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RESEARCH ARTICLE

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Correlations between Serum P2X7, Vitamin A, 25-hydroxy Vitamin D, and Mycoplasma Pneumoniae Pneumonia

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Abstract

Background: Identifying new molecular diagnostic markers for Mycoplasma Pneumoniae Pneumonia (MPP) has always been an essential topic since MPP cases have increased every year, especially among children. Here, we examined the correlation between serum level of Purinergic receptor P2X7, vitamin A, and 25-hydroxy vitamin D (25(OH)D) and the severity of MPP, aiming to identify molecules that have the potential to become diagnostic markers.

Methods: This study was conducted on 186 cases aged 1–14 (136 MPP and 50 non-MPP patients). Serum levels of Purinergic receptor P2X7, vitamin A, 25(OH)D, and multiple inflammatory and immune factors were measured, compared, and tested for statistical significance.

Results: Serum P2X7, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) levels were significantly increased in severe MPP patients, while serum vitamin A, 25(OH)D, IgA, and IgG levels were significantly decreased.

Conclusion: Our results demonstrated a positive correlation between serum P2X7 level and the severity of MPP, and negative correlations between serum levels of vitamin A and 25(OH)D and the severity of MPP, suggesting that high serum levels of P2X7 and low serum levels of vitamin A and 25(OH)D may indicate relatively severer MPP.

KEYWORDS

25-hydroxy vitamin D, Mycoplasma Pneumoniae Pneumonia, P2X7, vitamin A

Fanjun Meng and Ping Chen contributed equally to this study.

1 | INTRODUCTION

Mycoplasma Pneumoniae (MP) infection is a significant cause of community-acquired pneumonia (CAP), contributing to more than 40 percentages of CAP cases in children.[1] MP can be transmitted by droplets and is often associated with community/facility-based outbreaks, principally among school-aged children and young adults.[1] In the last decades, the number of children diagnosed with Mycoplasma Pneumoniae Pneumonia (MPP) has been continually increasing every year, and these children always suffer a prolonged course of the disease symptoms.[1] Typical MPP can cause fever, wheezing, breathing difficulties, chest pain, and chills, while severe MPP (sMPP) often leads to obstructive bronchiolitis, pleural effusion, pulmonary fibrosis, atelectasis, damage of other organs, and even death.[1,2] The severe and extensive impact of MPP on children's health emphasizes the importance of effectively identifying MPP cases and reducing MPP incidence, which largely depends on accurate diagnostic methods. Although the diagnosis of CAP (including MPP) is traditionally based on clinical feathers, molecular diagnosis has been given increasing attention. Nowadays, multiple molecular markers have been established and used to indicate adult CAP severity, such as procalcitonin (PCT) and C-reactive protein (CRP).[3] However, the molecular diagnosis of child MPP is still based on non-standardized approaches with few established makers.

Although mycoplasma has been identified and studied for decades, MP infection's pathogenesis is still unclear. One popular explanation is that MP adheres to airway epithelial cells through the membrane P protein, which will lead to immune and inflammatory responses, including producing autoantibodies, forming immune complexes, and activating complements.[4,5] The immune responses will trigger toxins released, damaging the respiratory and other target organs.[6] Inflammatory responses are essential in regulating the ebullient sequestration of peripheral neutrophils and the repairment of lung tissue.[7] Accumulation of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, and IL-10, has been observed after MP infection.[8] It has been established that children are more vulnerable to respiratory infections due to their insufficient immunity.[8] Deficiencies of vitamin A and 25-hydroxy vitamin D (25(OH)D), two essential nutrients for maintaining immunity, have been found to make children susceptible to respiratory infections, including MPP.[8-10] Although decreased levels of immune-related factors such as vitamin A and 25(OH)D have been found in many patients with respiratory infections, the correlations between these factors and the disease severity have not been examined. Besides, a more recent study has shown that purinergic receptor P2X7, a ligand-gated ion channel, plays a crucial role in inflammasome activation during microorganism infection by permitting the rapid efflux of potassium ions.[11] The depletion of the mouse *P2x7* gene will significantly damage the immune system and increase infection.[12] Since P2X7 plays essential roles related to the immune system and inflammation, it can be linked to respiratory infections such as MPP. However, whether levels of P2X7 change significantly during respiratory infections have not been examined. The potential correlation between P2X7 and MPP remains to be determined. In this study, we investigated how serum P2X7, vitamin A, and 25(OH)D levels correlated with the severity of MPP.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was conducted prospectively in the Hospital of Cardiovascular and Cerebrovascular Diseases affiliated to General Hospital of Ningxia Medical University (Ningxia, China) from September 2017 to May 2020. A total of 136 children aged 1–14 years with MPP and 50 children without MPP were enrolled. This study was conducted following the ethics and approved protocol of the Committee of the General Hospital of Ningxia Medical University. The written informed agreements were obtained from caregivers of all children.

Inclusion criteria: (I) aged from one to fourteen years; (II) meets the diagnostic criteria of MPP after inspection and bacterial culture experiment; (III) with clear and complete clinical record. Exclusion criteria: (I) with a history of infection in the past three months; (II) usage of other anti-infective drugs such as glucocorticoids within three months; (III) combined with congenital heart disease, tuberculosis, liver, and kidney disease; (IV) combined with immune system disease; (V) pneumonia caused by viruses or mixed pathogens; (VI) combined with asthma and other respiratory diseases.

According to the clinical pulmonary infection score (CPIS),[2] patients were divided into severe MPP patients (sMPPs) and non-severe MPP patients (nsMPPs) based on the severity of the disease. The CPIS system includes a total of seven items, body temperature, tracheal secretions, white blood cell count, oxygenation, chest X-rays, progress of lung infiltrates, and culture of tracheal aspirates. The total score is 12 points. Children with CPIS > 6 points are considered sMPPs, while children with CPIS ≤ 6 points are considered nsMPPs. Fifty children without MPP who underwent physical examination in the hospital during the same period were selected as the control group (CPs).

2.2 | Measurements of serum P2X7, Vitamin A, 25(OH)D, TNF-α, IL-1β, IgA, and IgG

Peripheral venous blood specimens were collected from the children on the next day of admission. Samples were centrifuged at 3000 r/min for 5 min, and the supernatants were collected. Each supernatant was divided into three parts and sent to different test centers for different measurements. Enzyme-linked immunosorbent assay was performed to measure serum levels of P2X7 (Human P2X Purinoceptor 7 (P2RX7) ELISA Kit; Catalog #: CSB-EL017325HU; CUSABIO; Detection Range: 25–1600 pg/ml; Intra-assay CV% <8%; Inter-assay CV% <10%), TNF-α (Human TNF-α ELISA Kit; Catalog #: PT518; Beyotime; Detection Range: >4.3 pg/ml; Intra-assay CV%

<10%; Inter-assay CV% <10%), and IL-1β (Human IL-1β ELISA Kit; Catalog #: PI305; Beyotime; Detection Range: >2.2 pg/ml; Intra-assay CV% <10%; Inter-assay CV% <10%). Chemiluminescence methods were used to measure serum levels of 25(OH)D (25-OH Vitamin D in Vitro Diagnostic Kits; Catalog #: 130211004 M; MAGLUMI; Detection Range: 3.0–150.0 ng/ml; Intra-assay CV% <15%; Inter-assay CV% <10%), IgG (RIDASCREEN® Mycoplasma pneumoniae IgG; Catalog #: K4321; R-biopharm; Detection Range: negative <23 U/ml, positive >31 U/ml; Intra-assay CV% <4.1%; Inter-assay CV%<4.4%), IgM (RIDASCREEN® Mycoplasma pneumoniae IgM; Catalog #: K4331; R-biopharm; Detection Range: negative <50 U/ml, positive >71 U/ml; Intra-assay CV%<6.2%; Inter-assay CV%<5.0%), and IgA (RIDASCREEN® Mycoplasma pneumoniae IgA; Catalog #: K4311; R-biopharm; Detection Range: negative <39 U/ml, positive >50 U/ml; Intra-assay CV% <4.7%; Inter-assay CV% <4.5%). High-performance liquid chromatography (HPLC) was performed to measure serum levels of vitamin A (Vitamin A & Vitamin E HPLC Assay; Catalog #: VAE31-H100; EAGLE, Detection Range: >0.01 mg/l; Intra-assay CV% <1.0%, Inter-assay CV% <4.4%). All the measurements were performed according to the manufacturer's instructions with technical triplicates. Average values were calculated.

2.3 | Statistical analysis

Data were analyzed using SPSS 17.0 statistical software (version 17.0, SPSS, Inc., Chicago, IL, USA). Mapping analysis was performed using Graph Pad Prism 7. Count data were shown with numbers and percentages. Normal distribution of data was performed as ($\bar{\chi} \pm s$). Student's t test, F test, and the chi-square test were performed. Comparisons between groups were evaluated by the chi-square test. Z-values were calculated through the Mann-Whitney U test (Wilcoxon Rank Sum Test). Spearman's correlation coefficients were used to assess the correlations among P2X7, vitamin A, 25(OH)D, TNF-α, IL-1β, IgA, and IgG. Significant factors that may predict the severity of MPP were chosen by multivariate logistic regression analysis. $p < 0.05$ was considered to show a statistically significant difference.

3 | RESULTS

3.1 | General comparison of CPs, nsMPPs, and sMPPs

In our study, fifty CPs with a median age of 7.59 years (interquartile range, 5.17–10.01 years) were enrolled, including 24 girls and 26 boys. Eighty-four nsMPPs with a median age of 7.81 years (interquartile range, 5.50–10.12 years) were enrolled, including 43 girls and 41 boys. Fifty-two sMPPs with a median age of 6.87 years (interquartile range, 4.73–9.01 years) were enrolled, including 27 girls and 25 boys. A summary of the cases (Table 1) showed that age, body mass index (BMI), or gender among the three groups had no significant difference ($p > 0.05$).

3.2 | Comparison of Serum levels of P2X7, Vitamin A, and 25(OH)D among CPs, nsMPPs, and sMPPs

To begin with, we examined the changes in P2X7, vitamin A, and 25(OH)D levels after MP infection. Serum levels of P2X7, vitamin A, and 25(OH)D in the CPs, nsMPPs, and sMPPs were measured as previously described. Student's t test was performed to test whether the observed differences between each pair of experimental groups were significant. Both the nsMPPs and sMPPs presented significantly ($p < 0.05$) increased levels of P2X7 with an average value of more than 128.55 ng/ml compared with the CPs (Figure 1A, Table 2). The sMPPs showed an even higher level of P2X7 compared with the nsMPPs with statistical significance. In contrast, serum levels of vitamin A and 25(OH)D decreased significantly ($p < 0.05$) in both the nsMPPs and sMPPs compared with the CPs (Figure 1B, C, Table 2). The levels of vitamin A and 25(OH)D were even significantly lower in the sMPPs than those in the nsMPPs. We next performed analysis of variance (ANOVA) and F test to test whether levels of P2X7, vitamin A, and 25(OH)D differed significantly among the three groups. The result of F test showed that the serum level of P2X7 increased along with the severity of MPP in the three groups with statistical significance ($p < 0.001$), while changes in vitamin A and 25(OH)D failed

TABLE 1 The general comparison of CPs, nsMPPs, and sMPPs

Group	Male/Total	Female/Total	Age (year)	Female/Male	BMI (kg/m^2)
CP	26/50	24/50	7.59 ± 2.42	24/26	15.14 ± 0.82
nsMPP	41/84	43/84	7.81 ± 2.31	43/41	14.92 ± 0.79
sMPP	27/52	27/52	6.87 ± 2.14[a,b]	27/25[c,d]	15.33 ± 0.86[e,f]

Abbreviations: BMI, body mass index. $p < 0.05$ was considered to show a statistically significant difference; CP, control group; nsMPP, non-severe Mycoplasma Pneumoniae Pneumonia; sMPP, severe Mycoplasma Pneumoniae Pneumonia.

[a] $Z = -1.587$, $p = 0.152$;
[b] $Z = -2.081$, $p = 0.257$;
[c] $\chi^2 = 0.589$, $p = 0.476$;
[d] $\chi^2 = 0.351$, $p = 0.413$;
[e] $\chi^2 = 0.757$, $p = 0.524$;
[f] $\chi^2 = 0.5797$, $p = 0.365$.

to be significant (p > 0.05) (Table 2). Together, both t test and F test suggested that serum levels of P2X7 positively correlated with the severity of MPP, while only t test suggested that serum levels of vitamin A and 25(OH)D negatively correlate with the severity of MPP.

3.3 | Comparison of serum levels of TNF-α, IL-1β, IgA, IgG, and IgM among CPs, nsMPPs, and sMPPs

We then examined the differences in the levels of multiple inflammatory factors, markers, and immunoglobulins among the three groups. Serum levels of TNF-α, IL-1β, IgA, IgG, and IgM in the CPs, nsMPPs, and sMPPs were measured as previously described. Student's t test and F test were performed to calculate statistical significance and the results accordant. Both the nsMPPs and sMPPs presented significantly (p < 0.001) increased levels of inflammatory markers (TNF-α, IL-1β) compared with the CPs (Table 3). The levels of TNF-α and IL-1β were even higher in the sMPPs compared with the nsMPPs with statistical significance, suggesting that levels of TNF-α and IL-1β positively correlate with the severity of MPP. In contrast, the levels of IgA and IgG were significantly (p < 0.001) decreased in the sMPPs and nsMPPs compared with the CPs (Table 3). The levels of IgA and IgG in the sMPPs were even lower, suggesting that IgA and IgG levels negatively correlate with the severity of MPP. IgM, on the other hand, showed very slight differences between the three groups, even though the differences are shown to be statistically significant by t test and F test.

3.4 | Correlations among P2X7, Vitamin A, 25(OH)D, inflammatory markers, and immunoglobulins were calculated.

We next examined whether there are correlations between serum levels of P2X7, vitamin A, and 25(OH)D and serum levels of inflammatory markers and immunoglobulins (Figure 2). Our correlation analysis showed that serum P2X7 levels had a significant and robust positive correlation with TNF-α (r = 0.74, p = 0.001) and with IL-1β (r = 0.775, p = 0.0001). Serum vitamin A levels showed a strong negative correlation with IgG (r = −0.76, p = 0.001) and a robust positive correlation with IgA (r = 0.70, p = 0.001). No significant correlation was observed between serum vitamin A levels and IgM (r = 0.02, p = 0.37). Similarly, serum 25(OH)D levels presented a robust negative correlation with IgG (r = −0.75, p = 0.001) and a substantial positive correlation with IgA (r = 0.68, p = 0.001). No significant link was recognized between serum 25(OH)D levels and IgM (r = 0.01, p = 0.33).

3.5 | Multivariate logistic regression analyses of P2X7, Vitamins A, 25(OH)D, and inflammatory markers.

We also performed multivariate logistic regression analyses to analyze the independent risk factors for P2X7, vitamins A, 25(OH)D, and inflammatory markers. Our analysis demonstrated that P2X7, vitamins A, 25(OH)D, TNF-α, IL-1β, IgA, and IgG are the significant factors (p < 0.05) of severe MPP (Table 4), confirming that immune and inflammatory responses were closely related to the pathogenesis of MPP. Our data suggested that those factors had the potential to become diagnostic markers indicating severe MPP.

4 | DISCUSSION

In the present study, we measured the serum levels of P2X7, inflammatory markers, vitamins A, and 25(OH)D in child patients with or without MPP. We explored their correlations with levels of inflammatory markers and the prevalence of nsMPP and sMPP. We established that both the nsMPPs and sMPPs had decreased serum levels of vitamin A and 25(OH)D but increased serum levels of P2X7, IgA, and IgG.

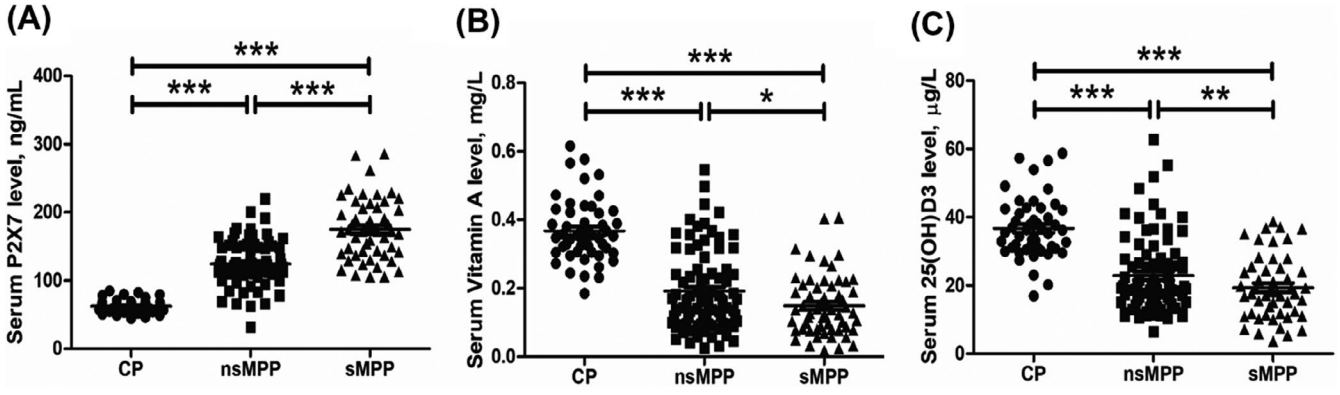

FIGURE 1 Evaluation of the serum levels of P2X7, vitamin A, and 25(OH)D3 among CP, nsMPP, and sMPP. The serum levels of P2X7 (Figure 1A), vitamin A (Figure 1B), and 25(OH)D (Figure 1C) were measured and compared among CPs, nsMPPs, and sMPPs. (n = 50 (CP), 84 (nsMPP) and 52 (sMPP). *<0.05; **<0.01; ***<0.001

MPP is primarily caused by MP infection. MP, like other invasive pathogens, will trigger inflammatory responses.[4,5,13] Recent studies revealed that severe MP infection is always combined with extreme inflammation reflected as increased inflammatory cytokines, such as TNF-α and IL-1β.[3,14,15] Inflammation responses caused by MP infection and other stimuli are complicated processes involving the communication and cooperation of multiple intercellular and exocellular macromolecules.[4] Recently, the P2X7 receptor, an ATP-mediated ion channel expressed by virtually all innate and adaptive immunity cells, has been identified to play an essential role in inflammation and immunity by mediating the ATP-dependent lysis of macrophages.[16] P2X7 mediates inflammasome activation, cytokine and chemokine release, T lymphocyte survival, and differentiation.[17] P2X7 has been reported to be upregulated during variate of infections and can become an anti-inflammatory target.[18] Previous studies about the relationship between P2X7 and respiratory system inflammation are mainly focused on chronic obstructive pulmonary disease, asthma, etc. with few studies in MPP, even though the fact that P2X7 can regulate TNF-α expression and inflammation suggests the possibility that the pathogenesis of MPP is associated with the upregulation of P2X7.[19-22] The correlation between P2X7 and inflammation during MP infection or MPP has not been evaluated until the present study.

TABLE 2 The serum levels of P2X7, vitamin A, and 25(OH)D among CPs, nsMPPs, and sMPPs

Group	P2X7(ng/mL)	Vitamin A(mg/L)	25(OH)D(μg/L)
CP	62.86 ± 11.89	0.5 ± 0.28	34.93 ± 10.62
nsMPP	128.55 ± 21.16[a]	0.19 ± 0.04[a]	28.94 ± 20.61[a]
sMPP	187.55 ± 26.42[a,b]	0.14 ± 0.05[a,b]	21.81 ± 15.32[a,b]
F	10.643	2.128	2.531
p	0.000	0.384	0.261

F, the result of F test; p, p-value of F test; CP, control group; nsMPP, non-severe Mycoplasma Pneumoniae Pneumonia; sMPP, severe Mycoplasma Pneumoniae Pneumonia. Data are shown as mean ± SD. n = 50 (CP), 84 (nsMPP) and 52 (sMPP). $p < 0.05$ was considered to show a statistically significant difference.

[a]Compared with CP, Student's t test, $p < 0.05$;

[b]Compared with nsMPP, Student's t test, $p < 0.05$.

Here, our data, for the first time, demonstrated the positive correlation between P2X7 and MPP, indicating the critical role of P2X7-mediated inflammasome activation during MPP. We also observed that serum levels of TNF-α and IL-1β in sMPPs were significantly higher than those in the non-serious group, which suggested that severe MPP is linked to robust inflammatory responses. Besides, we detected a positive correlation between the level of P2X7 and levels of TNF-α and IL-1β, which was expected since published data had established that P2X7 could activate IL-1β by reducing intracellular potassium levels and upregulate the level of TNF-α through ERK/p53-MAPK signaling pathway.[16] Based on our data, we proposed that serum P2X7 levels could be used as a new molecular marker for MPP diagnostics.

It has been well known that vitamins A and vitamin D are related to pathogen invasion, immune responses, and a variety of diseases.[23-25] Vitamin A is essential for the growth, differentiation, and maintenance of epithelial tissues. It plays a pleiotropic role in supporting the normal mucosal barrier, preventing invasive pathogens.[26] Vitamin A deficiency causes squamous metaplasia of the respiratory epithelium, impairment of the airway mucosal barrier, delay of repair, and reduced airway antioxidants.[26] Recent studies have shown that children with vitamin A deficiency are more vulnerable to respiratory tract infection.[9] Vitamin D, on the other hand, plays an essential role in innate immunity. It is involved in maintaining immune homeostasis, mainly through direct induction of the expression of antimicrobial peptides.[10,27] Vitamin D promotes the induction of T regulatory cells, stimulates the expression of inflammatory cytokines such as TNF-α.[10] Previous studies have demonstrated that vitamin D deficiency leads to an increased risk of pathogen infection, especially in respiratory tract.[28] Children with insufficient 25(OH)D, a metabolic form of vitamin D usually used for clinical testing with usually higher concentration and longer half-life,[29] are more vulnerable to pathogenic microbes such as influenza, respiratory viruses, airway pathogenic bacteria, and MP.[28,30] Although the linkage between vitamin A, 25(OH)D, and respiratory disease has been firmly formed, their correlations with the severity of MPP have not been studied until the present work. Here, we demonstrated the negative correlation between serum levels of vitamin A, 25(OH)D, and the severity of MPP based on the statistical significance calculated by Student's

TABLE 3 The serum levels of TNF-α, IL-1β, IgA, IgG, and IgM in CPs, nsMPPs, and sMPPs

Group	TNF-α(ng/mL)	IL-1β(ng/mL)	IgA(mg/mL)	IgG(mg/mL)	IgM(mg/mL)
CP	0.74 ± 0.18	0.23 ± 0.18	3.74 ± 1.36	14.80 ± 3.99	0.49 ± 0.38
nsMPP	1.39 ± 0.21[a]	0.64 ± 0.17[a]	1.37 ± 0.74[a]	8.77 ± 1.45[a]	0.46 ± 0.35[a]
sMPP	2.08 ± 0.0.27[a,b]	1.15 ± 0.22[a,b]	0.19 ± 0.18[a,b]	3.05 ± 0.96[a,b]	0.42 ± 0.28[a,b]
F	8.954	11.543	18.657	19.752	0.859
p	0.000	0.000	0.000	0.000	0.000

F, the result of F test; p, p-value of F test; CP, control group; nsMPP, non-severe Mycoplasma Pneumoniae Pneumonia; sMPP, severe Mycoplasma Pneumoniae Pneumonia. Data are shown as mean ± SD. n = 50 (CP), 84 (nsMPP) and 52 (sMPP). $p < 0.05$ was considered to show a statistically significant difference.

[a]Compared with CP, Student's t test, $p < 0.05$;

[b]Compared with nsMPP, Student's t test, $p < 0.05$.

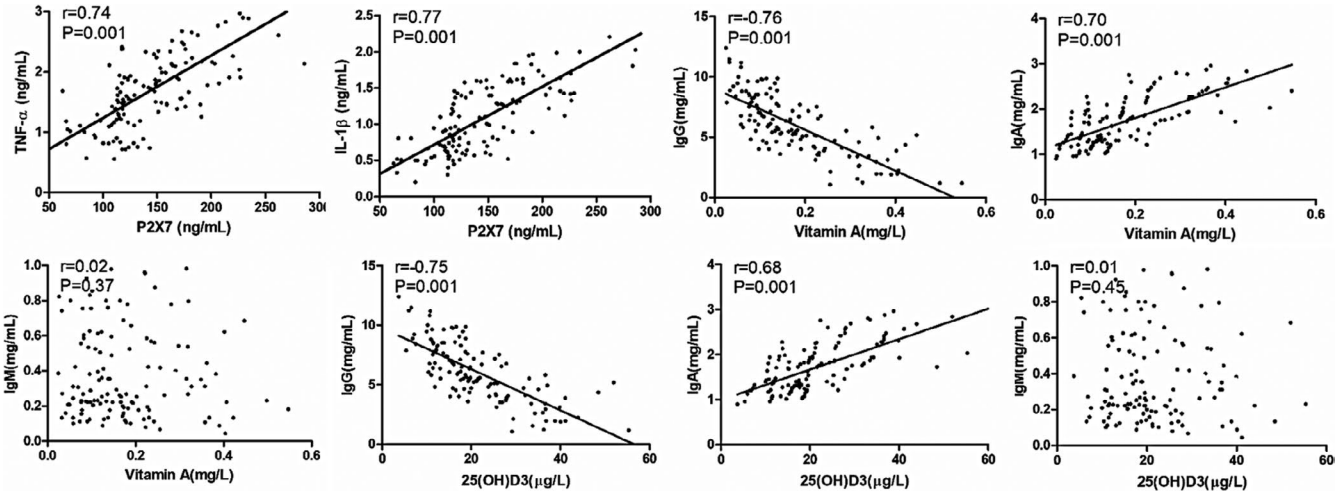

FIGURE 2 Scatter plot graphs of the Spearman correlation analysis. Correlation analysis between P2X7 and TNF-α, IL-1β; between vitamin A and IgG, IgA, IgM; between 25(OH)D and IgG, IgA, IgM; were performed. Each symbol represents the measurement of one case. The continuous line shows the least-square linear regression

TABLE 4 Multivariate logistic regression analyses of P2X7, IgA, IgG, Vitamins A, and 25-OH Vitamin D

Index	β	SE	Wald χ^2	OR (95% CI)	p	95%CI
P2X7	0.647	0.026	12.565	1.676	0.000	1.624–1.877
25-OH Vitamin D	−0.086	0.011	7.843	1.064	0.027	1.009–1.129
Vitamin A	−7.875	2.995	6.908	0.001	0.009	0.001–0.334
TNF-α	0.487	0.008	8.665	1.126	0.008	1.026–1.124
IL-1β	0.596	0.053	8.934	1.098	0.011	1.015–1.132
IgA	−0.263	0.062	11.658	1.656	0.000	1.433–1.721
IgG	0.477	0.045	14.533	1.743	0.000	1.699–1.923

Abbreviations: CI, confidence interval; OR, odds ratio; p, p-value. $p < 0.05$ was considered to show a statistically significant difference; SE, standard error around the coefficient for the constant; sMPP, severe Mycoplasma Pneumoniae Pneumonia; Wald, the Wald chi-square test value; β, the coefficient for the constant in the null model.

t test, indicating that severe MPP patients tend to have decreased serum vitamin A and 25(OH)D. We also detected that serum levels of vitamin A and 25(OH)D positively correlated with levels of IgA while negatively correlated with levels of IgG in MPP patients, suggesting that different immunoglobulins might act differently related to vitamin A and 25(OH)D during MPP. Also, no significant correlation with IgM was detected since changes in the serum level of IgM are very small during MP infection (Table 3).

MPP has always been a significant threat to the health of children and a significant cause of CAP. MP infection can cause pneumonia and, in severe cases, causes damage to multiple organs.[1,2] Although significant efforts have been taken, the etiology and pathogenesis of sMPP among children remain mostly unknown. It has been suggested that children's weakened immunity may be a significant contributing factor in inducing respiratory infections, and vitamin deficiencies among children, including vitamin A and vitamin D, are contributors to both insufficient immunity and pneumonia.[8,31] Based on this, it is reasonable to think that proteins related to immune responses may be related to MPP as well. Our multivariate logistic regression analyses suggested that P2X7, vitamins A, 25-OH vitamin D, TNF-α, IL-1β, IgA, and IgG were all factors that may predict the severity of MPP. IgA is a vital participant in local mucosal immunity.[32] IgG can control the phagocytosis of macrophages and plays a central role in resisting pathogen infection.[33] We found that the serum levels of IgA and IgG in MPPs were significantly decreased. Levels in sMPPs were even lower than nsMPPs. The levels of IgA and IgG conversely correlated with the levels of P2X7, TNF-α, and IL-1β, which agrees with the idea that severe MPP is associated with decreased immunity and increased inflammation.[4,5] Also, animal studies have demonstrated that lack of vitamins A and 25(OH)D can cause respiratory tract inflammation in mice model,[34] suggesting that all the factors we studied may be linked to MPP through the immune system and inflammation.[34] We suggested that all the factors we identified had the potential to be used as diagnostic markers of MPP or even the indicators of the severity of MPP.

Although the results of our study are promising, it does have three limitations. Firstly, even though we have excluded patients with a history of infection in the past three months or Pneumonia

caused by viruses or mixed pathogens, there is still possibility that unknown pathogens other than MP could influence that presented discoveries. Secondly, the presented study only focused on the serum level of P2X7, which may not represent the changes of P2X7 in the body since P2X7 is expressed in a variety of tissue and cells. Finally, all patients involved are Asian from the Ningxia areas in China, which may fail to represent a global trend.

5 | CONCLUSION

Our data show that children with severe MPP have higher serum levels of P2X7 and lower vitamin A and 25(OH)D, suggesting severer inflammatory reactions and weakened immunity. Our data proved the correlations between serum levels of P2X7, vitamin A, and 25(OH)D and the severity of MPP. We also identified P2X7, vitamins A, 25(OH)D, TNF-α, IL-1β, IgA, and IgG as factors that may predict the severity of MPP. We proposed that serum P2X7, vitamin A, and 25(OH)D levels can be used as diagnostic markers to indicate the severity of MPP in children.

CONFLICT OF INTEREST

Authors, corporators, and sponsors have no potential conflict of interest.

AUTHOR CONTRIBUTION

Fanjun Meng and Ping Chen involved in conceptualization, methodology, data curation, and software. **Xiaoru Li and Yuexuan Wu involved in** methodology, formal analysis, **and** visualization. **Huan Liu and Feng Jiang involved in** writing original draft **and** visualization. **Xiaolong Guo and Wenen Liu involved in** writing—review **and** editing. **Lixin Wang involved in** conceptualization, supervision, and writing—review **and** editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Lixin Wang, upon reasonable request.

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