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Epidemiological relationship between *Mycoplasma pneumoniae* Pneumonia and recurrent wheezing episode in children

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ABSTRACT

Objective: The relationship between Mycoplasma pneumoniae (MP) infection and initiation or exacerbation of childhood asthma remains controversial. This study was aimed to evaluate epidemiological and clinical relationship between MP infection and childhood recurrent wheezing episode (RWE).

Design: Retrospective case note review.

Setting: Paediatric department at a single Korean institution.

Participants: Consecutive admitted patients with MP pneumonia and RWE (0-15 years of age) between 2003 and 2014.

Methods: The retrospective medical records of patients with MP pneumonia (n=793 for epidemiological analysis and n=501 for clinical analysis) and those with RWE (n=384) from 2003 to 2014 were analyzed.

Results: During the study period, there were three MP pneumonia epidemics, but no corresponding increase of patients with RWE were seen in the epidemic years. In the 501 MP pneumonia patients, 52 (10.4%) had wheezing at presentation, 72 (14.4%) had allergic disease history, and 15 (3%) had RWE. The MP pneumonia patients with wheezing at presentation (n=52) were younger (3.7 years vs. 5.6 years, P<.001) and were more likely to have an allergic disease history than were those without wheezing (n=449). Among the patients with wheezing at presentation, 10 patients had previously RWE history. In a follow-up study, 13 patients (including 5 RWE) with initial wheezing and 25 patients (including 2 RWE) without wheezing had wheezy episodes after discharge. Among the total 501 patients, it was estimated that at least 31 MP pneumonia patients (6.2%) showed recurrent wheezing after initial MP infection.

Conclusions: A small part of children with MP pneumonia showed recurrent wheezing after MP pneumonia, and patients with RWE had a greater likelihood of experiencing wheezing when they had an initial MP. However, there were no increased admitted patients with RWE

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in MP pneumonia epidemic periods because of rarity of MP reinfection in children including patients with RWE or asthma.

Key words: asthma, children, epidemiology, *Mycoplasma pneumoniae,* pneumonia, recurrent wheezing

Strengths and limitations of this study

- This may be the first long-term epidemiological study regarding relationship between *Mycoplasma pneumoniae* (MP) infection and recurrent wheezing episode (RWE).
- Two-times examination for diagnosis of MP pneumonia strengthened accuracy for patient selection.
- During the study period, there were no significant changes in the diagnosis and treatment policies in our department.
- We discussed unsolved issues regarding MP infection and childhood asthma.
- There were limitations in this study in regards to the following: retrospective analysis, data collected from a single centre, and patient selection under our definition of recurrent wheezing episode (childhood asthma).

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INTRODUCTION

Mycoplasma pneumoniae (MP) is one of the major pathogens in respiratory tract infections in children and young adults, manifesting from asymptomatic infection to potential fatal pneumonia. MP pneumonia is an endemic disease in large communities, but it occurs in regional cyclic epidemics of 3-7 year intervals. Also, MP is associated with a wide spectrum of other organ-specific diseases including neurologic, dermatologic, hepatic, cardiac, musculoskeletal, hematologic diseases and possibly bronchial asthma.[1,2]

Childhood asthma is a complex disease process that can be classified into heterogeneous clinical phenotypes.[3] The diverse phenotypes of childhood asthma may be associated with host factors, including the developing immune and respiratory systems and individual genetic variations. Childhood asthma is also influenced by environmental factors such as socio-economic and/or cultural differences across populations.[3,4] Therefore, diagnosis of asthma in children, especially in children under 5 years of age, could not be defined definitely, and the phenotypes of childhood asthma, such as the prevalence of asthma and frequency of severe asthma, could differ among populations.[5,6] It has been suggested that initiation of asthma and acute exacerbation of asthma are associated with a variety of respiratory pathogens, including respiratory syncytial viruses (RSV), rhinoviruses and MP, although the exact reasons are still not fully understood.[7-9] In addition, it has been suggested that chronic MP infection is related to persistent bronchial hyper-responsiveness, chronic inflammation, and acute exacerbation in adult and child asthmatics.[10-13]

In Korea, MP pneumonia epidemics have been observed every 3 to 4 years from the mid-1980s to present. We recently found that during 2003-2012, there were three nationwide MP pneumonia epidemics, all of which had similar epidemiological patterns including age distribution and seasonal pattern.[14] In this study, we evaluated the epidemiological and clinical characteristics of patients with MP pneumonia and those with recurrent wheezing

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episode (RWE) admitted during the 2003-2014 period, and we compared the data in both diseases. Also, we discussed the relationship between MP infection and childhood asthma.

METHODS

Study design and setting

This study was a retrospective study conducted at Department of Paediatrics, The Catholic University of Korea Daejeon St. Mary's Hospital, a secondary referral hospital performing primary care for patients with MP pneumonia or RWE.

Patients and data collection

We performed a retrospective analysis of medical records of patients with MP pneumonia or RWE who were admitted to The Catholic University of Korea Daejeon St. Mary's Hospital from January 2003 to December 2014. In this study, diagnosis of MP pneumonia was based on chest radiographic findings and 2-times titration of IgM antibody (Serodia-Myco II, Fujirebio Inc., Tokyo, Japan; positive cutoff value ≥1:40) at presentation and before discharge, as previously described.[14,15] The subjects with RWE were collected through the diagnostic code number J459 (asthma, asthma nature, bronchial asthma, and infantile asthma). However, we used the term RWE in this study, because majority of patients were under 5 years of age and some patients over 6 years of age did not perform confirmative study for asthma such as pulmonary function test. An RWE patient was defined as one with expiratory wheezing at admission with at least one or more wheezing episodes within 6 months before admission, regardless of patient's age, and 37 cases of <2 years of age were included in the present study. Patients with first wheezing at admission for any respiratory tract infection, including MP pneumonia, were excluded from the RWE group. Readmitted patients with RWE in the same year or different year were included in this series (35 episodes). Clinical

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and laboratory parameters were evaluated and compared between the groups. After discharge, wheezing episodes were evaluated through the medical records in both patient groups who revisited the outpatient clinic or had readmission at least two or more times from the date of discharge to December 2014.

Statistical analysis

All calculations were performed with SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using the Student's t-test, chi-square test, and Fisher's exact test, and the data were expressed as mean ± standard deviation (SD) for continuous variables. A P value of <0.05 was considered statistically significant.

RESULTS

The subjects in the present study were 793 patients with MP pneumonia and 384 patients with RWE during the study period. For epidemiological comparison, a total 793 patients were used, as shown in previously published article.[14] For clinical comparison between the groups, a total of 501 MP pneumonia patients that were admitted during the recent epidemics (241 cases in 2007-2006, and 260 cases in 2011), and 384 patients with RWE were analyzed (Fig. 1). Most of MP pneumonia patients had follow-up period at least 3 years from last epidemic year of 2011, but the follow-up subjects satisfied with our selection criteria were 180 among 501 cases in the MP pneumonia group and 206 among 384 cases in the RWE group.

Epidemiological data of MP pneumonia and RWE from 2003 to 2014

In MP pneumonia patients, there were clear outbreaks of cases in 2003, 2006-2007, and 2011, with a few cases occurring in the inter-epidemic periods. There were relatively steady

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numbers of RWE cases every year during the study period. There were no corresponding increased cases in MP pneumonia epidemic years except in 2007 (Fig. 2). No MP pneumonia patient who was re-admitted due to MP reinfection across the epidemics was observed during study period. The mean age and age distribution were similar, and the peak age groups were 2-5 years of age in both groups (Fig. 3). In seasonality of both groups, MP pneumonia was prevalent in decreasing order of autumn, summer, winter, and spring, while patients with RWE were most prevalent in autumn, followed by spring, winter, and summer (Fig. 4).

Demographic and clinical characteristics of MP pneumonia and RWE groups

Demographic and clinical data in both groups are shown in Table 1. The mean age and age distribution were similar in both groups as shown in Fig. 2 (5.4 years vs. 5.6 years, $P = 0.312$). Both groups had male predominance, and there were more males in the RWE group. As expected, cases with wheezing at presentation, the proportion of patients with past history or family history of allergic diseases, and past history of wheezing were higher in the RWE group. Recurrent wheezing after discharge was observed in 38 of 180 patients with MP pneumonia and 141 of the 206 patients with RWE ($P < 0.001$). In laboratory indices, WBC count, eosinophil differential, IgG, and IgE were higher in the RWE group (Table 1).

In MP pneumonia patients, 52 of the total 501 patients (10.4%) had wheezing at presentation, 72 (14.4%) had a past history of allergic diseases, 15 (3%) had RWE according to our definition, and 40 (8%) had a family history of allergic diseases. When we divided the MP pneumonia patients into two groups of patients with wheezing (n=52) and without wheezing (n=449), the patients with wheezing were younger (3.7 years vs. 5.6 years, $P < 0.001$) and had higher proportions of past history allergic diseases and wheezing history and higher values of leukocyte count and eosinophil differential (Table 2). Among the 52 MP pneumonia patients with wheezing at admission, 10 were patient with RWE, while 42 had no

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previous RWE history. Thus, in this study, 66% (10 of 15) of patients with RWE history presented with wheezing, while 8.6% of patients without RWE history (42 of 486) were wheezing at presentation. In a follow-up study of patients who visited two or more times after discharge, 13 (including 5 cases of RWE) of 21 MP pneumonia patients with initial wheezing and 25 (including 2 cases of RWE) of 159 MP pneumonia patients without wheezing had at least one wheezing episode after discharge (Table 2). Although not all patients were followed-up, it was estimated that at least 31 of the total MP pneumonia patients (n=501, 6.2%) showed a recurrent wheezing after initial MP infection.

DISCUSSION

In this study, we found that patients with MP pneumonia and patients with RWE had a similar mean age and age distribution during the past decade. However, the annual number of cases in the two groups was quite different; there were relatively stable number of annual cases in patients with RWE, without a corresponding increase in cases during or after MP pneumonia epidemic years. Although reinfection with MP reported to be not uncommon in other studies based on polymerase chain reaction (PCR) or single serologic test-based studies,[16] we found that there were no serologically confirmed reinfected MP pneumonia patients among 793 patients during study period, except a few readmitted patients with complications of initial MP infection in each epidemic. It suggests that reattack of MP pneumonia in children is extremely rare, and once infected children seem to have immunity to other MP strains in future epidemics including macrolide-resistant MP strains.[14] During a MP epidemic, majority of susceptible children are asymptomatic or mild symptoms such as fever and sore throat, and only a small part of MP infected patients may affect pneumonia.[1,2] Moreover, since MP reinfection is also rare in asthmatics of all age, our epidemiological data

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might not be associated with the situations where asthmatics have reasons for increase of admission such as exacerbation or initiation of RWE or asthma.

MP pneumonia patients in this study showed a similar prevalence of allergic diseases compared to data from previous nationwide questionnaire-based studies in Korea: 3% (15/501) and 14.4% (72/501) of MP pneumonia patients had a past history of RWE (asthma) and a past history of allergic diseases, respectively. Whereas 5.8% of 6- to 7-year-old school children had asthma history in 2000, and 13.5% of children reported a diagnosis of atopic dermatitis in 2008-2011 in Korea.[17,18] In this study, among 52 MP pneumonia patients with initial wheezing, 10 were patients with RWE, while 42 had no previous wheezing history. Therefore, 66.6% (10 of 15) of patients with RWE, and 8.6% (42 of 486) of patients without RWE history were wheezing, and it suggests that patients with RWE or asthma with first-time MP infection might be prone to a wheezing episode as well as other respiratory virus infections as compounding factors of asthma exacerbation. Also, we found that after MP infection, at least 6% of MP pneumonia patients showed subsequent wheezing during at least over 3 years after initial MP infection. A large proportion of patients who experienced lower respiratory infections caused by RSV and rhinoviruses were reported to show recurrent wheezing after initial infection, especially when they were infected with other respiratory agents.[19,20] A study reported that approximately 50% of children affected with severe RSV bronchiolitis had a subsequent asthma diagnosis.[19] In Korea, the prevalent age in these respiratory viral infections are younger than that in MP infection, and it is possible that majority of MP infected patients experience RSV infection prior to MP infection as well as in other populations. On the other hand, some patients with severe MP pneumonia had prolonged anatomical abnormalities, suggestive of small airway obstruction,[21] and some patients had reduced pulmonary function after MP infection.[8] Thus, patients with severe lung

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injury caused by any respiratory pathogens may be prone to recurrent wheezing and subsequent diagnosis of asthma.

We found that WBC count, eosinophil differential, IgG, and IgE were higher in the RWE group than those in the MP pneumonia group which had similar mean age. It could in part be explained that different activation of immune system of the host against the insults from RWE which induces chronic or repeated inflammation may be reflected in these laboratory findings.

Previous studies have reported that asthma patients are more likely to be infected or colonized with MP compared to healthy controls, and MP infection in chronic asthmatics might be associated with persistent bronchial hyper-responsiveness and exacerbation.[10-13] MP pneumonia epidemics are cyclic in 3-4-year intervals, and the duration of an epidemic is generally limited to 1 year or occasionally 2 years, and during the inter-epidemic period of the 2-3 years there are a few patients as observed in this study.[14] There are many asymptomatic healthy MP carriers during the epidemic periods, especially young children, who may serve as reservoirs for the spread of MP infection.[22-24] It is natural that colonization of MP during MP pneumonia epidemics can occur in asthma patients and old persons as well as in healthy young children and adults since colonization does not mean the 'infection'. The prevalent rates of MP colonization between the asthma group and the control groups could be influenced by the MP epidemic year during the study period. Thus, patient selection bias could be considered if a study period is long and different in the both groups. Wood et al. reported that the rates of MP colonization in asthmatics and healthy children during the same study period were similar using a sensitive PCR method,[13] suggesting that colonization occurs equally in the two groups. One study reported that the status of asthma control and the lung function tests were not different between asthmatic patients with chronic MP infection and those without, indicating the similar severity of asthma between the group.[25]

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In this study, the numbers of patient with RWE during a recent decade did not change with a rather decreasing trend in recent years. Moreover, we have experienced that asthma severity might change to a milder phenotype in Korea over time. A recent population-based study in Korea also indicated that the prevalence of asthma was reduced in recent years.[26] Changing epidemiology in infectious disease and infection-related immune-mediated diseases, including scarlet fever, pandemic influenza, acute rheumatic fever, acute post-streptococcal glomerulonephritis, and Kawasaki disease, has been well documented in Korea; initially severe disease phenotypes have changed to milder phenotypes over time.[27-30] Because allergic diseases, including asthma, may be associated with various pathogen infections and environmental factors,[31,32] it is possible that the changing epidemiology of childhood asthma occurs over time in the populations.

There are some limitations to this retrospective study. We evaluated only admitted patient groups with a part of the follow-up subjects at a single hospital. History of allergic diseases was not as precise as in International Study of Asthma and Allergies in Childhood (ISAAC) studies. In our definition of RWE in the present study, the pneumonia patients with first wheezing, regardless of severity of respiratory symptoms, were not included in the asthma group. Considering the age distribution of patients, larger part of patients might be the transient recurrent wheezers and small part of true asthma patients who experience and progress recurrent wheezing into adulthood might be included in the RWE group. Further multicenter studies with prospective designs are needed for exact epidemiological relationship between MP infection and childhood asthma.

CONCLUSIONS

A small part of MP pneumonia patients had a subsequent wheezing after initial MP infection as well as those observed in other respiratory pathogen infections. Patients with RWE have a

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greater likelihood of experiencing wheezing than non-asthmatics when they had an initial MP infection, suggesting that MP infection is one of exacerbation factors in childhood asthma. However, we found no corresponding increase in the number of patients with RWE in MP pneumonia epidemic periods, and this finding may in part be explained that MP reinfection are very rare in children including patients with RWE or asthma as shown in this study.

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Contributors

KYL designed and conceptualized and the study, and drafted the manuscript. JUR participated in preliminary data collection, and wrote the initial manuscript. HMK and EAY analyzed data and revised the manuscript for critical content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Competing interests

None declared.

Ethic approval

The study was approved by the Institutional Review Board of The Catholic University of Korea Daejeon St. Mary's Hospital (DC17RESI0053).

Data sharing statement

Data of the study are available from the corresponding author (KYL).

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Table 1. Clinical and laboratory findings of the patients with MP pneumonia or recurrent wheezing episode (RWE)

	MP pneumonia (n = 501)	RWE (n = 384)	*P*
Clinical characteristics			
Age (y)	5.4 ± 3.4	5.6 ± 3.5	0.31
Male : female	255/246	156/128	<.001
Wheeze at presentation, n (%)	52 (10.4)	384 (100)	<.001
Past history of allergy*, n (%)	72 (14.4)	379 (98.7)	<.001
Past history of wheeze, n (%)	15 (3.0)	384 (100)	<.001
Family history of allergy, n (%)	40 (8.0)	134 (34.9)	<.001
Wheeze after discharge, n (%)	38/180 (21.1)	141/206 (68.4)	<.001
Laboratory findings			
Leukocyte (/μL)	8800 ± 4500	11100 ± 4200	<.001
Eosinophil (%)	2.2 ± 2.9	3.8 ± 3.9	<.001
IgG (mg/dL)	899 ± 235	946 ± 232	<.001
IgE (IU/mL)	267 ± 391	617 ± 673	<.001

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Table 2. Clinical and laboratory findings of MP pneumonia patients with wheezing and without wheezing

	Wheezing (n = 52)	No Wheezing (n = 449)	*P*
Clinical characteristics			
Age (y)	3.7 ± 3.0	5.6 ± 3.4	<.001
Male : female	32/20	223/226	0.11
Past history of allergy*, n (%)	16 (30.8)	56 (12.5)	0.01
Past history of wheeze, n (%)	10 (19.2)	5 (1.1)	<.001
Family history of allergy, n (%)	8 (15.4)	32 (7.1)	0.05
Wheeze after discharge, n (%)	13/21 (61.9)	25/159 (15.7)	<.001
Laboratory findings			
Leukocyte (x10^3//μL)	10400 ± 5400	8700 ± 4.3	0.03
Eosinophil (%)	3.0 ± 3.2	2.1 ± 2.9	0.08
IgG	873 ± 219	903 ± 238	0.43
IgE	407 ± 609	249 ± 350	0.11

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Figure legends

Figure 1. Flow diagram of the patient selection in this study

Figure 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

Figure 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

Figure 4. Seasonality in MP pneumonia and recurrent wheezing episode groups

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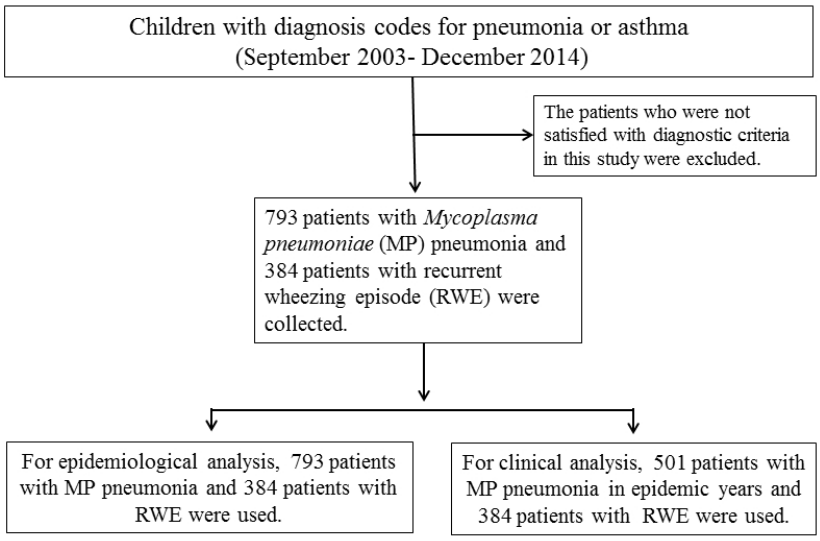

Fig. 1. Flow diagram of the patient selection in this study

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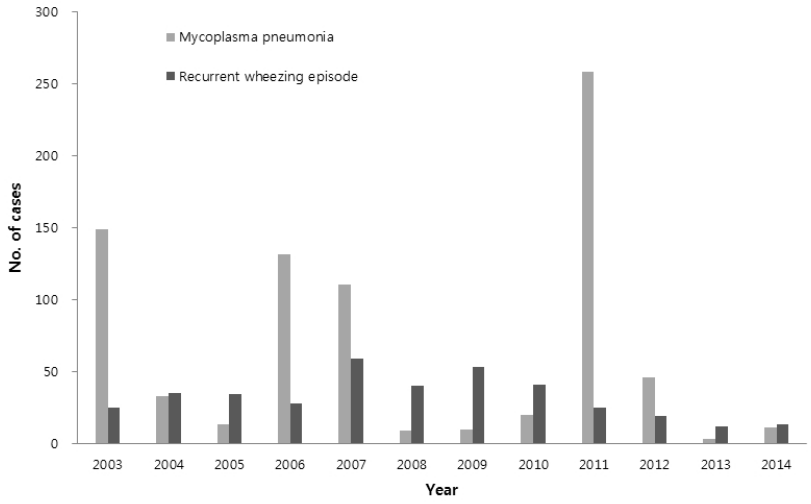

Fig. 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

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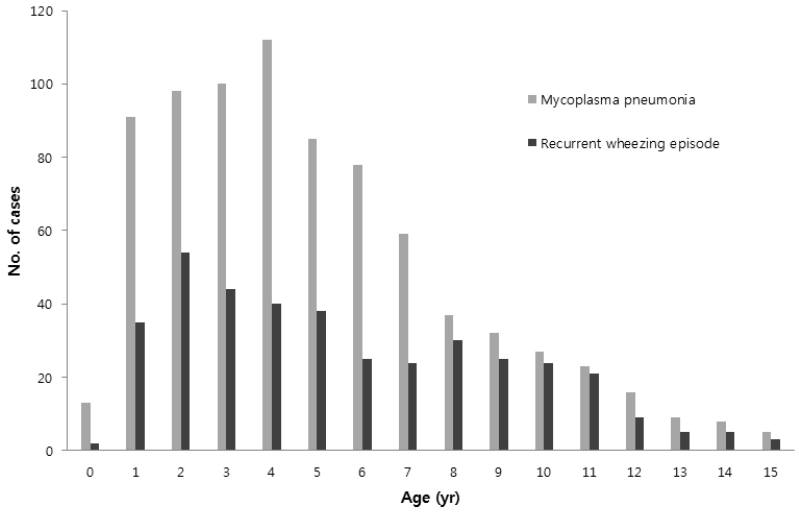

Fig. 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

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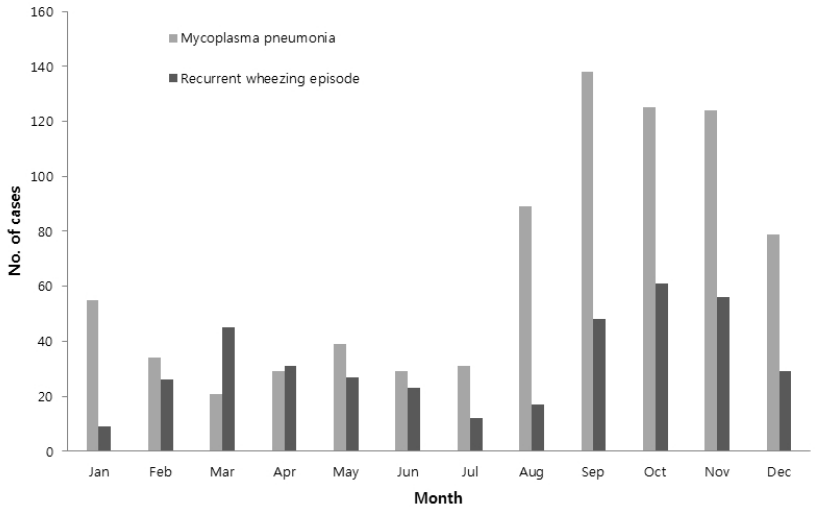

Fig. 4. Seasonality in MP pneumonia and recurrent wheezing episode groups

254x190mm (96 x 96 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any pre-specified hypotheses	P4-5
Methods			
Study design	4	Present key elements of study design early in the paper	P5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	P5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	P5
Study size	10	Explain how the study size was arrived at	P5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P6
		(*b*) Describe any methods used to examine subgroups and interactions	P6
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

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		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P6
		(b) Give reasons for non-participation at each stage	P6
		(c) Consider use of a flow diagram	P6 (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P6
		(b) Indicate number of participants with missing data for each variable of interest	P8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	P8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	P6-8-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P6-8
		(*b*) Report category boundaries when continuous variables were categorized	P6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	P8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	P8-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiological relationship between *Mycoplasma pneumoniae* Pneumonia and recurrent wheezing episode in children: an observational study at a single hospital in Korea

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Word count: 2,550 (except abstract and references)

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ABSTRACT

Objective: This study was aimed to evaluate epidemiological and clinical relationship between MP infection and childhood recurrent wheezing episode (RWE).

Design: Retrospective case note review.

Setting: Paediatric department at a single Korean institution.

Participants: Consecutive admitted patients with MP pneumonia and RWE (0-15 years of age) between 2003 and 2014.

Methods: The retrospective medical records of patients with MP pneumonia (n=793 for epidemiological analysis and n=501 for clinical analysis) and those with RWE (n=384) from 2003 to 2014 were analyzed. Diagnosis of MP pneumonia was made based on 2-times titration of IgM antibody during hospitalization. An RWE patient was defined as one with expiratory wheezing with at least one or more wheezing episodes based on medical records.

Results: During 3 MP pneumonia epidemics, there were no corresponding increases of patients with RWE in the epidemic years. In the 501 MP pneumonia patients, 52 (10.4%) had wheezing at presentation and 15 (3%) had RWE. The MP pneumonia patients with wheezing at presentation (n=52) were younger and were more likely to have an allergic disease history than those without wheezing (n=449). Among wheezing patients at presentation, 10 patients had previously RWE history. In a follow-up study, 13 patients (including 5 RWE) with initial wheezing and 25 patients (including 2 RWE) without wheezing had wheezy episodes after discharge. Among the total 501 patients, it was estimated that at least 31 MP pneumonia patients (6.2%) showed recurrent wheezing after initial MP infection.

Conclusions: A small part of children with MP pneumonia showed recurrent wheezing after MP pneumonia, and patients with RWE had a greater likelihood of experiencing wheezing when they had an initial MP infection. However, there were no increased admitted patients with RWE in MP pneumonia epidemic periods because of rarity of MP reinfection in children including patients with RWE or asthma.

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Key words: asthma, children, epidemiology, *Mycoplasma pneumoniae* pneumonia, recurrent wheezing

Strengths and limitations of this study

- This may be the first long-term epidemiological study regarding relationship between *Mycoplasma pneumoniae* (MP) infection and recurrent wheezing episode (RWE).
- Two-times examination of anti-MP IgM for diagnosis of MP pneumonia strengthened accuracy for patient selection.
- We discussed unsolved issues regarding MP infection and childhood asthma.
- There were limitations in this study in regards to the following: retrospective analysis, data collected from a single centre, and patient selection under our definition of RWE (or childhood asthma).

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INTRODUCTION

Mycoplasma pneumoniae (MP) is one of the major pathogens in respiratory tract infections in children and young adults, manifesting from asymptomatic infection to potential fatal pneumonia. MP pneumonia is an endemic disease in large communities, but it occurs in nationwide cyclic epidemics of 3-7 year intervals. Also, MP is associated with a wide spectrum of other organ-specific diseases including neurologic, dermatologic, hepatic, cardiac, musculoskeletal, hematologic diseases and possibly bronchial asthma.[1,2]

Childhood asthma is a complex disease process that can be classified into heterogeneous clinical phenotypes.[3] The diverse phenotypes of childhood asthma may be associated with host factors, including the developing immune and respiratory systems and individual genetic variations. Childhood asthma is also influenced by environmental factors such as socio-economic and/or cultural differences across populations.[3,4] Therefore, diagnosis of asthma in children, especially in children under 5 years of age, could not be defined definitely, and the phenotypes of childhood asthma, such as the prevalence of asthma and frequency of severe asthma, could differ among populations.[5,6] It has been suggested that initiation of asthma and acute exacerbation of asthma are associated with a variety of respiratory pathogens, including respiratory syncytial viruses (RSV), rhinoviruses and MP, although the exact reasons are still not fully understood.[7-9] In addition, it has been suggested that chronic MP infection is related to persistent bronchial hyper-responsiveness, chronic inflammation, and acute exacerbation in children and adults with asthma.[10-13]

In Korea, MP pneumonia epidemics have been observed every 3 to 4 years from the mid-1980s to present. We recently found that during 2003-2012, there were three nationwide MP pneumonia epidemics, all of which had similar epidemiological patterns including age distribution and seasonal pattern.[14] In this study, we evaluated the epidemiological and clinical characteristics of patients with MP pneumonia and those with recurrent wheezing

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episode (RWE) admitted during the 2003-2014 period, and we compared the data in both diseases. Also, we discussed the relationship between MP infection and childhood asthma.

METHODS

Study design and setting

This study was a retrospective study conducted at Department of Paediatrics, The Catholic University of Korea Daejeon St. Mary's Hospital, a secondary referral hospital performing primary care for patients with MP pneumonia or RWE.

Patients and public involvement

Patients and public were not involved in the study due to its retrospective nature.

Patients and data collection

We performed a retrospective analysis of medical records of patients with MP pneumonia or RWE who were admitted to our institution from January 2003 to December 2014. In this study, diagnosis of MP pneumonia was based on chest radiographic findings and 2-times titration of IgM antibody (Serodia-Myco II, Fujirebio Inc., Tokyo, Japan; positive cutoff value ≥1:40) at presentation and before discharge. Briefly, diagnoses of MP pneumonia were made when patients showed seroconversion (negative to positive), 4-fold or greater increase in IgM titres, or high titres of > 1:640 in initial and follow up examinations during the disease course. Patients who received the test once or those who did not increase or decreased titres were excluded when initial titres were < 1:320.[14,15]

The subjects with RWE were collected through the diagnostic code number J459 (asthma, asthma nature, bronchial asthma, and infantile asthma). However, we used the term RWE in this study, because majority of patients were under 5 years of age and some patients over 6 years of age did not perform confirmative study for asthma such as pulmonary function test. An RWE patient was defined as one with expiratory wheezing at admission with at least one

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or more wheezing episodes within 6 months before admission, regardless of patient's age based on the medical records. Patients with first wheezing at admission for any respiratory tract infection, including MP pneumonia, were excluded from the RWE group. Readmitted patients with RWE in the same year or different year were included in this series (35 episodes). Clinical and laboratory parameters were evaluated and compared between the groups. After discharge, wheezing episodes were evaluated through the medical records in both patient groups who revisited the outpatient clinic or had readmission at least two or more times from the date of discharge to December 2014.

Statistical analysis

All calculations were performed with SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using the Student's t-test, chi-square test, and Fisher's exact test, and the data were expressed as mean ± standard deviation (SD) for continuous variables. A *P* value of <0.05 was considered statistically significant.

RESULTS

The subjects in the present study were 793 patients with MP pneumonia and 384 patients with RWE during the study period. For epidemiological comparison, a total 793 patients were used, as shown in previously published article.[14] For clinical comparison between the groups, a total of 501 MP pneumonia patients that were admitted during the recent epidemics (241 cases in 2007-2006, and 260 cases in 2011), and 384 patients with RWE were analyzed (Fig. 1). Most of MP pneumonia patients had follow-up period at least 3 years from last epidemic year of 2011, but the follow-up subjects satisfied with our selection criteria were 180 among 501 cases in the MP pneumonia group and 206 among 384 cases in the RWE group.

Epidemiological data of MP pneumonia and RWE from 2003 to 2014

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In MP pneumonia patients, there were clear outbreaks of cases in 2003, 2006-2007, and 2011, with a few cases occurring in the inter-epidemic periods. There were relatively steady numbers of RWE cases every year during the study period. There were no corresponding increased cases in MP pneumonia epidemic years except in 2007 (Fig. 2). No MP pneumonia patient who was re-admitted due to MP reinfection across the epidemics was observed during study period. The mean age and age distribution were similar, and the peak age groups were 2-5 years of age in both groups (Fig. 3). In seasonality of both groups, MP pneumonia was prevalent in decreasing order of autumn, summer, winter, and spring, while patients with RWE were most prevalent in autumn, followed by spring, winter, and summer (Fig. 4).

Demographic and clinical characteristics of MP pneumonia and RWE groups

Demographic, clinical and laboratory data in both groups are shown in Table 1. The mean age and age distribution were similar in both groups as shown in Fig. 2 (5.4 years vs. 5.6 years, $P = 0.312$). Both groups had male predominance, and there were more males in the RWE group. As expected, cases with wheezing at presentation, the proportion of patients with past history or family history of allergic diseases, and past history of wheezing were higher in the RWE group. Recurrent wheezing after discharge was observed in 38 of 180 patients with MP pneumonia and 141 of the 206 patients with RWE ($P < 0.001$).

In MP pneumonia patients, 52 of the total 501 patients (10.4%) had wheezing at presentation, 72 (14.4%) had a past history of allergic diseases, 15 (3%) had RWE according to our definition, and 40 (8%) had a family history of allergic diseases. When we divided the MP pneumonia patients into two groups of patients with wheezing (n=52) and without wheezing (n=449), the patients with wheezing were younger (3.7 years vs. 5.6 years, $P < 0.001$) and had higher proportions of past history allergic diseases and wheezing history and higher values of leukocyte count and eosinophil differential (Table 2). Among the 52 MP pneumonia patients with wheezing at admission, 10 were patient with RWE, while 42 had no

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previous RWE history. Thus, in this study, 66% (10 of 15) of patients with RWE history presented with wheezing, while 8.6% of patients without RWE history (42 of 486) had wheezing at presentation. In a follow-up study of patients who visited two or more times after discharge, 13 (including 5 cases of RWE) of 21 MP pneumonia patients with initial wheezing and 25 (including 2 cases of RWE) of 159 MP pneumonia patients without wheezing had at least one wheezing episode after discharge (Table 2). Although not all patients were followed-up, it was estimated that at least 31 of the total MP pneumonia patients (n=501, 6.2%) showed a recurrent wheezing after initial MP infection.

DISCUSSION

In this study, we found that patients with MP pneumonia and patients with RWE had a similar mean age and age distribution during the past decade. However, the annual number of cases in the two groups was quite different; there were relatively stable number of annual cases in patients with RWE, without a corresponding increase in cases during or after MP pneumonia epidemic years. Although reinfection with MP reported to be not uncommon in other studies based on polymerase chain reaction (PCR) or single serologic test-based studies,[16] we found that there were no serologically confirmed reinfected MP pneumonia patients among 793 patients during study period, except a few readmitted patients with complications of initial MP infection in each epidemic. It suggests that reattack of MP pneumonia in children is extremely rare, and once infected children seem to have immunity to other MP strains in future epidemics including macrolide-resistant MP strains.[14] During a MP epidemic, the majority of susceptible children are asymptomatic or have mild symptoms such as fever and sore throat, and only a small part of MP infected patients may affect pneumonia.[1,2] Moreover, since MP reinfection is also rare in asthmatics of all age, our

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epidemiological data might not be associated with the situations where asthmatics have reasons for increase of admission such as exacerbation or initiation of RWE or asthma.

MP pneumonia patients in this study showed a similar prevalence of allergic diseases compared to data from previous nationwide questionnaire-based studies in Korea: 3% (15/501) and 14.4% (72/501) of MP pneumonia patients had a past history of RWE (asthma) and a past history of allergic diseases, respectively. Whereas 5.8% of 6- to 7-year-old school children had asthma history in 2000, and 13.5% of children reported a diagnosis of atopic dermatitis in 2008-2011 in Korea.[17,18] In this study, among 52 MP pneumonia patients with initial wheezing, 10 were patients with RWE, while 42 had no previous wheezing history. Therefore, 66.6% (10 of 15) of patients with RWE, and 8.6% (42 of 486) of patients without RWE history were wheezing, and it suggests that patients with RWE or asthma with first-time MP infection might be prone to a wheezing episode as well as other respiratory virus infections as compounding factors of asthma exacerbation. Also, we found that after MP infection, at least 6% of MP pneumonia patients showed subsequent wheezing during at least over 3 years after initial MP infection. A large proportion of patients who experienced lower respiratory infections caused by RSV and rhinoviruses were reported to show recurrent wheezing after initial infection, especially when they were infected with other respiratory agents.[19,20] A study reported that approximately 50% of children affected with severe RSV bronchiolitis had a subsequent asthma diagnosis.[19] In Korea, since the majority of children might affect RSV during infancy and early childhood period as well as in other populations, it is possible that majority of MP infected patients experience RSV infection prior to MP infection. On the other hand, other authors reported that some patients with severe MP pneumonia had prolonged anatomical abnormalities, suggestive of small airway obstruction,[21] and some patients had reduced pulmonary function after MP infection.[8] Thus,

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patients with severe lung injury caused by any respiratory pathogens may be prone to recurrent wheezing and subsequent diagnosis of asthma.

We found that WBC count, eosinophil differential, IgG, and IgE values were higher in the RWE group than those in the MP pneumonia group which had similar mean age. It could in part be explained that different activation of immune system of the host against the insults from RWE which induces chronic or repeated inflammation may be reflected in these laboratory findings.

Previous studies have reported that asthma patients are more likely to be infected or colonized with MP compared to healthy controls, and MP infection in chronic asthmatics might be associated with persistent bronchial hyperresponsiveness and exacerbation.[10-13] MP pneumonia epidemics are cyclic in 3-4-year intervals, and the duration of an epidemic is generally limited to 1 year or occasionally 2 years, and during the inter-epidemic period of the 2-3 years there are a few patients as observed in this study.[14] There are many asymptomatic healthy MP carriers during the epidemic periods, especially young children, who may serve as reservoirs for the spread of MP infection.[22-24] It is natural that colonization of MP during MP pneumonia epidemics can occur in asthma patients and old persons as well as in healthy young children and adults since colonization does not mean the 'infection'. The prevalent rates of MP colonization between the asthma group and the control groups could be influenced by the MP epidemic year during the study period. Thus, patient selection bias could be considered if a study period is long and different in both groups. Wood et al. reported that the rates of MP colonization in asthmatics and healthy children during the same study period were similar using a sensitive PCR method,[13] suggesting that colonization occurs equally in the two groups. One study reported that the status of asthma control and the lung function tests were not different between asthmatic patients with chronic MP infection and those without, indicating the similar severity of asthma between the group.[25]

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In this study, the numbers of patient with RWE during a recent decade did not change with a rather decreasing trend in recent years. Moreover, we have experienced that asthma severity might change to a milder phenotype in Korea over time. A recent population-based study in Korea also indicated that the prevalence of asthma was reduced in recent years.[26] Changing epidemiology in infectious disease and infection-related immune-mediated diseases, including scarlet fever, pandemic influenza, acute rheumatic fever, acute post-streptococcal glomerulonephritis, and Kawasaki disease, has been well documented in Korea; initially severe disease phenotypes have changed to milder phenotypes over time.[27-30] Because allergic diseases, including asthma, may be associated with various pathogen infections and environmental factors,[31,32] it is possible that the changing epidemiology of childhood asthma occurs over time in the populations.

There are some limitations to this retrospective study. We evaluated only admitted patient groups with a part of the follow-up subjects at a single hospital. History of allergic diseases was not as precise as in International Study of Asthma and Allergies in Childhood (ISAAC) studies. In our definition of RWE in the present study, the pneumonia patients with first wheezing, regardless of severity of respiratory symptoms, were not included in the asthma group. Considering the age distribution of patients, larger part of patients might be the transient recurrent wheezers and small part of true asthma patients who experience and progress recurrent wheezing into adulthood might be included in the RWE group. Further multicenter studies with prospective designs are needed for exact epidemiological relationship between MP infection and childhood asthma.

In conclusions, a small part of MP pneumonia patients had a subsequent wheezing after initial MP infection as well as those observed in other respiratory pathogen infections. Patients with RWE have a greater likelihood of experiencing wheezing than non-asthmatics when they had an initial MP infection, suggesting that MP infection is one of exacerbation

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factors in childhood asthma. However, we found no corresponding increase in the number of patients with RWE in MP pneumonia epidemic periods, and this finding may in part be explained that MP reinfection are very rare in children including patients with RWE or asthma as shown in this study.

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Contributors

KYL designed and conceptualized and the study, and drafted the manuscript. JUR participated in preliminary data collection, and wrote the initial manuscript. HMK and EAY analyzed data and revised the manuscript for critical content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Competing interests

None declared.

Ethic approval

The study was approved by the Institutional Review Board of The Catholic University of Korea Daejeon St. Mary's Hospital (DC17RESI0053).

Data sharing statement

Data of the study are available from the corresponding author (KYL).

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Table 1. Clinical and laboratory findings of the patients with MP pneumonia or recurrent wheezing episode (RWE)

	MP pneumonia (n = 501)	RWE (n = 384)	*P*
Clinical characteristics			
Age (y)	5.4 ± 3.4	5.6 ± 3.5	0.31
Male : female	255/246	256/128	<.001
Wheeze at presentation, n (%)	52 (10.4)	384 (100)	<.001
Past history of allergy*, n (%)	72 (14.4)	379 (98.7)	<.001
Past history of wheeze, n (%)	15 (3.0)	384 (100)	<.001
Family history of allergy, n (%)	40 (8.0)	134 (34.9)	<.001
Wheeze after discharge, n (%)	38/180 (21.1)	141/206 (68.4)	<.001
Laboratory findings			
Leukocyte (/μL)	8800 ± 4500	11100 ± 4200	<.001
Eosinophil (%)	2.2 ± 2.9	3.8 ± 3.9	<.001
IgG (mg/dL)	899 ± 235	946 ± 232	<.001
IgE (IU/mL)	267 ± 391	617 ± 673	<.001

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Table 2. Clinical and laboratory findings of MP pneumonia patients with wheezing and without wheezing

	Wheezing (n = 52)	No Wheezing (n = 449)	*P*
Clinical characteristics			
Age (y)	3.7 ± 3.0	5.6 ± 3.4	<.001
Male : female	32/20	223/226	0.11
Past history of allergy*, n (%)	16 (30.8)	56 (12.5)	0.01
Past history of wheeze, n (%)	10 (19.2)	5 (1.1)	<.001
Family history of allergy, n (%)	8 (15.4)	32 (7.1)	0.05
Wheeze after discharge, n (%)	13/21 (61.9)	25/159 (15.7)	<.001
Laboratory findings			
Leukocyte (x10^3//μL)	10400 ± 5400	8700 ± 4.3	0.03
Eosinophil (%)	3.0 ± 3.2	2.1 ± 2.9	0.08
IgG	873 ± 219	903 ± 238	0.43
IgE	407 ± 609	249 ± 350	0.11

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Figure legends

Figure 1. Flow diagram of the patient selection in this study

Figure 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

Figure 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

Figure 4. Seasonality in MP pneumonia and recurrent wheezing episode groups

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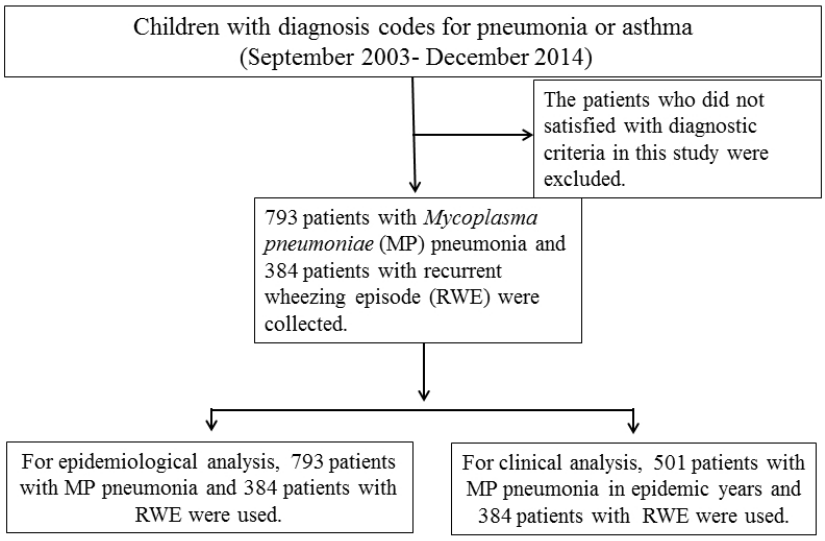

Figure 1. Flow diagram of the patient selection in this study

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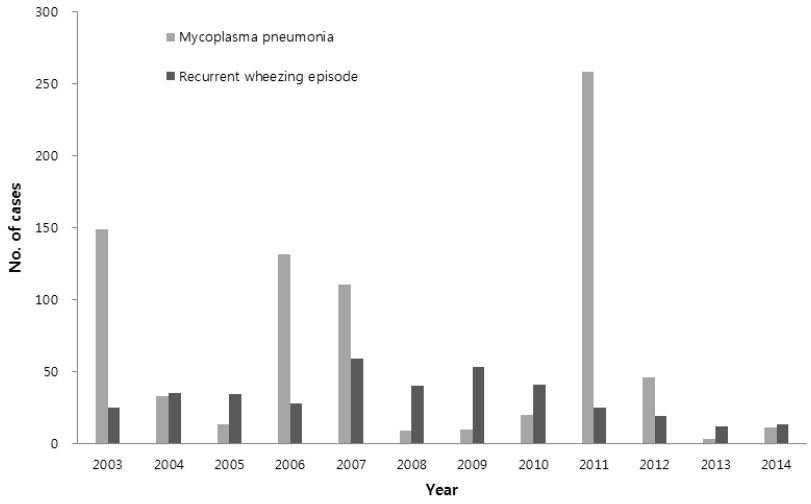

Fig. 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

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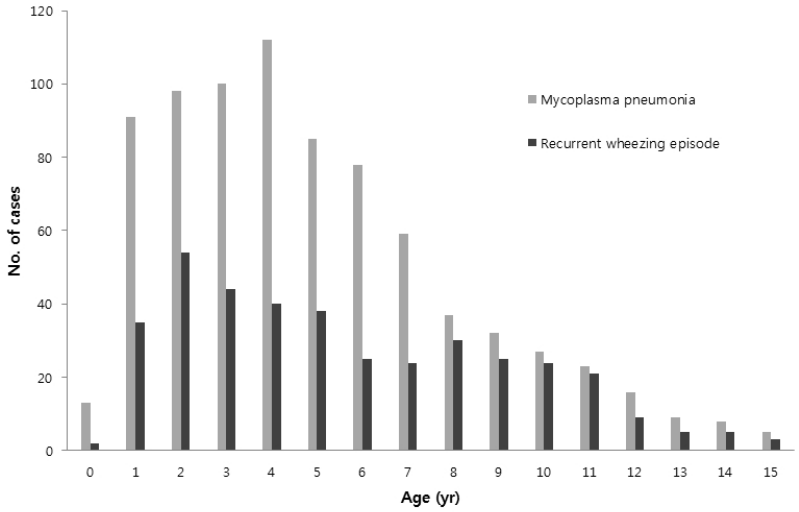

Fig. 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

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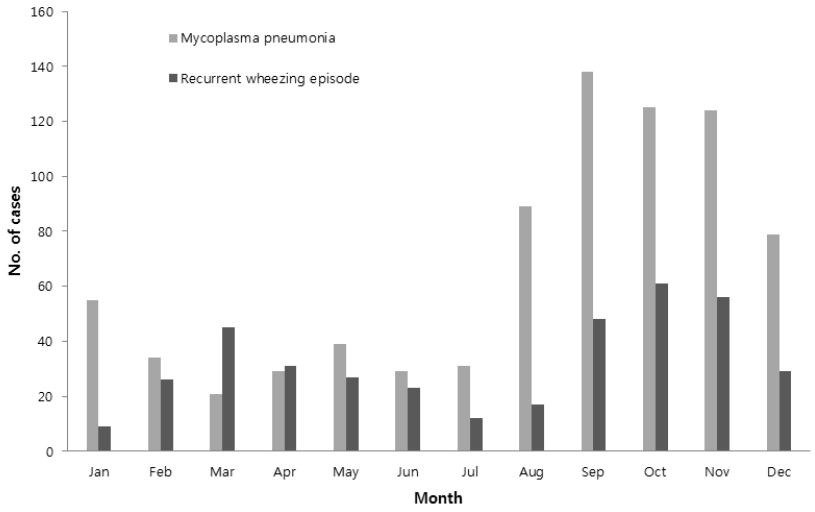

Fig. 4. Seasonality in MP pneumonia and recurrent wheezing episode groups

254x190mm (96 x 96 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any pre-specified hypotheses	P4-5
Methods			
Study design	4	Present key elements of study design early in the paper	P5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	P5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	P5
Study size	10	Explain how the study size was arrived at	P5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P6
		(*b*) Describe any methods used to examine subgroups and interactions	P6
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

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		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P6
		(b) Give reasons for non-participation at each stage	P6
		(c) Consider use of a flow diagram	P6 (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P6
		(b) Indicate number of participants with missing data for each variable of interest	P8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	P8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	P6-8-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P6-8
		(*b*) Report category boundaries when continuous variables were categorized	P6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	P8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	P8-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiological relationship between *Mycoplasma pneumoniae* pneumonia and recurrent wheezing episode in children: an observational study at a single hospital in Korea

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ABSTRACT

Objective: This study was aimed to evaluate epidemiological and clinical relationship between MP infection and childhood recurrent wheezing episode (RWE).

Design: Retrospective case note review.

Setting: Paediatric department at a single Korean institution.

Participants: Consecutive admitted patients with MP pneumonia and RWE (0-15 years of age) between 2003 and 2014.

Methods: The retrospective medical records of patients with MP pneumonia (n=793 for epidemiological analysis and n=501 for clinical analysis) and those with RWE (n=384) from 2003 to 2014 were analyzed. Diagnosis of MP pneumonia was made based on 2-times titration of IgM antibody during hospitalization. An RWE patient was defined as one with expiratory wheezing with at least one or more wheezing episodes based on medical records.

Results: During 3 MP pneumonia epidemics, there were no corresponding increases of patients with RWE in the epidemic years. In the 501 MP pneumonia patients, 52 (10.4%) had wheezing at presentation and 15 (3%) had RWE. The MP pneumonia patients with wheezing at presentation (n=52) were younger and were more likely to have an allergic disease history than those without wheezing (n=449). Among wheezing patients at presentation, 10 patients had previously RWE history. In a follow-up study, 13 patients (including 5 RWE) with initial wheezing and 25 patients (including 2 RWE) without wheezing had wheezy episodes after discharge. Among the total 501 patients, it was estimated that at least 31 MP pneumonia patients (6.2%) showed recurrent wheezing after initial MP infection.

Conclusions: A small part of children with MP pneumonia showed recurrent wheezing after MP pneumonia, and patients with RWE had a greater likelihood of experiencing wheezing when they had an initial MP infection. However, there were no increased admitted patients with RWE in MP pneumonia epidemic periods because of rarity of MP reinfection in children including patients with RWE or asthma.

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Key words: asthma, children, epidemiology, *Mycoplasma pneumoniae* pneumonia, recurrent wheezing

Strengths and limitations of this study

- This may be the first long-term epidemiological study regarding relationship between *Mycoplasma pneumoniae* (MP) infection and recurrent wheezing episode (RWE).
- Two-times examination of anti-MP IgM for diagnosis of MP pneumonia strengthened accuracy for patient selection.
- We discussed unsolved issues regarding MP infection and childhood asthma.
- There were limitations in this study in regards to the following: retrospective analysis, data collected from a single centre, and patient selection under our definition of RWE (or childhood asthma).

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INTRODUCTION

Mycoplasma pneumoniae (MP) is one of the major pathogens in respiratory tract infections in children and young adults, manifesting from asymptomatic infection to potential fatal pneumonia. MP pneumonia is an endemic disease in large communities, but it occurs in nationwide cyclic epidemics of 3-7 year intervals. Also, MP is associated with a wide spectrum of other organ-specific diseases including neurologic, dermatologic, hepatic, cardiac, musculoskeletal, hematologic diseases and possibly bronchial asthma.[1,2]

Childhood asthma is a complex disease process that can be classified into heterogeneous clinical phenotypes.[3] The diverse phenotypes of childhood asthma may be associated with host factors, including the developing immune and respiratory systems and individual genetic variations. Childhood asthma is also influenced by environmental factors such as socio-economic and/or cultural differences across populations.[3,4] Therefore, diagnosis of asthma in children, especially in children under 5 years of age, could not be defined definitely, and the phenotypes of childhood asthma, such as the prevalence of asthma and frequency of severe asthma, could differ among populations.[5,6] It has been suggested that initiation of asthma and acute exacerbation of asthma are associated with a variety of respiratory pathogens, including respiratory syncytial viruses (RSV), rhinoviruses and MP, although the exact reasons are still not fully understood.[7-9] In addition, it has been suggested that chronic MP infection is related to persistent bronchial hyper-responsiveness, chronic inflammation, and acute exacerbation in children and adults with asthma.[10-13]

In Korea, MP pneumonia epidemics have been observed every 3 to 4 years from the mid-1980s to present. We recently found that during 2003-2012, there were three nationwide MP pneumonia epidemics, all of which had similar epidemiological patterns including age distribution and seasonal pattern.[14] In this study, we evaluated the epidemiological and clinical characteristics of patients with MP pneumonia and those with recurrent wheezing

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episode (RWE) admitted during the 2003-2014 period, and we compared the data in both diseases. Also, we discussed the relationship between MP infection and childhood asthma.

METHODS

Study design and setting

This study was a retrospective study conducted at Department of Paediatrics, The Catholic University of Korea Daejeon St. Mary's Hospital, a secondary referral hospital performing primary care for patients with MP pneumonia or RWE.

Patients and public involvement

Patients and public were not involved in the study due to its retrospective nature.

Patients and data collection

We performed a retrospective analysis of medical records of patients with MP pneumonia or RWE who were admitted to our institution from January 2003 to December 2014. In this study, diagnosis of MP pneumonia was based on chest radiographic findings and 2-times titration of IgM antibody (Serodia-Myco II, Fujirebio Inc., Tokyo, Japan; positive cutoff value ≥1:40) at presentation and before discharge. Briefly, diagnoses of MP pneumonia were made when patients showed seroconversion (negative to positive), 4-fold or greater increase in IgM titres, or high titres of > 1:640 in initial and follow up examinations during the disease course. Patients who received the test once or those who did not increase or decreased titres were excluded when initial titres were < 1:320.[14,15]

The subjects with RWE were collected through the diagnostic code number J459 (asthma, asthma nature, bronchial asthma, and infantile asthma). However, we used the term RWE in this study, because majority of patients were under 5 years of age and some patients over 6 years of age did not perform confirmative study for asthma such as pulmonary function test.

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An RWE patient was defined as one with expiratory wheezing at admission with at least one or more wheezing episodes within 6 months before admission, regardless of patient's age based on the medical records. Patients with first wheezing at admission for any respiratory tract infection, including MP pneumonia, were excluded from the RWE group. Readmitted patients with RWE in the same year or different year were included in this series (35 episodes). Clinical and laboratory parameters were evaluated and compared between the groups. After discharge, wheezing episodes were evaluated through the medical records in both patient groups who revisited the outpatient clinic or had readmission at least two or more times from the date of discharge to December 2014.

Statistical analysis

All calculations were performed with SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using the Student's t-test, chi-square test, and Fisher's exact test, and the data were expressed as mean ± standard deviation (SD) for continuous variables. A P value of <0.05 was considered statistically significant.

RESULTS

The subjects in the present study were 793 patients with MP pneumonia and 384 patients with RWE during the study period. For epidemiological comparison, a total 793 patients were used, as shown in previously published article.[14] For clinical comparison between the groups, a total of 501 MP pneumonia patients that were admitted during the recent epidemics (241 cases in 2007-2006, and 260 cases in 2011), and 384 patients with RWE were analyzed (Fig. 1). During the study period, the total number of admitted patients at our department was a total 25,911 (except nursery patients). There were approximately 2,100 admitted patients per year with some variations in each year.

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Most of MP pneumonia patients had follow-up period at least 3 years from last epidemic year of 2011, but the follow-up subjects satisfied with our selection criteria were 180 among 501 cases in the MP pneumonia group and 206 among 384 cases in the RWE group.

Epidemiological data of MP pneumonia and RWE from 2003 to 2014

In MP pneumonia patients, there were clear outbreaks of cases in 2003, 2006-2007, and 2011, with a few cases occurring in the inter-epidemic periods. There were relatively steady numbers of RWE cases every year during the study period. There were no corresponding increased cases in MP pneumonia epidemic years except in 2007 (Fig. 2). No MP pneumonia patient who was re-admitted due to MP reinfection across the epidemics was observed during study period. The mean age and age distribution were similar, and the peak age groups were 2-5 years of age in both groups (Fig. 3). In seasonality of both groups, MP pneumonia was prevalent in decreasing order of autumn, summer, winter, and spring, while patients with RWE were most prevalent in autumn, followed by spring, winter, and summer (data not shown).

Demographic and clinical characteristics of MP pneumonia and RWE groups

Demographic, clinical and laboratory data in both groups are shown in Table 1. The mean age and age distribution were similar in both groups as shown in Fig. 3 (5.4 years vs. 5.6 years, $P = 0.312$). Both groups had male predominance, and there were more males in the RWE group. As expected, cases with wheezing at presentation, the proportion of patients with past history or family history of allergic diseases, and past history of wheezing were higher in the RWE group. Recurrent wheezing after discharge was observed in 38 of 180 patients with MP pneumonia and 141 of the 206 patients with RWE ($P < 0.001$).

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In MP pneumonia patients, 52 of the total 501 patients (10.4%) had wheezing at presentation, 72 (14.4%) had a past history of allergic diseases, 15 (3%) had RWE according to our definition, and 40 (8%) had a family history of allergic diseases. When we divided the MP pneumonia patients into two groups of patients with wheezing (n=52) and without wheezing (n=449), the patients with wheezing were younger (3.7 years vs. 5.6 years, $P < 0.001$) and had higher proportions of past history allergic diseases and wheezing history and higher values of leukocyte count and eosinophil differential (Table 2). Among the 52 MP pneumonia patients with wheezing at admission, 10 were patient with RWE, while 42 had no previous RWE history. Thus, in this study, 66% (10 of 15) of patients with RWE history presented with wheezing, while 8.6% of patients without RWE history (42 of 486) had wheezing at presentation. In a follow-up study of patients who visited two or more times after discharge, 13 (including 5 cases of RWE) of 21 MP pneumonia patients with initial wheezing and 25 (including 2 cases of RWE) of 159 MP pneumonia patients without wheezing had at least one wheezing episode after discharge (Table 2). Although not all patients were followed-up, it was estimated that at least 31 of the total MP pneumonia patients (n=501, 6.2%) showed a recurrent wheezing after initial MP infection.

DISCUSSION

In this study, we found that patients with MP pneumonia and patients with RWE had a similar mean age and age distribution during the past decade. However, the annual number of cases in the two groups was quite different; there were relatively stable number of annual cases in patients with RWE, without a corresponding increase in cases during or after MP pneumonia epidemic years. Although reinfection with MP reported to be not uncommon in other studies based on polymerase chain reaction (PCR) or single serologic test-based

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studies,[16] we found that there were no serologically confirmed reinfected MP pneumonia patients among 793 patients during study period, except a few readmitted patients with complications of initial MP infection in each epidemic. It suggests that reattack of MP pneumonia in children is extremely rare, and once infected children seem to have immunity to other MP strains in future epidemics including macrolide-resistant MP strains.[14] During a MP epidemic, the majority of susceptible children are asymptomatic or have mild symptoms such as fever and sore throat, and only a small part of MP infected patients may affect pneumonia.[1,2] Moreover, since MP reinfection is also rare in asthmatics of all age, our epidemiological data might not be associated with the situations where asthmatics have reasons for increase of admission such as exacerbation or initiation of RWE or asthma.

MP pneumonia patients in this study showed a similar prevalence of allergic diseases compared to data from previous nationwide questionnaire-based studies in Korea: 3% (15/501) and 14.4% (72/501) of MP pneumonia patients had a past history of RWE (asthma) and a past history of allergic diseases, respectively. Whereas 5.8% of 6- to 7-year-old school children had asthma history in 2000, and 13.5% of children reported a diagnosis of atopic dermatitis in 2008-2011 in Korea.[17,18] In this study, among 52 MP pneumonia patients with initial wheezing, 10 were patients with RWE, while 42 had no previous wheezing history. Therefore, 66.6% (10 of 15) of patients with RWE, and 8.6% (42 of 486) of patients without RWE history were wheezing, and it suggests that patients with RWE or asthma with first-time MP infection might be prone to a wheezing episode as well as other respiratory virus infections as compounding factors of asthma exacerbation. Also, we found that after MP infection, at least 6% of MP pneumonia patients showed subsequent wheezing during at least over 3 years after initial MP infection. A large proportion of patients who experienced lower respiratory infections caused by RSV and rhinoviruses were reported to show recurrent wheezing after initial infection, especially when they were infected with other respiratory

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agents.[19,20] A study reported that approximately 50% of children affected with severe RSV bronchiolitis had a subsequent asthma diagnosis.[19] In Korea, since the majority of children might affect RSV during infancy and early childhood period as well as in other populations, it is possible that majority of MP infected patients experience RSV infection prior to MP infection. On the other hand, other authors reported that some patients with severe MP pneumonia had prolonged anatomical abnormalities, suggestive of small airway obstruction,[21] and some patients had reduced pulmonary function after MP infection.[8] Thus, patients with severe lung injury caused by any respiratory pathogens may be prone to recurrent wheezing and subsequent diagnosis of asthma.

We found that WBC count, eosinophil differential, IgG, and IgE values were higher in the RWE group than those in the MP pneumonia group which had similar mean age. It could in part be explained that different activation of immune system of the host against the insults from RWE which induces chronic or repeated inflammation may be reflected in these laboratory findings.

Previous studies have reported that asthma patients are more likely to be infected or colonized with MP compared to healthy controls, and MP infection in chronic asthmatics might be associated with persistent bronchial hyperresponsiveness and exacerbation.[10-13] MP pneumonia epidemics are cyclic in 3-4-year intervals, and the duration of an epidemic is generally limited to 1 year or occasionally 2 years, and during the inter-epidemic period of the 2-3 years there are a few patients as observed in this study.[14] There are many asymptomatic healthy MP carriers during the epidemic periods, especially young children, who may serve as reservoirs for the spread of MP infection.[22-24] It is natural that colonization of MP during MP pneumonia epidemics can occur in asthma patients and old persons as well as in healthy young children and adults since colonization does not mean the 'infection'. The prevalent rates of MP colonization between the asthma group and the control groups could be

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influenced by the MP epidemic year during the study period. Thus, patient selection bias could be considered if a study period is long and different in both groups. Wood et al. reported that the rates of MP colonization in asthmatics and healthy children during the same study period were similar using a sensitive PCR method,[13] suggesting that colonization occurs equally in the two groups. One study reported that the status of asthma control and the lung function tests were not different between asthmatic patients with chronic MP infection and those without, indicating the similar severity of asthma between the group.[25]

In this study, the numbers of patient with RWE during a recent decade did not change with a rather decreasing trend in recent years. Moreover, we have experienced that asthma severity might change to a milder phenotype in Korea over time. A recent population-based study in Korea also indicated that the prevalence of asthma was reduced in recent years.[26] Changing epidemiology in infectious disease and infection-related immune-mediated diseases, including scarlet fever, pandemic influenza, acute rheumatic fever, acute post-streptococcal glomerulonephritis, and Kawasaki disease, has been well documented in Korea; initially severe disease phenotypes have changed to milder phenotypes over time.[27-30] Because allergic diseases, including asthma, may be associated with various pathogen infections and environmental factors,[31,32] it is possible that the changing epidemiology of childhood asthma occurs over time in the populations.

There are some limitations to this retrospective study. We evaluated only admitted patient groups with a part of the follow-up subjects at a single hospital. History of allergic diseases was not as precise as in International Study of Asthma and Allergies in Childhood (ISAAC) studies. In our definition of RWE in the present study, the pneumonia patients with first wheezing, regardless of severity of respiratory symptoms, were not included in the asthma group. Considering the age distribution of patients, larger part of patients might be the transient recurrent wheezers and small part of true asthma patients who experience and

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progress recurrent wheezing into adulthood might be included in the RWE group. Further multicenter studies with prospective designs are needed for exact epidemiological relationship between MP infection and childhood asthma.

In conclusions, a small part of MP pneumonia patients had a subsequent wheezing after initial MP infection as well as those observed in other respiratory pathogen infections. Patients with RWE have a greater likelihood of experiencing wheezing than non-asthmatics when they had an initial MP infection, suggesting that MP infection is one of exacerbation factors in childhood asthma. However, we found no corresponding increase in the number of patients with RWE in MP pneumonia epidemic periods, and this finding may in part be explained that MP reinfection are very rare in children including patients with RWE or asthma as shown in this study.

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Contributors

KYL designed and conceptualized and the study, and drafted the manuscript. JUR participated in preliminary data collection, and wrote the initial manuscript. HMK and EAY analyzed data and revised the manuscript for critical content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Competing interests

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None declared.

Ethic approval

The study was approved by the Institutional Review Board of The Catholic University of Korea Daejeon St. Mary's Hospital (DC17RESI0053).

Data sharing statement

Data available from the Dryad Digital Repository: *https://doi.org/10.5061/dryad.3kt7fc2*

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Table 1. Clinical and laboratory findings of the patients with MP pneumonia or recurrent wheezing episode (RWE)

	MP pneumonia (n = 501)	RWE (n = 384)	*P*
Clinical characteristics			
Age (y)	5.4 ± 3.4	5.6 ± 3.5	0.31
Male : female	255/246	256/128	<.001
Wheeze at presentation, n (%)	52 (10.4)	384 (100)	<.001
Past history of allergy*, n (%)	72 (14.4)	379 (98.7)	<.001
Past history of wheeze, n (%)	15 (3.0)	384 (100)	<.001
Family history of allergy, n (%)	40 (8.0)	134 (34.9)	<.001
Wheeze after discharge, n (%)	38/180 (21.1)	141/206 (68.4)	<.001
Laboratory findings			
Leukocyte (/μL)	8800 ± 4500	11100 ± 4200	<.001
Eosinophil (%)	2.2 ± 2.9	3.8 ± 3.9	<.001
IgG (mg/dL)	899 ± 235	946 ± 232	<.001
IgE (IU/mL)	267 ± 391	617 ± 673	<.001

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Table 2. Clinical and laboratory findings of MP pneumonia patients with wheezing and without wheezing

	Wheezing (n = 52)	No Wheezing (n = 449)	*P*
Clinical characteristics			
Age (y)	3.7 ± 3.0	5.6 ± 3.4	<.001
Male : female	32/20	223/226	0.11
Past history of allergy*, n (%)	16 (30.8)	56 (12.5)	0.01
Past history of wheeze, n (%)	10 (19.2)	5 (1.1)	<.001
Family history of allergy, n (%)	8 (15.4)	32 (7.1)	0.05
Wheeze after discharge, n (%)	13/21 (61.9)	25/159 (15.7)	<.001
Laboratory findings			
Leukocyte (x10^3//μL)	10400 ± 5400	8700 ± 4.3	0.03
Eosinophil (%)	3.0 ± 3.2	2.1 ± 2.9	0.08
IgG	873 ± 219	903 ± 238	0.43
IgE	407 ± 609	249 ± 350	0.11

* Number of patients who had history to be diagnosed as having asthma, allergic rhinitis, and/or atopic dermatitis

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Figure legends

Figure 1. Flow diagram of the patient selection in this study

Figure 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

Figure 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

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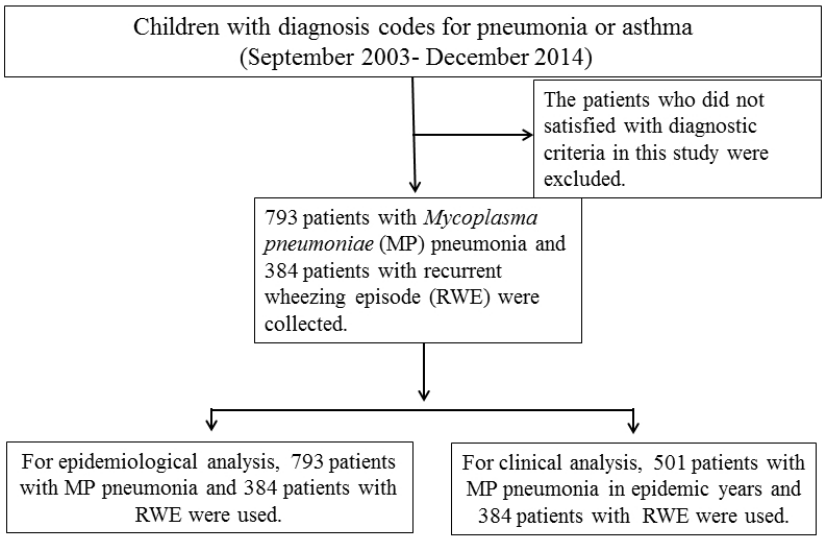

Figure 1. Flow diagram of the patient selection in this study

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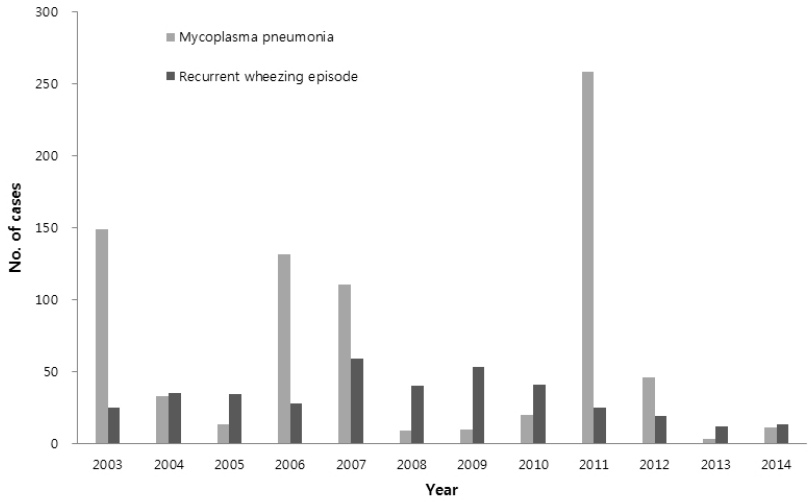

Fig. 2. Annual cases of MP pneumonia and recurrent wheezing episode during 2003-2014

254x190mm (96 x 96 DPI)

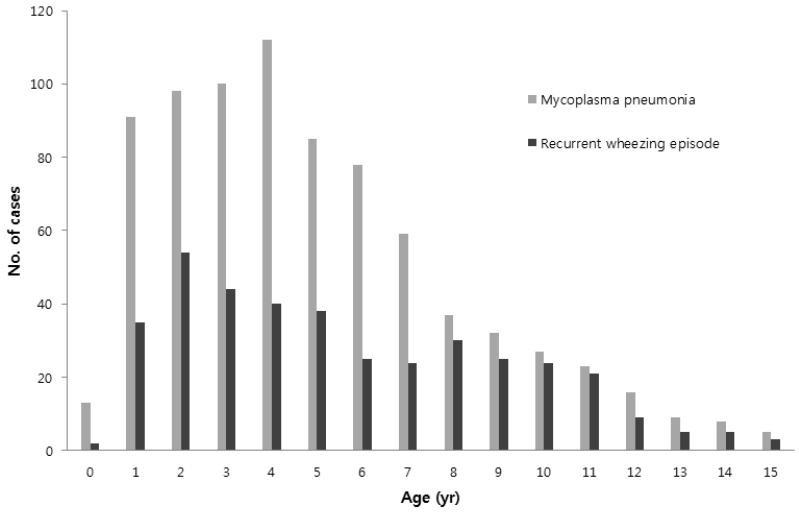

Fig. 3. Age distribution in MP pneumonia and recurrent wheezing episode groups

254x190mm (96 x 96 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P4
Objectives	3	State specific objectives, including any pre-specified hypotheses	P4-5
Methods			
Study design	4	Present key elements of study design early in the paper	P5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	P5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P5
Bias	9	Describe any efforts to address potential sources of bias	P5
Study size	10	Explain how the study size was arrived at	P5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P6
		(*b*) Describe any methods used to examine subgroups and interactions	P6
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	-

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		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	-
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P6
		(b) Give reasons for non-participation at each stage	P6
		(c) Consider use of a flow diagram	P6 (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P6
		(b) Indicate number of participants with missing data for each variable of interest	P8
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	P8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	P6-8-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P6-8
		(*b*) Report category boundaries when continuous variables were categorized	P6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			
Key results	18	Summarise key results with reference to study objectives	P8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P9-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	P8-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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