Association of *Mycoplasma pneumoniae* infection with increased risk of asthma in children

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Abstract. The present study was conducted to investigate the relationship between *Mycoplasma pneumoniae* (MP) infection and the risk of asthma among children by detecting the rate of MP immunoglobulin M (MP-IgM) and the eosinophil (EOS) count. A total of 139 asthmatic children were enrolled as the case group and assigned into three groups: Group A (aged <3 years, n=42), group B (aged 3-8 years, n=45) and group C (aged >8 years, n=52). Additionally, 115 healthy children were enrolled in the control group. Enzyme-linked immunosorbent assay was used to measure the MP-IgM-positive rate. EOS count was detected in the experimental and control groups by using a hemocytometer analyzer. A meta-analysis was performed by using the Comprehensive Meta-Analysis version 2.0 software. The positive rates of the MP-IgM and EOS count in the experimental group were significantly higher than those in control group (both P<0.001). Furthermore, the asthmatic children in group C had a higher MP-IgM-positive rate and EOS count as compared to those in groups A and B, respectively (all P<0.05). Results from groups A and B were not statistically significant (all P>0.05). The meta-analysis further confirmed that asthmatic children had a higher MP-IgM-positive rate as compared to the healthy controls (P<0.001). Age-stratified analysis revealed that the MP-IgM-positive rate in asthmatic children aged ≥8 and <8 years was significantly higher than that in the healthy controls (P=0.003 and P<0.001). Asthmatic children had a higher MP-IgM-positive rate and EOS count as compared with controls, suggesting that the MP infection may be closely associated with the risk of asthma. Additionally, the positive rate of MP-IgM may indicate an important biological marker in predicting the development of asthma.

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Introduction

Asthma is a common chronic inflammatory disease of the airways, and is characterized by reversible airflow obstruction, bronchospasm and various recurring symptoms, including wheezing, chest tightness, coughing and shortness of breath (1). Furthermore, asthma affects both adults and children, and may occur in all populations and locations across the globe (2). Statistics showed that approximately 300 million people suffered from asthma worldwide, and approximately 250,000 annual mortalities are attributed to asthma, most of which are preventable (3). It has been reported that the prevalence of asthma in the population aged 0-17 years is 9.5%, which is higher than that of adults aged 18 and over (7.7%) for the period 2008-2010 in the United States (4). Additionally, research has also shown that 11% of 12-year-old schoolchildren may suffer from severe asthma (4). Currently, there are no specific treatments for asthma and long-term standard treatment may reduce the recurrence of asthma, but evidence has also suggested that early intervention is essential for the patients with asthma (5-8). However, the diagnosis of asthma in children is difficult and easily misdiagnosed, which may result in under treatment of asthmatics or overtreatment of transient wheezes and lead to lung function impairment (9). Therefore, a correct and timely diagnosis of asthma in children is the first step towards effective management and treatment of asthma, which may reduce the potential harm due to misdiagnosis (10).

Previous findings showed that the presence of inflammation has become one of the most important markers in the diagnosis of asthma (11). Moreover, a variety of phenotypes of chronic asthma with persistent inflammation have been recognized, and a link between bacterial infections and refractory asthma has emerged (12,13). *Mycoplasma pneumoniae* (MP), an extracellular pathogen lacking a cell wall, is a common causative agent of pneumonia in children and young adults (14). Most MP infections are limited to respiratory tract, with 3-10% of patients developing clinical pneumonia; approximately 25% of patients with MP infections also suffer a variety of extra-pulmonary manifestations (15). Although the mechanism of MP pathogenesis remains to be elucidated, one of the known important components thereof is the induction of various cytokines and chemokines (16). Host immune factors may influence the outcome of infection, and previous findings showed that asthmatic children were lacking

in cellular and humoral responses to MP infection (17,18). MP may infect the upper and lower respiratory tract, causing pneumonia or bronchitis, and may be involved in the initiation and recurrence of asthma exacerbations (19,20). It has been suggested that MP infection leads to changes of a variety of serum immune parameters, such as MP immunoglobulin M (MP-IgM), MP immunoglobulin E (MP-IgE), interleukin-18, eosinophils (EOS) count and others (21-23). Prior evidence has also shown that MP may link to new-onset asthma, exacerbations of asthma, chronic worsening of asthma and long-term decrements in pulmonary function (24,25). However, defining a clear relationship between MP infection and asthma has been difficult, and the precise role and pathogenic mechanisms of MP in asthma risk are unclear.

In the current case-control investigation, we analyzed the serum-specific MP-IgM antibody levels and EOS count in asthmatic children in order to investigate the association between MP infection and the risk of asthma among children. Furthermore, we conducted a meta-analysis to test and verify the results of the case-control study.

Materials and methods

Ethics statement. The protocol of this study was carried out with the approval of the Institutional Review Board of Linyi People's Hospital (Shandong, China). Children were enrolled in this study after obtaining informed written consents from their parents. All the study procedures were in line with the Declaration of Helsinki (26).

Study participants. Between January 2013 and December 2014, a total of 139 children (87 males and 52 females; mean age, 6.65±2.28 years; range, 0-15 years) previously diagnosed with asthma were recruited from the Respiratory Care Department of Linyi People's Hospital and assigned into an experimental group. All the asthma patients were divided into three groups according to age: Group A (age <3 years, n=42); group B (age range, 3-8 years, n=45) and group C (age >8 years, n=52). In addition, 115 healthy children (68 males and 47 females; mean age, 6.39±2.34 years; range, 1-12 years), without any history of respiratory tract infection, from the Medical Center were enrolled as the control group. The diagnosis of asthma was based on physician assessment according to the national guidelines published by the Subspecialty Group of Respiratory Diseases Society of Pediatrics of Chinese Medical Association in 2008 (27). The disease course of asthma in the patients was 6-20 days. The inclusion criteria were the following: i) Patients with recurring wheezing, short breath, chest distress or cough, which were mostly related to allergen contact, cold air, physical or chemical stimulation, viral respiratory tract infections and exercise; ii) patients that had wheezing breath sounds and a prolonged or interrupted expiratory flow; and iii) patients that had a notable curative effect on inhaled bronchodilators. Patients were excluded if they had severe heart disease, severe pulmonary disease and kidney disease, or had a history of allergic bronchial asthma. The diagnostic criteria of MP infection include: MP-IgM antibodies were positive; MP immunoglobulin G (MP-IgG)-negative antibodies changed into positive or the IgG antibody titers were elevated >4-fold (28).

Sample collection and detection (serum MP-IgM expression detection and EOS counting). Venous blood (2 ml) was drawn in the morning after an overnight fast at 7th day of the disease course of asthma from all the children in the experimental and control groups. The collected samples were centrifuged at 1,500 x g for 15 min to separate the serum. Enzyme-linked immunosorbent assay (ELISA) was used to measure the positive rate of MP-IgM in children, using a Serodia Myco II kit (Fujirebio Inc., Tokyo, Japan). Serum IgM level (>750 U/ml) was considered to be a positive MP-IgM antibody level, suggesting MP infection. The EOS count was detected by a routine blood cell analyzer.

Treatments. Children with MP-IgM negative antibodies received β-lactam antibiotics (penicillin or cephalosporin). Patients with acute asthma were treated with bronchodilators or hormone therapy. Additionally to routine therapy, children with MP-IgM-positive antibodies received sequential therapy including 10 mg/kg/day azithromycin. Patients took azithromycin continuously for 3-5 days until the body temperature dropped. After the clinical symptoms and signs were largely dissipated, patients were treated with an oral administration of 10 mg/kg/day azithromycin continuously for 3 days a week, over a course of 3 weeks. Blood and urine routine examinations, as well as liver function test were performed during the administration. When any unusual symptoms appeared, the medicine causing the effect was stopped.

Serum parameters of MP infection. The positive rate of MP-IgM and EOS count in the experimental and control groups were recorded. The comparison on the differences of MP-IgM-positive rate and EOS count between two groups was performed. The comparison on the differences of MP-IgM-positive rate and EOS count of asthma patients among the different age groups was performed.

Statistical analysis. Categorical data are expressed as numbers and percentages, and were analyzed using χ^2 test. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD). The t-test was used to measure the differences between two groups of continuous variables. P<0.05 was considered to indicate a statistically significant difference.

A comprehensive search was conducted for relevant studies published in PubMed (1966-2014), Cochrane Library (2005-2014), Wanfang database (1990-2014), Google Scholar and Chinese National Knowledge Infrastructure (1990-2014) databases using the following search terms: ('*Mycoplasma pneumoniae*' or 'MP') and ('asthma' or 'bronchial asthma') and ('child' or 'children'). The references of the eligible articles or textbooks were also reviewed to check through manual searches to identify other potential studies. Data from published studies were extracted independently by two authors to provide the necessary information. Any disagreement was resolved by discussion between the authors. A fixed or random effect model was used to measure the odds ratios (ORs) and its 95% confidence intervals (CIs) to evaluate the associations between MP infection and asthma. The significance of the pooled estimate was made using the Z-test (29). Cochran's Q-statistic was applied to estimate the heterogeneity

Table I. The demographic characteristics of the children with asthma and healthy controls.

Groups	Case group (n=139)	Control group (n=115)	P-value
Gender (M/F)	87/52	68/47	0.574
Age (years)	6.65±2.28	6.39±2.34	0.372
BMI (kg/cm^2)	19.8±2.1	20.2±2.5	0.167
ETS, n (%)	91 (65.5%)	66 (57.4%)	0.187
Family history of asthma, n (%)	20 (14.4%)	8 (7.0%)	0.060
Urban family, n (%)	85 (61.2%)	62 (53.9%)	0.245
Exposure to dust environment, n (%)	33 (23.7%)	24 (20.9%)	0.418

M, male; F, female; BMI, body mass index; ETS, environmental tobacco smoke.

among studies, and P<0.05 was considered to be statistically significant (30). The I^2 test was also used to quantify the heterogeneity (range from 0 to 100%) (31). Random-effect model (DerSimonian-Laird method) was used when P<0.05 or I^2 >50%. Fixed-effects model (Mantel-Haenszel method) was used when there was no statistical heterogeneity (32). Data for the meta-analysis were analyzed by the Comprehensive Meta-Analysis version 2.0 software (Biostat Inc., Englewood, NJ, USA). Begg's funnel plot and Egger's test were performed to assess the publication bias of articles in all comparison models.

Results

Baseline characteristics of study population. Table I shows the baseline characteristics of subjects in the experimental and control groups. No significant difference was observed in gender, age, body mass index (BMI), and exposure to environmental tobacco smoke (ETS) between children with asthma and healthy controls (all P>0.05). Children with asthma had a higher rate of family history of asthma when compared with the control group, but with no significant difference (14.4 vs. 7.0%, P=0.060). Furthermore, the rates of children in urban families and the rate of exposure to dust in the experimental group were higher than those in the control group, but with no significant difference (urban family: 61.2 vs. 53.9%, P=0.245; exposure to dust: 23.7 vs. 20.9%, P=0.418).

Positive rate of serum IgM antibodies. Of all subjects, 54.7% (76/139) of asthma patients and 15.7% (18/115) of the control group were found to be positive for MP-IgM antibodies. The positive rate of MP-IgM in the experimental group was significantly higher than that in the control group (54.7 vs. 15.7%, χ^2 41.11, P<0.001) (Fig. 1). ELISA results showed that 17 (40.5%) patients in group A, 22 (48.9%) patients in group B and 37 (71.2%) patients in group C were detected to be MP-IgM antibody-positive. There was no significant difference in the positive rate of MP-IgM between groups A and B (40.5 vs. 48.9%, χ^2 0.62, P=0.430). However, the positive rates of MP-IgM in groups A and B were significantly lower
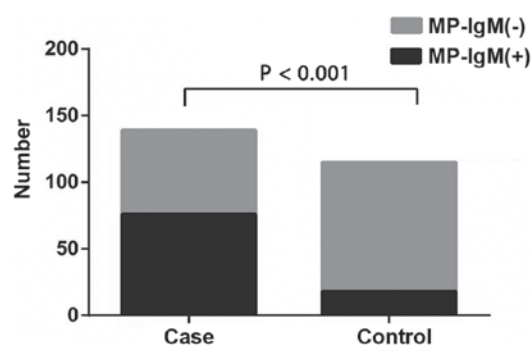

Figure 1. The positive rates of MP-IgM in asthmatic children (case group) and healthy controls (control group). The MP-IgM-positive rate in asthmatic children was significantly higher than that in the healthy controls (P<0.001). MP-IgM, *Mycoplasma pneumoniae* immunoglobulin M.
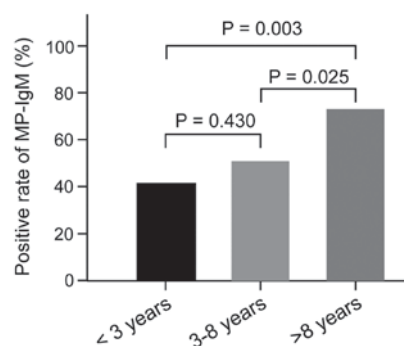

Figure 2. The positive rates of MP-IgM of asthmatic children in different age groups. The asthmatic children aged more than 8 years had a higher positive rate of MP-IgM than the asthmatic children aged <3 years, and the asthmatic children aged 3-8 years, respectively. MP-IgM, *Mycoplasma pneumoniae* immunoglobulin M.
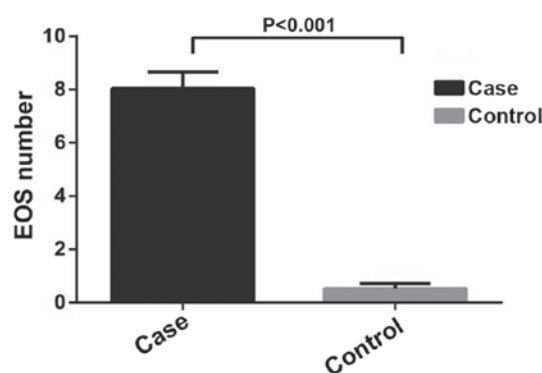
Figure 3. The EOS count in asthmatic children (case group) and healthy controls (control group). The EOS count in asthmatic children was significantly higher than that in the healthy controls (P<0.001). EOS, eosinophils.

than those in group C, respectively (group A vs. group C: 40.5 vs. 71.2%, χ^2=8.95, P=0.003; group B vs. group C: 48.9 vs. 71.2%, χ^2=5.02, P=0.025) (Fig. 2).

Association of EOS count with asthma risk. The results showed that the blood EOS count in asthma patients was markedly higher than those in healthy controls (8.04±0.62 10^9/l vs. 0.52±0.20 10^9/l, t=124.8, P<0.001) (Fig. 3). According to the different age groups of asthma patients, we found that the

Figure 4. The EOS count of asthmatic children in different age groups. The asthmatic children aged more than 8 years had an increased EOS count as compared to that in the asthmatic children aged <3 years and the asthmatic children aged 3-8 years, respectively. EOS, eosinophils. P<0.05.
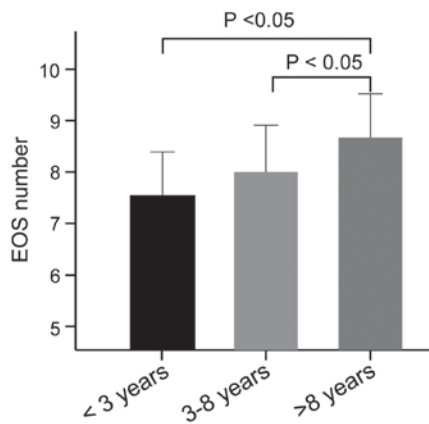

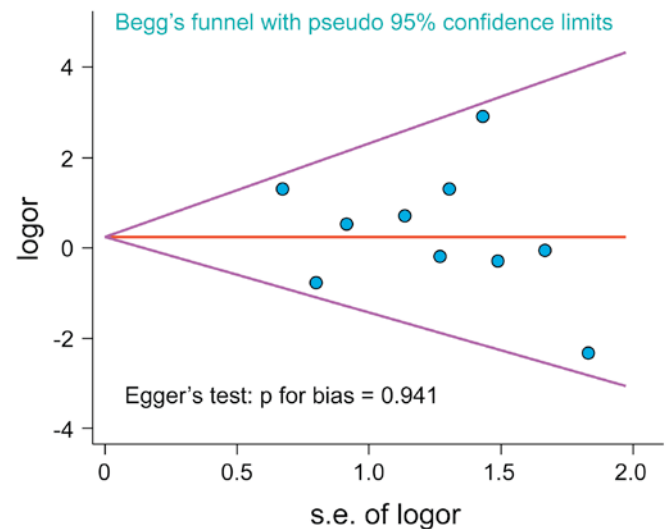Figure 5. Flow diagram of the studies identified.

Figure 6. Publication bias were evaluated by Begg's funnel plot and Egger's test. The shape of the funnel plots were symmetrical and P-value for Egger's test was 0.941, suggesting that there was no evidence of publication bias among these 10 included studies.
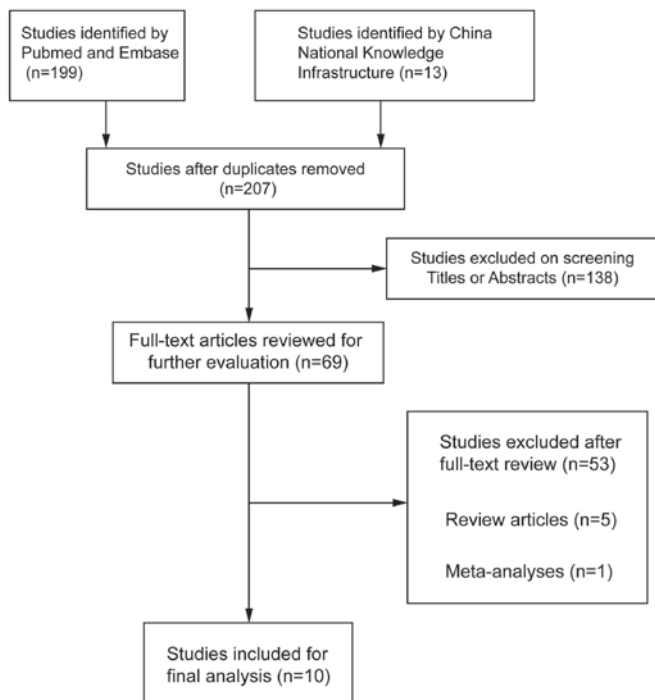
blood EOS count in groups A, B and C was (7.56±1.62) 10^9/l, (8.01±1.32) 10^9/l and (8.65±1.09) 10^9/l, respectively. No significant difference in the blood EOS count was observed between groups A and B (t=1.43, P=0.158). Nevertheless, the blood EOS count in groups A and B were significantly lower than that in group C, respectively (group A vs. C: t=3.89, P<0.05; group B vs. C: t=2.62, P<0.05) (Fig. 4).

Results of meta-analysis. A total of 207 articles associated with the searched keywords were identified. Of these studies, 138 were excluded based on the titles and abstracts; another 59 studies were eliminated after reviewing the full text. Eventually, 10 studies met our inclusion criteria for this meta-analysis (25,33-41): H.-E. Zeng-Hong, Correlation Between Mycoplasma Pnenumonia Infection and Pediatric Asthma, 2011; Jiang Hua, *Mycoplasma pneumoniae* Infection and Bronchial Asthma in Children, 2013; Zhao Zhi-Yun, Observation on Infection Results and Correlation Between Childhood Asthma and *Mycoplasma pneumoniae*, 2014; Liu Chunxiang, Association of *Mycoplasma pneumoniae* Infection and Pediatric Asthma, 2009) (Fig. 5). Egger's test and Begg's funnel plot were used to statistically assess the funnel plot symmetry. The results suggested no evidence of publication bias for the included 10 studies (Fig. 6). These 10 case-control studies were composed of 795 asthma patients and 630 healthy controls. Publication year of articles included ranged from 2000 to 2014. There was significant heterogeneity observed (I^2=63.95%, P_h<0.05); thereby the random effects model was carried out. Meta-analysis results showed that the MP-IgM-positive rate in asthma patients were markedly higher than that of healthy controls (OR=3.496, 95% CI=2.060-5.933, P<0.001) (Fig. 7). Age-stratified analysis presented that the MP-IgM-positive rate in both children ≥8 and <8 years were significantly higher than that of healthy controls (average age, ≥8 years: OR=3.074, 95% CI=1.455-6.494, P=0.003; average age, <8 years: OR=4.762, 95% CI=2.570-8.822, P<0.001) (Fig. 8).

Discussion

MP is a highly specialized bacterium that functions as an obligate parasite in the respiratory tract of humans, which may be involved in establishing a chronic infection (36). Furthermore, research has shown that MP infects the upper and lower respiratory tract which may be associated with the occurrence of bronchitis and asthma; however, the underlying mechanism is not completely understood (42,43). In this regard, we performed a case-control study and a meta-analysis to explore the correlation of MP infection with the increased risk of asthma. This study found that MP-IgM-positive rate was higher in asthmatic children when compared with healthy controls, indicating that elevated MP-IgM-positive rate may
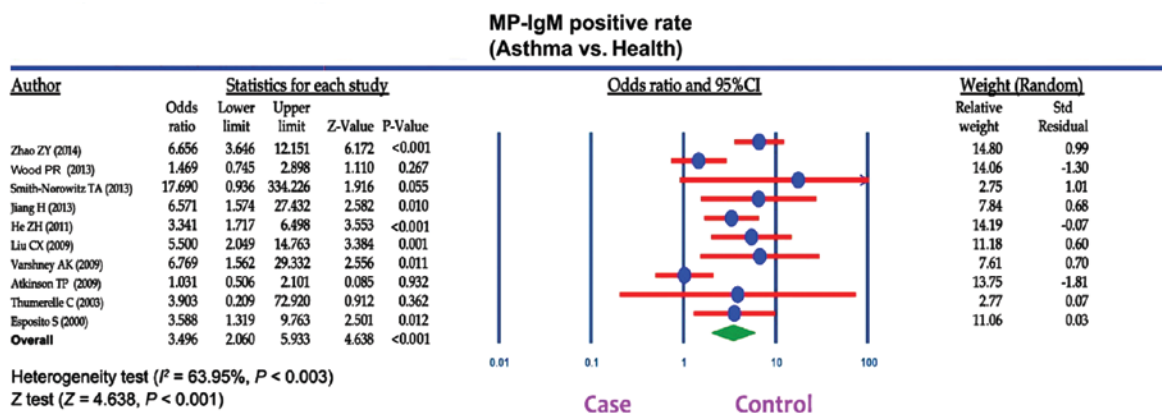
MP-IgM positive rate
(Asthma vs. Health)

Author	Odds ratio	Lower limit	Upper limit	Z-Value	P-Value	Odds ratio and 95%CI	Relative weight	Std Residual
Zhao ZY (2014)	6.656	3.646	12.151	6.172	<0.001		14.80	0.99
Wood PR (2013)	1.469	0.745	2.898	1.110	0.267		14.06	-1.30
Smith-Norowitz TA (2013)	17.690	0.936	334.226	1.916	0.055		2.75	1.01
Jiang H (2013)	6.571	1.574	27.432	2.582	0.010		7.84	0.68
He ZH (2011)	3.341	1.717	6.498	3.553	<0.001		14.19	-0.07
Liu CX (2009)	5.500	2.049	14.763	3.384	0.001		11.18	0.60
Varshney AK (2009)	6.769	1.562	29.332	2.556	0.011		7.61	0.70
Atkinson TP (2009)	1.031	0.506	2.101	0.085	0.932		13.75	-1.81
Thumerelle C (2003)	3.903	0.209	72.920	0.912	0.362		2.77	0.07
Esposito S (2000)	3.588	1.319	9.763	2.501	0.012		11.06	0.03
Overall	3.496	2.060	5.933	4.638	<0.001			

Heterogeneity test (*I²* = 63.95%, *P* < 0.003)
Z test (Z = 4.638, *P* < 0.001)

Case   Control

Figure 7. Forest plots of relationship of *Mycoplasma pneumoniae* immunoglobulin M (MP-IgM)-positive rate with the risk of asthma among children.

MP-IgM positive rate
(Ages: Asthma vs. Health)

Group by Age	Author	Odds ratio	Lower limit	Upper limit	Z-Value	P-Value	Odds ratio and 95%CI	Relative weight	Std Residual
<8 years	04 Jiang H (2013)	6.571	1.574	27.432	2.582	0.010		18.62	0.49
<8 years	06 Liu CX (2009)	5.500	2.049	14.763	3.384	0.001		39.00	0.37
<8 years	09 Thumerelle C (2003)	3.903	0.209	72.920	0.912	0.362		4.44	-0.14
<8 years	10 Esposito S (2000)	3.588	1.319	9.763	2.501	0.012		37.94	-0.70
<8 years		4.762	2.570	8.822	4.960	<0.001			
≥8 yeays	01 Zhao ZY (2014)	6.656	3.646	12.151	6.172	0.000		21.18	1.05
≥8 yeays	02 Wood PR (2013)	1.469	0.745	2.898	1.110	0.267		20.42	-0.98
≥8 yeays	03 Smith-Norowitz TA (2013)	17.690	0.936	334.226	1.916	0.055		5.12	1.07
≥8 yeays	05 He ZH (2011)	3.341	1.717	6.498	3.553	<0.001		20.56	0.11
≥8 yeays	07 Varshney AK (2009)	6.769	1.562	29.332	2.556	0.011		12.63	0.79
≥8 yeays	08 Atkinson TP (2009)	1.031	0.506	2.101	0.085	0.932		20.09	-1.44
≥8 yeays	**Overall**	3.074	1.455	6.494	2.943	0.003			

<8 years: Z test (Z = 4.960, *P* < 0.001)
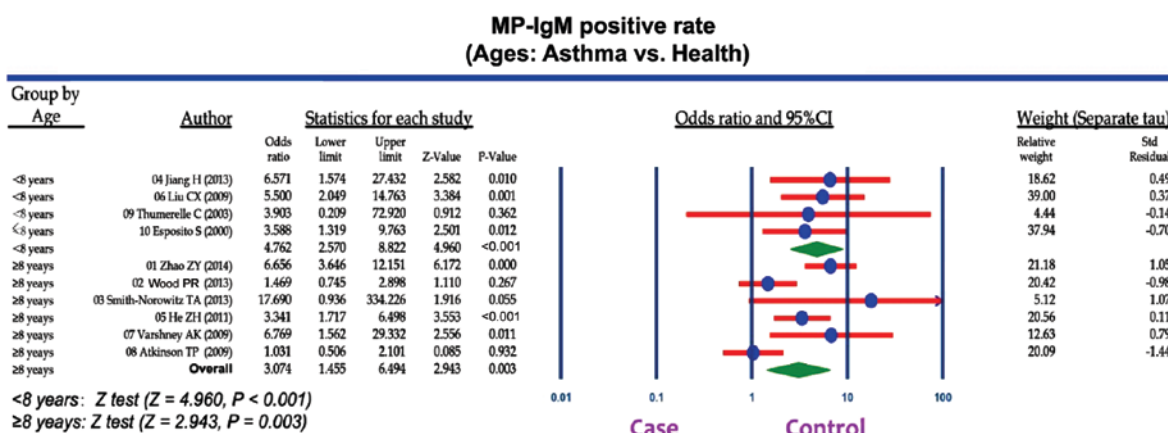≥8 yeays: Z test (Z = 2.943, *P* = 0.003)

Case   Control

Figure 8. Stratified analysis for the difference of *Mycoplasma pneumoniae* immunoglobulin M (MP-IgM)-positive rate between the asthmatic children (case group) and the healthy controls (control group).

be involved in the development of asthma among children. Generally, the elevated MP-IgM-positive rate can be used as an early diagnostic criterion of the initial acute MP infection (44). As a specific antigen, MP can induce immediate or delayed hypersensitivity reaction, and may result in allergic airway inflammation and induce the production of IgE and IgM, which may mediate type 1 allergic reaction. Therefore, MP may lead to the development of asthma (45). Additionally, MP infection is associated with significant specific IgM responses in addition to specific IgG and IgE responses; thus, the production of specific MP-IgM may have a role in the exacerbation of asthma (17,46). High positive levels of MP-IgM antibodies were found in patients who were infected with MP during asthma exacerbation (37). We suspected that the possible explanations of how MP affects the airway included the production of specific MP-IgM antibodies in addition to direct effects on airway epithelium, inflammatory reaction on airways and alteration in the autonomic nervous system (36). Smith-Norowitz *et al* found that the IgM-positivity was apparently higher in asthmatic patients as compared to the healthy controls, and suggested that the increased specific MP-IgM responses may play an important role in MP infection in asthma development (35). Previous report by Varshney *et al* highlighted that MP may play a crucial role in the onset of asthma in predisposed children and be a trigger for recurrent

wheezing (37). Consistent with previous studies, our study also demonstrated that the MP infection accompanied with higher MP-IgM-positive rate may be involved in the development of asthma. Additionally, we conducted a meta-analysis to confirm the associations and found that the asthmatic children had a higher MP-IgM-positive rate, indicating that a higher MP-IgM-positive rate may be associated with an increased susceptibility to asthma.

Another important finding in our study revealed that the levels of EOS in asthmatic children were significantly higher than those in the non-asthmatic controls. This leads us to believe that patients with asthma may suffer from allergy inflammation and the elevated EOS count may be crucial in the pathogenesis of asthma. EOS is thought to have a key pro-inflammatory role in the pathogenesis of asthma as its mediators are identified in asthmatic, but not healthy lungs. The suppression of eosinophil infiltration in various clinical diseases by glucocorticoids may be correlated with an amelioration of symptoms and disordered airway function (47). Characterized as an eosinophilic airway inflammation, asthma is often considered to be associated with a higher peripheral blood EOS count, and the EOS may play an important role in the pathogenesis of bronchial asthma (48,49). Additionally, airway hyper-responsiveness has been reported to be closely correlated with the presence of EOS (50,51). Early studies found that the respiratory tract infections with a

higher EOS count had a higher risk of asthma when compared with the respiratory tract infectors with normal EOS count, suggesting that the EOS count is an important clinical index in predicting the development of asthma (52,53). Furthermore, we found that the asthmatic children aged >8 years had higher MP-IgM-positive rate and EOS count as compared to the asthmatic children aged <3 years and asthmatic children aged 3-8 years. We suspected that these differences may be correlated with the clinically significant alterations in lung function. However, the underlying mechanism involved remains unclear; therefore, further studies based on the involvement of mechanistic pathways in asthma in children of different ages should be conducted.

In conclusion, the present study suggests that asthmatic children have a higher MP-IgM-positive rate and EOS count when compared with non-asthmatic controls, indicating that MP infection may promote the production of IgM in asthmatic children. Deficient host defense mechanisms in children with asthma may contribute to a higher prevalence of MP-IgM, which likely reflects an increased susceptibility to asthma. However, with our own limitations, such as small sample size and the inclusion and exclusion criterion of the meta-analysis may affect the statistical power of this study. Future large-scale prospective studies are needed to confirm the correlations and predictive value of MP infection with asthma risk.

Acknowledgements

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