Journal of Tropical Pediatrics, 2020, 00, 1–5
doi: 10.1093/tropej/fmaa109
Original Paper

Time to *Mycoplasma Pneumoniae* RNA Clearance for Wheezy vs. Non-Wheezy Young Children with Community-Acquired Pneumonia

Jiande Chen,[1#] MD, Fengjuan Ji,[1,2#] MM, Yong Yin,[1] MD, *and* Shuhua Yuan,[1] PhD

[1]Department of Respiratory Medicine, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, Pudong, Shanghai 200127, China
[2]Department of Neonatology, Women's Hospital School of Medicine, Zhejiang University, Shangcheng, Hangzhou, Zhejiang 310006, China
[#]The first two authors contributed equally to this work.
Correspondence: Yong Yin, Department of Respiratory Medicine, Shanghai Children's Medical Center Affiliated to Shanghai Jiao Tong University School of Medicine, No.1678 Dongfang Road, Pudong, Shanghai 200127, China. Tel/Fax: +86-21-38625703.
E-mail: yinyong9999@163.com.

ABSTRACT

Objectives: We sought to investigate the dynamics of *Mycoplasma pneumoniae* (Mp) RNA in hospitalized young children with community-acquired pneumonia (CAP) and to explore whether Mp RNA clearance differed for wheezy and non-wheezy group after the onset of azithromycin treatment.
Methods: We included hospitalized young children (1–72 months of age) with CAP caused by Mp infection. Mp RNA was detected as soon as the patient was admitted and the dynamics of Mp-RNA were monitored after the beginning of azithromycin treatment on Days 4, 7, 14 and 28.
Results: Among 40 hospitalized young children with *Mycoplasma pneumoniae* pneumonia (Mpp), 16 had wheezing. Time to first positive Mp-RNA confirmation after symptom onset of Mpp was similar for the wheezy group (median 7 days, interquartile range 7–10.5) and the non-wheezy group (median 7 days, interquartile range 5.8–8.3). The duration of positive Mp-RNA detection after the onset of azithromycin treatment was shorter among the wheezy group than in the non-wheezy group (median 4 vs. 7 days; hazard ratio 2.083; 95% confidence interval: 1.023–4.244).
Conclusions: Mp-RNA clearance was significantly faster among Mpp young children with wheezing than in those without wheezing after the onset of azithromycin treatment.

LAY SUMMARY

We sought to investigate the dynamics of *Mycoplasma pneumoniae* (Mp) RNA in hospitalized young children with community-acquired pneumonia and to explore whether Mp RNA clearance differed for wheezy and non-wheezy group after the onset of azithromycin treatment. Our study suggested

that Mp-RNA clearance was significantly faster among *Mycoplasma pneumoniae* pneumonia young children with wheezing than in those without wheezing after the onset of azithromycin treatment.

KEYWORDS: community-acquired pneumonia, *Mycoplasma pneumoniae*, RNA, wheezing, dual amplification

INTRODUCTION

Mycoplasma pneumoniae (Mp) was one of the most commonly detected bacterial pathogen among hospitalized children with community-acquired pneumonia (CAP) [1, 2]. Although it was thought that Mp was more commonly detected in children over 5 years old, Mp may play an important role as a cause of respiratory tract infections also in younger children. The prevalence of *Mycoplasma pneumoniae* pneumonia (Mpp) among hospitalized young children has been found to be 17% [3]. In pediatric practice, wheezing is likely to be developed in Mpp patients [1, 4, 5] and has been observed to be significantly associated with Mpp [5]. Although the duration of Mp DNA in hospitalized children with Mpp has been investigated among children aged 3 months to 14 years [6], the duration of Mp RNA in hospitalized young children with Mpp is unknown. The aim of this study was to investigate the dynamics of Mp RNA in hospitalized young children with Mpp and to explore whether Mp-RNA clearance was different between wheezy and non-wheezy group after the onset of azithromycin treatment.

MATERIALS AND METHODS

Study design

We monitored on the time for Mp-RNA negative conversion after receiving intravenous azithromycin (10 mg/kg/day for 5 consecutive days) on Days 4, 7, 14 and 28. This study was conducted in Shanghai Children's Medical Center and was in accordance with the guidelines of the Declaration of Helsinki. The study was approved by the Ethics Committee of Shanghai Children's Medical Center. Informed consent was obtained from the legal guardian/s.

Study subjects

We included hospitalized young children (1–72 months of age) with CAP caused by Mp infection between August 2017 and January 2018. CAP was diagnosed by the presence of fever, acute respiratory symptoms (cough, tachypnea, difficult breathing) or both, plus presence of new infiltrate on chest radiography or consolidation [7]. According to the Guidelines for management of CAP in children (the revised edition of 2013) (II) [8], non-severe CAP patients did not present any of the following items: bad general health status, dehydration, inability to drink, disturbance of consciousness, significantly increased respiratory rate, cyanosis, dyspnea, multi lobes or $\geq$2/3 of the lung involvement, pleural effusion, blood oxygen saturation $\leq$92% and extra pulmonary complications. Mp infection was defined as positive detection of Mp RNA. Diagnosis of Mpp was based on diagnosis of CAP and Mp infection. Children with no evidence of Mpp, other co-infected pathogen(s) (respiratory viruses, bacteria and *Chlamydia pneumonia*), chronic pulmonary disease, poor compliance (defined as inability to come in for follow-up visits) or immunodeficiency were excluded. Wheezing referred to a continuous high-pitched sound coming from the chest during expiration [9], and was determined by respiratory physicians' careful auscultation in the study.

Pathogen RNA detection

RNA of pathogens (including respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza virus, *C.pneumonia* and Mp) was detected using MultiResPathogen Nucleic Acid Assay Kit (Dual Amplification) (Wuhan Zhongzhi Biotechnologies Inc., Hubei, China) according to the manufacturer's instructions. Briefly, throat swab samples were collected by sterile cotton-tipped sticks and placed into a transport vial. Released pathogen nucleic acids from lysed cells were reverse transcribed to cDNA using reverse transcriptase followed by being transcribed to RNA using T7 RNA polymerase. The amplified RNA products were captured by the capture probes and anchored in the micropores. One end of the specific probe was bound to the RNA

Table 1. Characteristics of included children in this study

Characteristic	Wheezing ($n = 16$)	Non-wheezing ($n = 24$)	Total ($n = 40$)
Age (months), median (interquartile range)	27 (10.75–39)	28.5 (15.75–36)	28.5 (11.25–36)
Male/female	11/5	12/12	23/17
Length of hospital stay (days), median (interquartile range)	6 (5–9.25)	7 (6–9)	7 (6–9)

product, and the other end was combined with the amplification probe which was subsequently linked to streptavidin–horseradish peroxidase (HPR) conjugates. Chemiluminescence substrate of HPR was added to the capture probe—RNA amplification product—specific probe—amplification probe—streptavidin–HPR complex and the signal was detected. Patients underwent pathogen RNA detection as soon as they were admitted. After the beginning of azithromycin treatment which was given immediately when Mp infection was confirmed, consecutive Mp-RNA detection was performed on Days 4, 7, 14 and 28.

Statistical analysis

Sex comparison between the groups was performed using the χ^2 test. Age comparison and comparison of the length of hospital stay were performed using Mann–Whitney *U* test. Times to positivity for Mp-RNA detection were described with median, interquartile range and range values. We used the Mantel Haenszel hazard ratio (HR) with 95% confidence interval (CI) to compare the time of Mp-RNA negative conversion on the basis of wheezing. Statistical significance was determined as a two-sided p-value < 0.05. Statistical analyses were performed by using SPSS software 25.0 (IBM SPSS Statistics, Armonk, NY, USA).

RESULTS

Clinical characteristics

During the 6-month study period, we included 40 non-severe CAP participants with Mp infection; of these, 16 (40%) had wheezing. None of the included participants needed the use of oxygen or had a history of asthma. The characteristics of included children were listed in Table 1. No significant difference was observed in sex, age and the length of hospital stay between participants with wheezing and without wheezing.

Mp-RNA confirmation and clearance

Time to first positive Mp-RNA confirmation after symptom onset of Mpp was similar for the wheezy group (median 7 days, interquartile range 7–10.5) and the non-wheezy group (median 7 days, interquartile range 5.8–8.3) (Fig. 1). Mp-RNA clearance after the beginning of azithromycin treatment ranged from 4 to 28 days with a median of 7 days (interquartile range 4–8.75). When stratified by wheezing, the duration of positive Mp-RNA detection after the beginning of azithromycin treatment was shorter among the wheezy group than in the non-wheezy group (median 4 vs. 7 days; HR 2.083; 95% CI: 1.023–4.244; Fig. 2).

DISCUSSION

This is the first study that shows the dynamics of Mp RNA in hospitalized young children with Mpp after the onset of azithromycin treatment.

Several factors have been confirmed to affect Mp clearance. For example, the duration of positive Mp-DNA detection was correlated with the length of hospital stay and was longer in children with severe Mpp than in those with mild disease [6]. Asthmatic and non-asthmatic airways might differ in Mp clearance mechanisms [10], which may also lead to different duration of Mp-DNA or RNA positivity. In our study, these were well balanced between wheezy and non-wheezy groups.

Time to pathogen confirmation after symptom onset of Mpp was similar for the wheezy and the non-wheezy groups. This suggests that from the date of symptom onset of CAP, wheezing does not affect the first positive detection time of Mp RNA and the

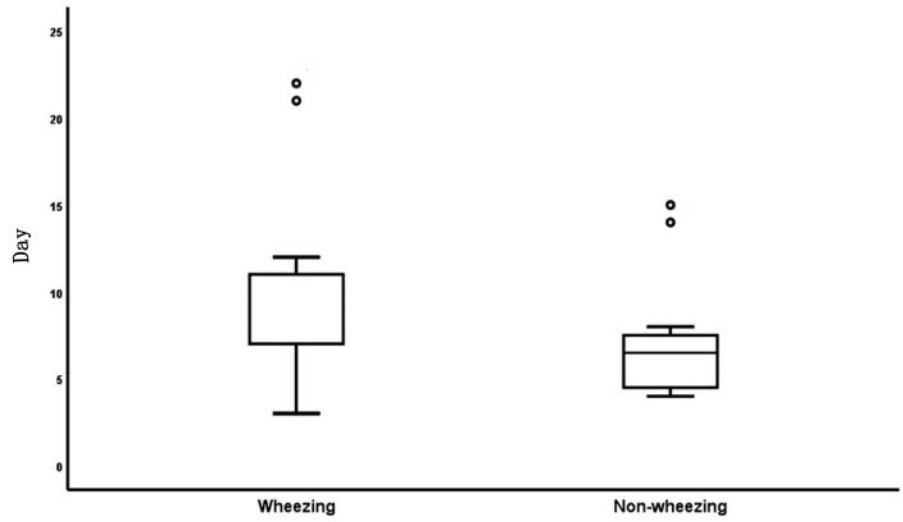

Fig. 1. Time to Mp-RNA confirmation for the wheezy and the non-wheezy groups.

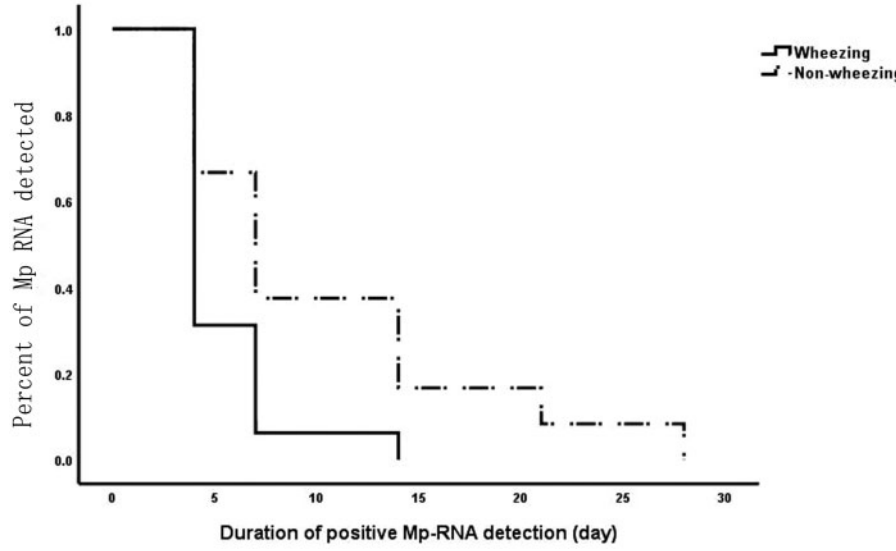

Fig. 2. Kaplan–Meier survival analysis of the duration of positive Mp-RNA detection for the wheezy and the non-wheezy groups.

time to diagnose Mpp. And this resulted in the same baseline for the dynamics of Mp RNA before the onset of azithromycin treatment among the wheezy and the non-wheezy groups.

In other studies, the median duration of Mp-DNA positivity was 5–7 weeks after disease onset, although patients received macrolides treatment [6, 11]. However, the duration of Mp-RNA positivity after the onset of azithromycin treatment in our study was shorter (within 4 weeks). What's more, real-time PCR is incapable of distinguishing live bacteria from the dead ones, and its products are DNA, suggesting an increased risk of laboratory contamination and false-positive results. Given these

drawbacks, Mp-RNA testing might be more suitable than Mp-DNA testing for monitoring Mp clearance in future studies.

The finding of a shorter duration of positive Mp-RNA detection in the wheezy group after the onset of azithromycin treatment when compared with the non-wheezy group is intriguing and suggests an adequate antibiotic treatment could shorten the period of persistence of Mp RNA in wheezy patients. One study has reported that wheezing at presentation was associated with a low bacterial load [12], which may partly explain why the same azithromycin treatment could shorten the duration of positive Mp-RNA detection in the wheezy group when compared with the non-wheezy group. And the severity of Mpp depends on the Mp bacterial load [13]. Based on this, wheezing may not worsen Mpp, and macrolide therapy should be shorter in treating wheezy patients.

The findings of the present study have a limitation. Bacterial load of Mp in hospitalized young children with Mpp was not determined because the method of Mp-RNA detection in this study was qualitative, but not quantitative.

The present results show that after treatment with azithromycin, Mp-RNA clearance was significantly faster among Mpp young children with wheezing than in those without wheezing.

ACKNOWLEDGEMENTS

The authors would like to thank Wuhan Zhongzhi Biotechnologies Inc. for the excellent technical assistance and to thank the support from Pfizer China.

FUNDING

This study was funded by Scientific research project of Shanghai Pudong New Area Health Committee (No. PW2017E-1), Scientific research projects of Shanghai Science and Technology Commission (No. 19441909000), and Investigation and study on the prevalence of Mycoplasma pneumoniae infection and macrolides resistance in outpatient and hospitalized children with acute respiratory illness in China (Shanghai Children s Medical Center).

REFERENCES

1. Kutty PK, Jain S, Taylor TH, *et al. Mycoplasma pneumoniae* among children hospitalized with community-acquired pneumonia. Clin Infect Dis 2019;68:5–12.
2. Jain S, Williams DJ, Arnold SR, *et al.* Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med 2015;372:835–45.
3. Bezerra PG, Britto MC, Correia JB, *et al.* Viral and atypical bacterial detection in acute respiratory infection in children under five years. PLoS One 2011;6: e18928.
4. Han MS, Yun KW, Lee HJ, *et al.* Contribution of co-detected respiratory viruses and patient age to the clinical manifestations of *Mycoplasma pneumoniae* pneumonia in children. Pediatr Infect Dis J 2018;37:531–6.
5. Medjo B, Atanaskovic-Markovic M, Radic S, *et al. Mycoplasma pneumoniae* as a causative agent of community-acquired pneumonia in children: clinical features and laboratory diagnosis. Ital J Pediatr 2014;40:104.
6. Han X, Li S, Lu S, *et al.* Amplification of 16S rDNA by nested PCR for measurement of *Mycoplasma pneumoniae* DNA over time: clinical application. J Med Microbiol 2012;61:426–30.
7. McIntosh K. Community-acquired pneumonia in children. N Engl J Med 2002;346:429–37.
8. Subspecialty Group of Respiratory Diseases of the Society of Pediatrics of Chinese Medical Association, the Editorial Board of Chinese Journal of Pediatrics. Guidelines for management of community acquired pneumonia in children(the revised edition of 2013) (II). Zhonghua Er Ke Za Zhi 2013;51:856–62.
9. Kudo K, Takasaki J, Manabe T, *et al.* Systemic corticosteroids and early administration of antiviral agents for pneumonia with acute wheezing due to influenza A(H1N1)pdm09 in Japan. PLoS One 2012;7:e32280.
10. Ledford JG, Mukherjee S, Kislan MM, *et al.* Surfactant protein-A suppresses eosinophil-mediated killing of *Mycoplasma pneumoniae* in allergic lungs. PloS One 2012; 7:e32436.
11. Nilsson AC, Bjorkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute *Mycoplasma pneumoniae* infection and reveals a high rate of persistent infection. BMC Microbiol 2008;8:93.
12. Jiang W, Yan Y, Ji W, *et al.* Clinical significance of different bacterial load of *Mycoplasma* pneumoniae in patients with *Mycoplasma pneumoniae* pneumonia. Braz J Infect Dis 2014; 18:124–8.
13. Nilsson AC, Bjorkman P, Welinder-Olsson C, *et al.* Clinical severity of *Mycoplasma pneumoniae* (MP) infection is associated with bacterial load in oropharyngeal secretions but not with MP genotype. BMC Infect Dis 2010;10:39.