PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Epidemiological relationship between Mycoplasma pneumoniae pneumonia and recurrent wheezing episode in children: an observational study at a single hospital in Korea
AUTHORS	Rhim, Jung-Woo; Kang, Hyun-Mi; Yang, Eun-Ae; Lee, Kyung-Yil

VERSION 1 - REVIEW

REVIEWER	Arianna Dondi Pediatric Unit AOU S.Orsola-Malpighi Bologna
REVIEW RETURNED	29-Sep-2018

GENERAL COMMENTS	The paper is a retrospective study in which the authors seek a relationship between M. pneumoniae pneumonia and recurrent wheezing. The subject is interesting, and the number of enrolled subjects is quite high. However, I have some major concerns about several points. 1) My main concern is about the methodology of the study. Patients admitted between 2003 and 2014 for MP pneumonia or recurrent wheezing episodes were enrolled retrospectively. However, I cannot find clearly: a. Inclusion and exclusion criteria b. The clinical and laboratory information that was considered important for this study and the way it was collected (hospital records I guess, but please specify) c. How MP pneumonia was treated; did any MP patient require ICU admission or have serious complication? Were there differences in subsequent wheezing episodes in different treatment groups? d. How were previous wheezing episodes diagnosed? Were they documented by previous medical records? 2) Authors state that subsequent wheezing episodes were evaluated through medical records of outpatients' visits and readmissions, but no direct patient interview is mentioned, so that this information might be seriously biased (several wheezing episodes might have been missed and some patients might have chosen other clinics for follow-up visits). 3) Choosing to only include RWE requiring admission might also cause a bias in the number of the wheezing episodes, because the majority of patients who are visited in the ER for wheezing does not require hospital admission. The authors should explain why they decided to evaluate only children admitted for wheezing. 4) Another big concern is about the findings of the study. Authors state quite honestly that MP epidemics did not cause an increase in hospital admissions for recurrent wheezing episodes. A

	relationship was found between recurrent wheezing and wheezing as a symptom of MP pneumonia, but this is not really surprising, as it is well known that patients with recurrent wheezing will probably have exacerbations during respiratory infections. Minor points: - Abstract: a definition of recurrent wheezing should be included, as well as the way in which subsequent wheezing episodes were recorded. - Page 2 line 38: delete “were” - Page 4 line 46: “adults and children with asthma” - Page 5 methods: specify how MP pneumonia was excluded in patients affected by RWE - Page 5 lines 49-51: “…and 37 cases… present study.” Probably this part of the sentence should be deleted. - Page 6, results: the number of total admissions during the study period should be reported. - Page 6 lines 30-32: the number of MP pneumonia patients is much higher than that of RWE patients and this should be explained; is it maybe due to the fact that most of the patients with wheezing are not admitted to the hospital ward? - Page 7 lines 36-39: differences in these laboratory findings do not really add important information to the work and reporting them in table 1 is enough. I would delete this sentence (“In laboratory indices…RWE group”). - Page 8 line 6: change “were” with “had” - Page 8 line 50: add “the” before “majority of susceptible…” and add “have” before “mild symptoms” - Page 9 lines 43-45: “…the prevalent age… than that in MP infection” English language sounds incorrect, please check - Page 9 line 49: “On the other hand…” please add “other authors reported that some patients…” - Page 10 line 45: “in both groups”, delete “the” - Table 1: how was the difference in male to female ratio between the 2 groups calculated? I tried the chi square test and I could not find a statistical significance. It is strange that all the results are s.s. with $p<0.001$ - Figure 1: “The patients who were not satisfied with…” change into “The patients who did not satisfy…” The entire paper should be checked for the English language.

REVIEWER	Raymond Josette Hôpital Cochin, Paris, France
REVIEW RETURNED	23-Oct-2018

GENERAL COMMENTS	The aim of this study was to evaluate epidemiological and clinical relationship between MP infection and childhood recurrent wheezing episode (RWE). It was a retrospective study. One of the major problems of this study was the diagnostic criteria used to define M. pneumoniae infection. Radiological signs are not reliable . In addition, serology has its limits since IGM anti-M. pneumoniae can persist for a very long time (1 year or more). Diagnosis of M. pneumoniae infection is based on detection of M. pneumoniae by PCR in rhynopharyngeal aspirations and seroconversion.

	Specic comments: - The methods of diagnostic are not specified in the abstract - In the methods section: the diagnostic by "2 times titration of igM antibodies" need to be better explained The discussion could be shortened and better structured - Figure 2 and f 4 can be deleted

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

The paper is a retrospective study in which the authors seek a relationship between M. pneumoniae pneumonia and recurrent wheezing. The subject is interesting, and the number of enrolled subjects is quite high. However, I have some major concerns about several points.

1) My main concern is about the methodology of the study. Patients admitted between 2003 and 2014 for MP pneumonia or recurrent wheezing episodes were enrolled retrospectively. However, I cannot find clearly:

a. Inclusion and exclusion criteria

b. The clinical and laboratory information that was considered important for this study and the way it was collected (hospital records I guess, but please specify)

c. How MP pneumonia was treated; did any MP patient require ICU admission or have serious complication? Were there differences in subsequent wheezing episodes in different treatment groups?

d. How were previous wheezing episodes diagnosed? Were they documented by previous medical records?

A: We appreciate you request for clarity on the following points.

a, b & d. As shown previous our studies (references. 14, 15), Mycoplasma pneumoniae (MP) pneumonia patients were diagnosed and selected with a confirmed diagnosis via titration of anti-MP IgM at initial admission, and follow up during the course of the disease. Recurrent wheezing episode (RWE) was diagnosed based on medical records. We added sentences to include these issues, including exclusion criteria of MP pneumonia, in the Abstract and Method sections.

c. As for therapy, we have reported the effectiveness of early corticosteroids for the treatment of patients with MP pneumonia, including antibiotic non-responsive cases, since the nationwide epidemics in 2003. The therapy was effective, irrespective of the antibiotics used, and therefore we believe that the number of patients that ultimately needed treatment in the intensive care unit since 2003 have decline. There were no adverse reactions following steroid treatment (references, Lee KY, et al. Pediatr Pulmonol 2006;41:263-8, Youn YS, et al. BMC Pediatr 2010;10:48, and Youn YS, et al. Infect Chemother 2014;46:239-47).

2) Authors state that subsequent wheezing episodes were evaluated through medical records of outpatients' visits and readmissions, but no direct patient interview is mentioned, so that this information might be seriously biased (several wheezing episodes might have been missed and some patients might have chosen other clinics for follow-up visits).

A: We agree with the reviewer's comment, and certainly agree that a certain amount of selection bias is present which is a major disadvantage of retrospective studies. Therefore, we have included statements on this issue in the limitations section of the study within the manuscript.

3) Choosing to only include RWE requiring admission might also cause a bias in the number of the wheezing episodes, because the majority of patients who are visited in the ER for wheezing does not require hospital admission. The authors should explain why they decided to evaluate only children admitted for wheezing.

A. We again agree with the reviewer's comment on RWE patients. However, this study is a retrospective chart review study, and the admission policy on children presenting with wheezing in the ER or outpatient department was the same during the study period. RWE after MP infection was checked through search of medical records including outpatient records, as described in the Method section.

4) Another big concern is about the findings of the study. Authors state quite honestly that MP epidemics did not cause an increase in hospital admissions for recurrent wheezing episodes. A relationship was found between recurrent wheezing and wheezing as a symptom of MP pneumonia, but this is not really surprising, as it is well known that patients with recurrent wheezing will probably have exacerbations during respiratory infections.

A: Thank you for your comments on our results.

Minor points:

- Abstract: a definition of recurrent wheezing should be included, as well as the way in which subsequent wheezing episodes were recorded.

A: We added the definition of recurrent wheezing, and the way in which subsequent wheezing episodes were recorded in the Abstract section, as recommended.

- Page 2 line 38: delete "were"

- Page 4 line 46: "adults and children with asthma"

- Page 5 methods: specify how MP pneumonia was excluded in patients affected by

RWE

- Page 5 lines 49-51: "…and 37 cases… present study." Probably this part of the sentence should be deleted.

A: We corrected these points, as indicated

- Page 6, results: the number of total admissions during the study period should be reported.

A: According to the electronic data of our hospital, the total number of admitted patients during the study period at our department was a total 25,911, which was approximately 2,100 admitted patients per year. However, we did not add these findings in the manuscript because of the possibility of uncertainty, and we ask for your kind understanding on this point.

- Page 6 lines 30-32: the number of MP pneumonia patients is much higher than that of RWE patients and this should be explained; is it may be due to the fact that most of the patients with wheezing are not admitted to the hospital ward?

A: As described in the Discussion section, the numbers of patient with RWE during the recent decade did not change, with a rather decreasing trend in the recent years. Moreover, the severity of asthma

presenting in children has changed to a milder phenotype in Korea over time, during an observation period of 3 decades at a single hospital. The severity of asthma may be different in the populations, and admission policies of MP pneumonia and RWE may be somewhat different in Korea and Western countries.

- Page 7 lines 36-39: differences in these laboratory findings do not really add important information to the work and reporting them in table 1 is enough. I would delete this sentence ("In laboratory indices…RWE group").

A: We deleted this sentence.

- Page 8 line 6: change "were" with "had"

- Page 8 line 50: add "the" before "majority of susceptible…" and add "have" before "mild symptoms"

- Page 9 lines 43-45: "…the prevalent age… than that in MP infection" English language sounds incorrect, please check

Page 9 line 49: "On the other hand…" please add "other authors reported that some patients…"

- Page 10 line 45: "in both groups", delete "the"

A: In Korea, since a majority of children may have been affected by RSV infection during infancy and early childhood as well as in other populations, it is possible that majority of MP infected patients have experienced RSV infection prior to MP infection. We corrected the errors and changed the sentences as you indicated.

- Table 1: how was the difference in male to female ratio between the 2 groups calculated? I tried the chi square test and I could not find a statistical significance. It is strange that all the results are s.s. with $p<0.001$

A; We found that there was a mistake in recording of number in Table 1 (number of 156 instead of 256). We have re-performed statistical analyses, and there were no faults in the statistical data, and corrected the mistake in Table 1.

- Figure 1: "The patients who were not satisfied with…"change into "The patients who did not satisfy…"

The entire paper should be checked for the English language.

A: We corrected as you have indicated, and the revised manuscript has been re-checked by a native English speaker.

Reviewer: 2

The aim of this study was to evaluate epidemiological and clinical relationship between MP infection and childhood recurrent wheezing episode (RWE). It was a retrospective study. One of the major problems of this study was the diagnostic criteria used to define M. pneumoniae infection. Radiological signs are not reliable. In addition, serology has its limits since IgM anti-M. pneumoniae can persist for a very long time (1 year or more). Diagnosis of M. pneumoniae infection is based on detection of M. pneumoniae by PCR in rhinopharyngeal aspirations and seroconversion.

A: Thank you for your insightful comments on our article and the unsolved issues regarding early diagnosis in MP infection.

Comments:

- The methods of diagnostic are not specified in the abstract

A: We added diagnosis criteria in the Abstract:

- In the methods section: the diagnostic by "2 times titration of igM antibodies" need to be better explained

A: We add sentences regarding diagnosis of MP infection as follows.

Briefly, diagnoses of MP pneumonia were made when patients showed seroconversion (negative to positive), 4-fold or greater increase in IgM titers, or high titers 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 titers were excluded when initial titers were < 1:320.

- The discussion could be shortened and better structured

- Figure 2 and f 4 can be deleted

A: We revised the sentences for a more clear discussion on the objective of the study.

We thought that these figures may be helpful, and ask for your understanding in including the figures in our manuscript.

VERSION 2 – REVIEW

REVIEWER	Arianna Dondi AOU S.Orsola-Malpighi Hospital Pediatric Emergency Unit Bologna, Italy
REVIEW RETURNED	04-Dec-2018

GENERAL COMMENTS	The paper has improved after revision. I would still add the number of total admissions in the study period, even if the authors prefer not to (see page 6, results, previous review).

REVIEWER	Josette Raymond Université Paris 5, Hôpital Cochin, Paris
REVIEW RETURNED	13-Dec-2018

GENERAL COMMENTS	The definition of Mycoplasma pneumoniae infection is still a problem. If a seroconversion (IGM negative to positive) supports the diagnosis of infection, elevated levels of IgG in two different specimens without IgM can not confirm the infection. In addition, the authors did not remove the two figures as requested

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

The paper has improved after revision. I would still add the number of total admissions in the study period, even if the authors prefer not to (see page 6, results, previous review).

R: We appreciate your comments. We add the sentences as you indicated: 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.

Reviewer 2

The definition of Mycoplasma pneumoniae infection is still a problem. If a seroconversion (IGM negative to positive) supports the diagnosis of infection, elevated levels of IgG in two different specimens without IgM cannot confirm the infection. In addition, the authors did not remove the two figures as requested.

R: We appreciate your comments.

During the early stage of initial MP infection, anti-MP IgM antibodies appear first, and anti-MP IgG antibodies follow soon after IgM appearance, and IgM and IgG levels gradually increase in the acute stage of MP infection, as well as in other systemic viral infections. Thus, we thought that elevated levels of IgG in two different specimens without IgM cannot confirm the initial MP infection, and it may interpret as past MP infection.

We confirmed these findings in recent 2015-16 epidemic, using 2 serologic tests: Serodia-Myco II (microparticle agglutination test) using in this study, and an enzyme-linked immunoassay (EIA) for anti-IgG and IgM, 2-times examinations during hospitalization. All patient who had initially IgM negative showed all IgG negative, and in the 2nd examination, majority of patients showed IgG sero-conversion (negative to positive), including initially IgM positive with lower titers. Additionally, we found that the positive numeral values of IgM in EIA were correlated with titers of Serodia-Myco II through 2-times examination (unpublished observation). It is well known that the diagnosis of MP pneumonia in the early stage is problematic because of anti-MP IgM, as well as anti-IgG, negative in some patients, especially severely affected patients (Please, refer to Ref. 15). Further, we have experienced that PCR positive rates are less than 50% in each epidemic, compared to 2-times serologic test. Thus, we believe that two-times examination of anti-MP IgM in the early stage of MP infection may be reasonable for diagnosis of MP pneumonia and patient selection.

- We believe that figure 2 is an essential part in this epidemiologic study, and we removed figure 4 as you indicated. We hope your kind understandings.