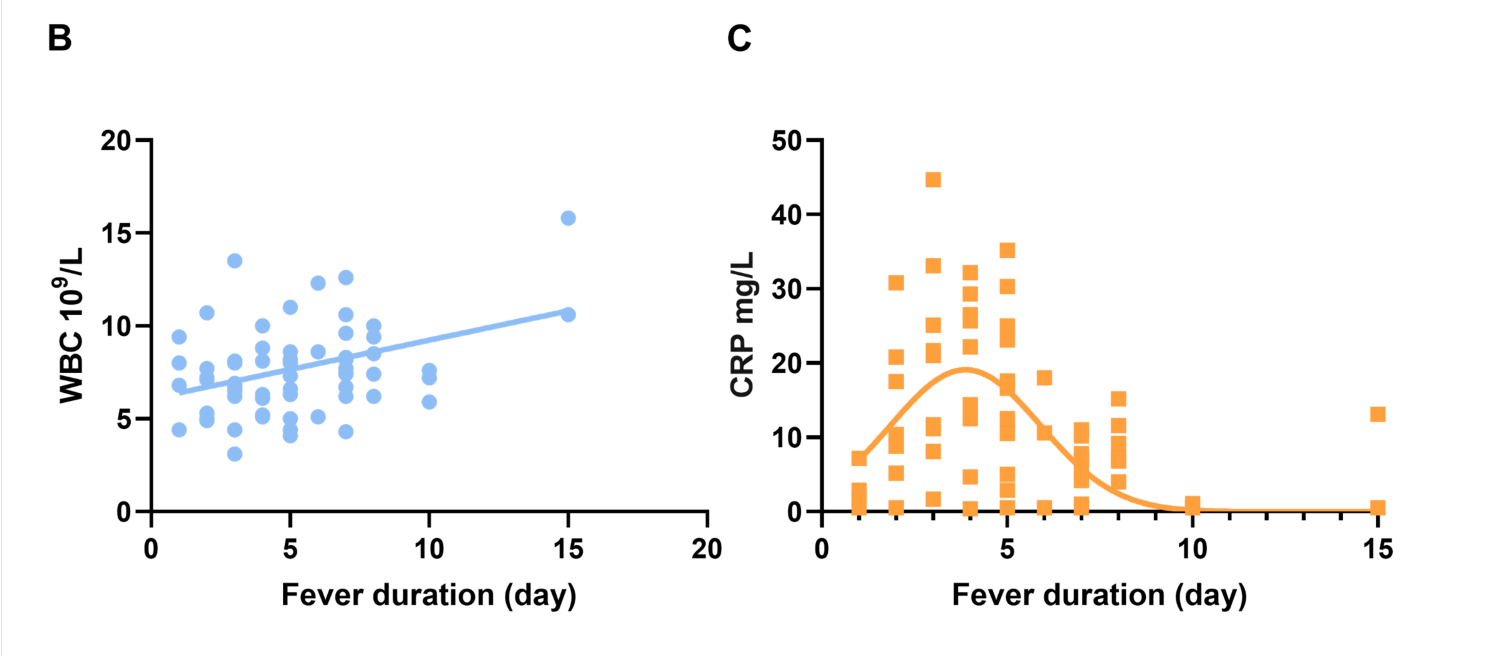


Figure 3

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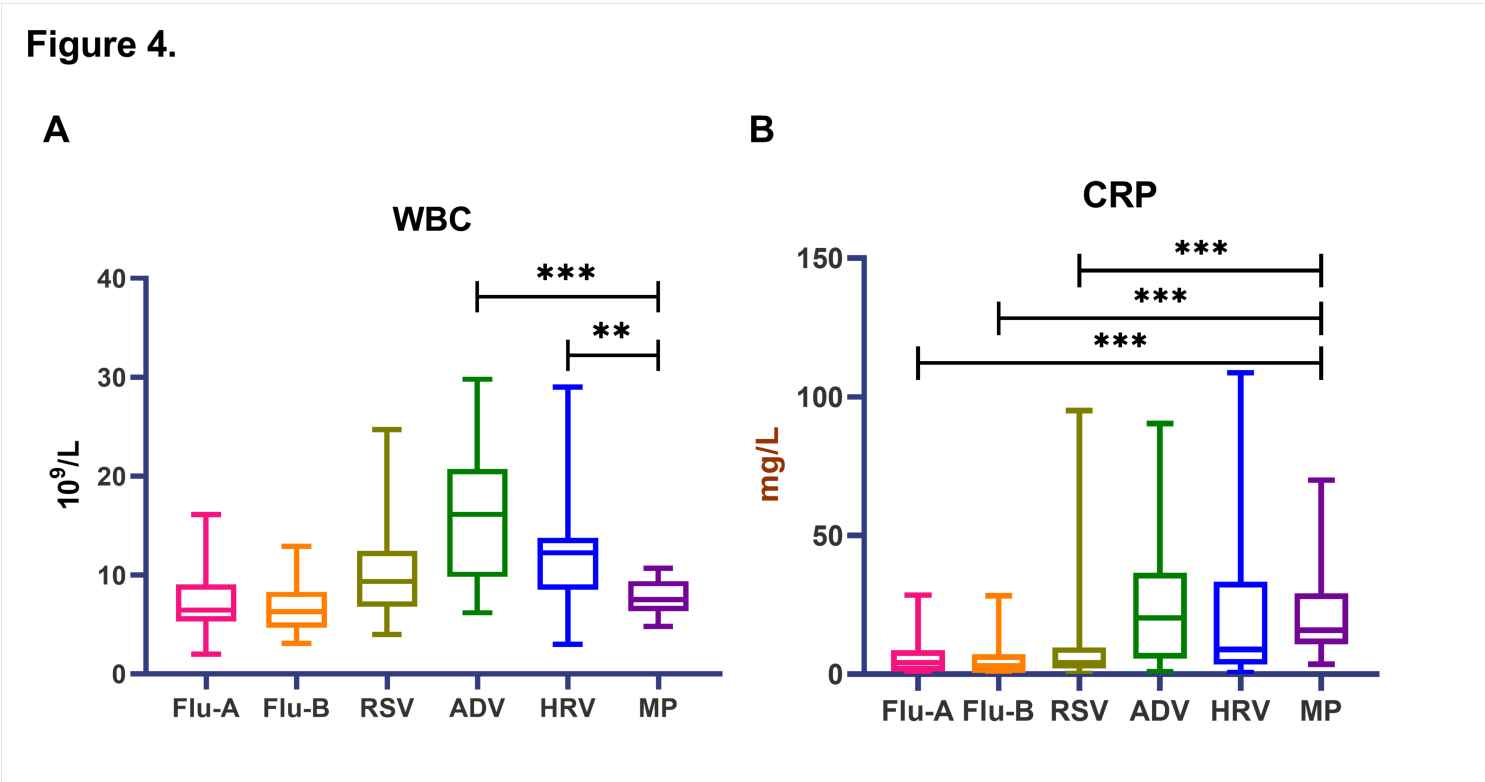


Figure 4

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Figure 5.

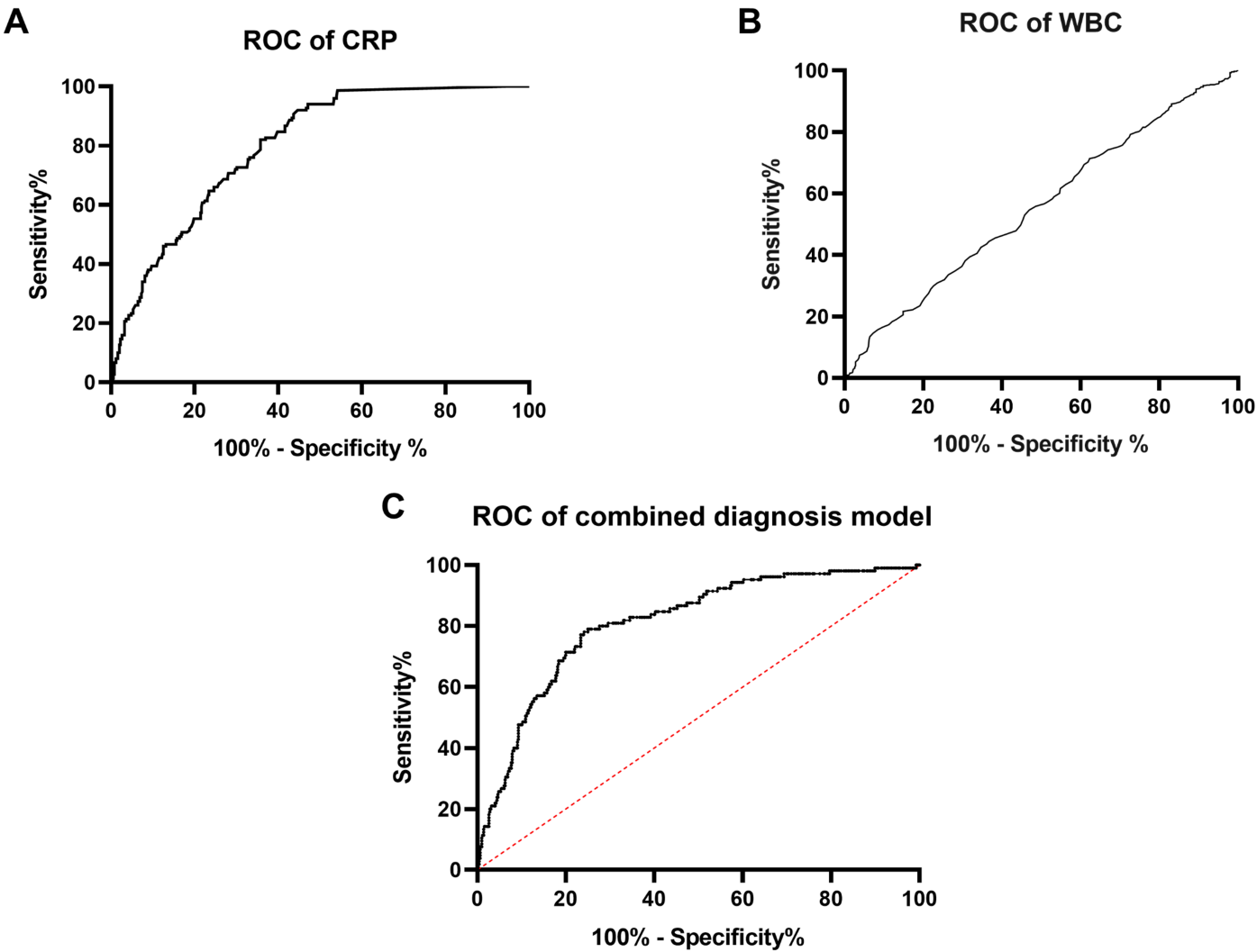


Figure 5

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