BMJ Open

Is presence of bronchial mucus plugs associated with longer radiographic resolution in patients with refractory Mycoplasma Pneumoniae pneumonia? A prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	Draft
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Huang, Lizhen ; Children's Hospital of Soochow University, Department of Respiratory Medicine; First People's Hospital of Wujiang District Huang, Xia ; Children's Hospital of Nanjing Medical University Jiang, Wujiang; Children's Hospital of Soochow University, Department of Respiratory Medicine Zhang, Rong; Children's Hospital of Soochow University, Department of Respiratory Medicine Yan, Yongdong; Children's Hospital of Soochow University, ; Huang, Li ; Children's Hospital of Soochow University, Department of Respiratory Medicine
Keywords:	Mucus plugs, refractory Mycoplasma Pneumoniae pneumonia, radiographic resolution

Title page

Is presence of bronchial mucus plugs associated with longer radiographic resolution in patients with refractory Mycoplasma Pneumoniae pneumonia? A prospective cohort study

Lizhen Huang[1,*], Xia Huang[2,*], Wujun Jiang[2], Rong Zhang[2], Yongdong Yan[2], Li Huang[2]

[1] Children's Hospital of Soochow University, Suzhou, China; First People's Hospital of Wujiang District, Suzhou, China.

[2] Children's Hospital of Nanjing Medical University, Nanjing, China

[3] Children's Hospital of Soochow University, Suzhou, China

*These authors contributed equally to this work.

Corresponding author: Yongdong Yan, E-mail: suzhoupulmonology@163.com, Tel +86 512 80698312, Fax +86 512 80698312, or Li Huang, E-mail: szdalv@163.com, Tel +86 512 80698312, Fax +86 512 80698312.

Abstract

Objectives: To examine prospectively the radiographic clearance of Refractory Mycoplasma Pneumoniae Pneumonia (RMPP) in immunocompetent children, and to identify independent predictors of time to complete radiographic resolution in patients with RMPP.

Design and setting: RMPP patients treated with bronchoscopy were prospectively enrolled in the study between Jan 2011 and Dec 2015. Multivariate logistic regression was performed to identify independent predictors of time to complete radiographic resolution.

Results: Of the 187 RMPP patients, Bronchial mucus plugs formation was detected in 73 (39.0%) patients. C-reaction protein (CRP) ≥50 mg/L, lactate dehydrogenase (LDH) ≥480 U/L, Fever duration ≥10 days and presence of mucus plugs were associated with a longer time to radiographic clearance (all $P < 0.01$). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

Conclusions: Clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic clearance in patients with RMPP.

Strengths and limitations of this study:

- This is the first study to analyze prospectively the risk factors associated with longer time to radiographic clearance in patients with RMPP.

- Serial posteroanterior and lateral chest radiographs were obtained every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.
- Our study was a single-center based study, which might have potential biases and a multi-center study is needed in the future.

Key words: Mucus plugs; refractory Mycoplasma Pneumoniae pneumonia; radiographic resolution.

Text

Mycoplasma pneumoniae (M. pneumoniae) is a common etiology of childhood community-acquired pneumonia (CAP) [1, 2]. M. pneumoniae infections are usually mild, while in recent decades, pediatricians are facing increasing numbers of refractory Mycoplasma Pneumoniae pneumonia (RMPP) patients. RMPP often show no improvement in clinical and radiological findings despite of appropriated macrolides treatment. Corticosteroids has been proved to be effective in treating RMPP [3, 4]. However, despite the use of corticosteroids, some patients with RMPP still have fever persisting and radiological deterioration. They required investigation using bronchoscopy under which mucus plug formation is often detected[5, 6].

We encountered several cases of RMPP who had mucus plug formation under bronchoscopy. RMPP, especially RMPP with mucus plug may have a longer radiographic resolution time. Some patients may even complicated by longstanding pulmonary sequelae such as bronchiectasis[7, 8]. No investigations have been reported with careful statistical consideration given to the prognostic significance of factors in the radiographic resolution of RMPP. we sought to examine prospectively the radiographic clearance of RMPP in immunocompetent children. The risk factors associated with longer time to radiographic clearance in RMPP patients were analyzed.

Methods

Cohort Description

Patients with CAP who hospitalized in Department of Respiratory Medicine in Children's Hospital of Soochow University from January 1, 2012 to December 31, 2015, were evaluated prospectively for identifying patients who met criteria for RMPP. RMPP was considered when (1). Cough, fever or auscultatory findings together with pulmonary infiltrates on chest radiograph, (2). A significant rise in M. pneumoniae IgG or seroconversion in paired sera, together with M. pneumoniae DNA detected in nasopharyngeal aspirates, (3). Fever persisting (>38.5°C) and radiological deterioration after the therapy of macrolide for 7 days or more. Bronchoscopy was indicated when lobar consolidation or atelectasis persisted on chest X-ray film after corticosteroid theray for 1 week. Patients aged from 1 months to 14 years were eligible for participation. The following patients were excluded from the study: (1) patients with bronchopulmonary dysplasia, congenital heart diseases, immunodeficiency and heredity neurological disorders; (2) those have evidence of co-infection with other pathogens. The study was approved by the Institutional Review Board of Suzhou University, and informed consent was obtained for all participants or their parents. Chest X rays films were followed up after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.

Diagnostic tests for M. pneumoniae

Nasopharyngeal aspirates were obained within 1 day after the patients admitted. As described previously, specimens were tested to amplify the M. pneumoniae P1 adhesin gene by using PCR analysis[6]. A quantitative M. pneumoniae DNA diagnostic

kit (DaAn Gene Co., Ltd. Guangzhou, China) was used. The target specific for M. pneumoniae genome is 16S rRNA gene.

The paired serum samples were taken at admission and at least two weeks after the first serum. The serum samples were tested for IgM and IgG antibodies against M. pneumoniae using a ELISA kit (Serion ELISA MP IgG/IgM, Institute Virion/Serion, Germany). The cut-off value was 0.5×mean optical density (OD) of control serum of the kit. As described previously, a significant rise in IgG titre was defined as a doubling of the OD value above the cut-off. A sero-conversion was defined as the first serum was negative, and the second serum had an OD at least twice the cut-off [6].

Data Collection and Interpretation of Radiographs

Serial posteroanterior and lateral chest radiographs were obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved. All radiographs were evaluated independently by two radiologists (PP and WLG), who did not know the patients' clinical condition. Chest radiographs were reviewed by two radiologists in sequence with the prior films for comparison. If differences in interpretation of radiographs occurred, it would be resolved by joint consensus between the two radiologists. The radiographs were reviewed for the presence of consolidation, atelectasis and pleural disease (effusion or thickening). Consolidation, atelectasis and pleural disease were defined by standard radiographic criteria[9].

Clinical and laboratory data regarding gender, age, fever duration, length of hospital stay, White blood cell (WBC) count, percentage of neutrophils (%

neutrophils), platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction protein (CRP) were collected.

Statistical Analyses

For continuous variables, comparison of means was conducted by using the t test. For ordinally scaled data, the Wilcoxon rank sum test was used. For categorical variables, the chi-square or Fisher exact test were used. A univariate analysis for 8 influence factors (age, sex, WBC, CRP, LDH, number of involved lobes [unilobar vs multilobar involvement], presence of pleural effusion and presence of mucus plug) was performed. Multiple regression analysis was performed to select the variables associated with time to complete radiographic resolution. Probabilities of .05 or less were considered significant.

Results

A total of 223 RMPP patients were qualified for enrollment in the study. Twenty-one (9.4%) refused to participate, and 15 (6.7%) agreed to participate but did not return for their follow-up chest radiographs. Finally, 187 patients were recruited and received follow-up chest radiographs. These patients will be referred to as the study group. The patients who were eligible but excluded (n = 36) demonstrated no statistically significant difference in age and sex compared with the studied patients (n=187, both P>0.05). There was also no significant difference in presence of mucus plug in the study group and unenrolled group (P=0.44).

The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2y. Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar

involvement, while 88 (47.1) had multilobar involvement. Forty- six (24.6%) had pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%) patients (Figure 1). The median fever duration was 10 (7, 13) days and the median length of hospital stay was 11 (9, 17) days.

Approximately half of the patients had complete radiographic clearance by 4 weeks, and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and eighty-three (97.9%) had complete clearance at the end of the study period, and four (0.5%) had persistent abnormalities at 24 weeks (Table 2). The median time to radiographic clearance of all participants was 4 weeks (IQR:4–8 weeks). Twenty-seven percent of the subjects had a time to radiographic clearance for >8 weeks. In unadjusted analysis, time to radiographic clearance for >8 weeks was associated with % neutrophils, CRP, LDH, pleural effusion, mucus plug, fever duration when compared with those with time to radiographic clearance ≤8 weeks (all $P < 0.01$; Table 3). Other variables (sex, age, lobar involvement, WBC) showed no difference.

The multivariable logistic regression model for time to radiographic clearance for >8 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant predictors of a longer time to radiographic clearance were C-reaction protein ≥50 mg/L, LDH ≥480 U/L, Fever duration ≥10 days and presence of mucus plugs. (all $P < 0.01$). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

Discussion

This is the first study, to our knowledge, to analyze prospectively the risk factors associated with longer time to radiographic clearance in patients with RMPP. CRP, LDH, fever duration and presence of mucus plugs were independently associated with longer time to radiographic clearance.

M. pneumoniae infection is a common respiratory disease in children[10]. In recent years, an increasing number of RMPP patients are being reported, especially in the Asian countries[4, 11-14]. The role of mucus plug in refractory RMPP have been extensively studied recently[5, 6, 15]. Xu et al. identified age, fever duration, LDH and CRP as independent risk factors for mucus plug formation[5]. Wang et al. found that in the bronchoscopic imaging, the mucus plug served as a promising predictor for early RMPP diagnosis for pediatric patients with large pulmonary lesions[15]. Our previous study also found that RMPP patients with mucus plug were prone to be corticosteroid-resistant, and had a longer fever duration and hospital stay[6]. Our study further highlighted the role of mucus plugs in the time to radiographic clearance in RMPP patients.

In our study, we found that presence of mucus plugs was associated with longer time to radiographic clearance in RMPP patients. Liang and her colleagues found that MPP with severe cilia abnormalities was associated with a longer time to radiographic clearance[8]. In Liang's study, they mainly discussed cilia abnormalities, while they did not focus on the mucus plugs in the bronchoscopic findings. Mucus plugs formation was actually a manifestation of severe cilia abnormalities. Severe cilia abnormalities

disrupt the mucociliary clearance and reduce the airway immune function, causing mucus plug that is responsible for the development of atelectasis and delayed radiographic resolution[10, 16]. In RMPP patients, atelectasis is quite common radiographically. The persistent presence of atelectasis lead to a longer radiographic resolution time and even longstanding pulmonary sequelae such as bronchiectasis or bronchiolitis obliterans[17-19]. Thus, careful management and follow-up is needed for the patients with mucus plugs. Additional therapeutic interventions of bronchoscopy may be acquired to remove the mucous plug completely.

The study has some limitations. First, our study was a single-center based study, which might have potential biases and a multi-center study is needed in the future. Second, there might be some patients who had a co-infection with other pathogens which could not be detected and might therefore lead to longer radiographic clearance. Third, the serums and nasopharyngeal samples were not collected on the same day after disease onset, which might produce bias.

In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic clearance in patients with RMPP.

Acknowledgements: We thank all participants of this study.

Contributors: YDY conceived and designed the study. RZ and WJJ conducted the study. LZH and XH analyzed the data and interpreted the data. LH provided guidance on the data analysis. All authors drafted the manuscript and read, edited and approved

the final version of the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

This work was supported by the Science and Technology Program of Suzhou (grant numbers SYS201641 and SYS201558); Science and Technology Projects for the Youth of Suzhou (grant numbers KJXW2015013) and Research project of provincial health and Family Planning Commission (grant numbers H201622).

Competing interests: None declared.

Patient consent: Parental/guardian consent obtained.

Ethics approval: This research project was reviewed and approved by the Institutional Review Board of Suzhou University.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

Reference

1. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. *FEMS Microbiol Rev* 2008;32:956-73.

2. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev* 2004;17:697-728.

3. Lee KY, Lee HS, Hong JH, et al. Role of prednisolone treatment in severe Mycoplasma pneumoniae pneumonia in children. *Pediatr Pulmonol* 2006;41:263-8.

4. You SY, Jwa HJ, Yang EA, Kil HR, Lee JH. Effects of Methylprednisolone Pulse Therapy on Refractory Mycoplasma pneumoniae Pneumonia in Children. *Allergy Asthma Immunol Res* 2014;6:22-6.

5. Xu Q, Zhang L, Hao C, et al. Prediction of Bronchial Mucus Plugs Formation in Patients with Refractory Mycoplasma Pneumoniae Pneumonia. *J Trop Pediatr* 2017;63:148-54.

6. Yan Y, Wei Y, Jiang W, Hao C. The clinical characteristics of corticosteroid-resistant refractory Mycoplasma Pneumoniae pneumonia in children.

Sci Rep 2016;6:39929.

7. Zhang Y, Chen Y, Chen Z, et al. Effects of bronchoalveolar lavage on refractory Mycoplasma pneumoniae pneumonia. *Respir Care* 2014;59:1433-9.

8. Liang H, Jiang W, Han Q, Liu F, Zhao D. Ciliary ultrastructural abnormalities in Mycoplasma pneumoniae pneumonia in 22 pediatric patients. *Eur J Pediatr* 2012;171:559-63.

9. Milne EN. Correlation of physiologic findings with chest roentgenology. *Radiol Clin North Am* 1973;11:17-47.

10. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the Respiratory Tract and Beyond. *Clin Microbiol Rev* 2017;30:747-809.

11. Wang M, Wang Y, Yan Y, et al. Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. *Int J Infect Dis* 2014;29:18-23.

12. Yang M, Meng F, Wang K, et al. Interleukin 17A as a good predictor of the severity of Mycoplasma pneumoniae pneumonia in children. *Sci Rep* 2017;7:12934.

13. Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The Clinical Characteristics and Predictors of Refractory Mycoplasma pneumoniae Pneumonia in Children. *PLoS One* 2016;11:e0156465.

14. Lu A, Wang C, Zhang X, Wang L, Qian L. Lactate Dehydrogenase as a Biomarker for Prediction of Refractory Mycoplasma pneumoniae Pneumonia in Children. *Respir Care* 2015;60:1469-75.

15. Wang L, Lu S, Feng Z, et al. The early examination of combined serum and imaging data under flexible fiberoptic bronchoscopy as a novel predictor for

refractory Mycoplasma pneumoniae pneumonia diagnosis. *Medicine (Baltimore)* 2017;96:e9364.

16. Prince OA, Krunkosky TM, Sheppard ES, Krause DC. Modelling persistent Mycoplasma pneumoniae infection of human airway epithelium. *Cell Microbiol* 2018;20.

17. Zhao C, Liu J, Yang H, Xiang L, Zhao S. Mycoplasma pneumoniae-Associated Bronchiolitis Obliterans Following Acute Bronchiolitis. *Sci Rep* 2017;7:8478.

18. Metaxas EI, Balis E, Papaparaskevas J, Spanakis N, Tatsis G, Tsakris A. Bronchiectasis exacerbations: The role of atypical bacteria, respiratory syncytial virus and pulmonary function tests. *Can Respir J* 2015;22:163-6.

19. Kim CK, Chung CY, Kim JS, Kim WS, Park Y, Koh YY. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. *Pediatrics* 2000;105:372-8.

Figure legends

Figure 1 Bronchoscopic findings of the patient with mucus plug.

Tables

Table 1 Descriptive Analysis of Demographic, Laboratory, Radiographic, and Bronchoscopic Findings of the Study Population

Variables	
Male to female ratio	102/85
Age in years, mean ± SD	6.1 ± 2.2
Unilobar disease	99 (52.9)
Multilobar disease	88 (47.1)
Pleural effusion, n (%)	46 (24.6)
White cell count, median (quartile), $\times 10^9$/L	7.8 (6.1, 11.0)
% neutrophils, median (quartile)	75.5 (61.1, 82.4)
C-reaction protein, median (quartile), mg/L	32.9 (12.4, 59.7)

Lactic dehydrogenase, median (quartile), U/L	669.5 (486.5, 789.1)
Bronchial mucus plugs formation, N%	73 (39.0)
Fever duration, median (quartile), days	10 (7, 13)
Length of hospital stay, median (quartile), days	11 (9, 17)

Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae* pneumonia

Period (week)	Remaining patients* (n=187)	Mucus plug group (n=73)	Non-mucus plug group (n=114)
0	187	73	114
4	91	48	43
8	53	28	25
12	18	13	5
16	6	5	1
20	4	4	0
24	4	4	0

*Patients remaining with abnormal radiographic findings.

Table 3 Demographic, laboratory, radiographic, and bronchoscopic findings of children with refractory Mycoplasma pneumoniae pneumonia, according to time

to radiographic clearance

Characteristics	Time to radiographic clearance≤ 8wk (n=134)	Time to radiographic clearance> 8wk (n=53)	P
Male/female	73/61	30/23	0.79
Age in years, mean±SD	5.7±2.5	6.3±2.9	0.47
White cell count, median (quartile), $\times 10^9$/L	8.4 (6.3,11.2)	8.54 (6,11.8)	0.91
% neutrophils, median (quartile)	60.3 (21.5, 66.4)	67.4 (22.1, 72.5)	<0.01
C-reaction protein, median (quartile), mg/L	11.8 (4.9,28.7)	22.9 (9.7,66.4)	<0.01
Lactic dehydrogenase, median (quartile), U/L	396 (326.6, 506.2)	515.9 (329.5,688.9)	<0.01
Multilobar disease, N%	11 (8.2)	6 (11.3)	0.51
Pleural effusion, N%	24 (17.9)	17 (32.0)	<0.01
Bronchial mucus plugs formation, N%	13 (9.7)	21 (39.6)	<0.01
Fever duration ≥10d, N%	26 (19.4)	23 (43.4)	<0.01

Table 4 Multivariable predictors of time to radiographic clearance > 8 weeks among 402 children with refractory Mycoplasma pneumoniae pneumonia

Characteristics	OR (95% CI)	P
C-reaction protein ≥50 mg/L	3.1 (1.7-5.2)	<0.01
Lactic dehydrogenase ≥480 U/L	2.8 (1.5-4.5)	<0.01
Fever duration ≥10d	13.5 (7.8~41.4)	<0.01

Bronchial mucus plugs formation	11.5 (2.5-45.7)	<0.01

354

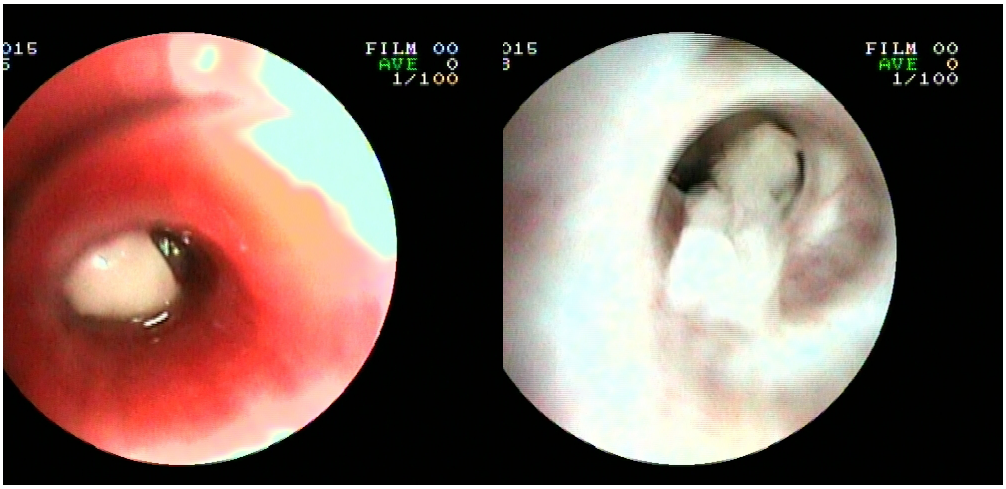


Figure1. Bronchoscopic findings of the patient with mucus plug.

352x169mm (72 x 72 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	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	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5 , 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5 , 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4 , 5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6 , 7
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	*6 , 7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	7
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	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7

		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7
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	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
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	8
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	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
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	10

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

BMJ Open

Is presence of bronchial mucus plugs associated with longer radiographic resolution in patients with refractory Mycoplasma Pneumoniae pneumonia? A prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023719.R1
Article Type:	Research
Date Submitted by the Author:	25-Jul-2018
Complete List of Authors:	Huang, Lizhen ; Children's Hospital of Soochow University, Department of Respiratory Medicine; First People's Hospital of Wujiang District Huang, Xia ; Children's Hospital of Nanjing Medical University Jiang, Wujiang; Children's Hospital of Soochow University, Department of Respiratory Medicine Zhang, Rong; Children's Hospital of Soochow University, Department of Respiratory Medicine Yan, Yongdong; Children's Hospital of Soochow University, ; Huang, Li ; Children's Hospital of Soochow University, Department of Respiratory Medicine
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Infectious diseases
Keywords:	Mucus plugs, refractory Mycoplasma Pneumoniae pneumonia, radiographic resolution

Title page

Is presence of bronchial mucus plugs associated with longer radiographic resolution in patients with refractory Mycoplasma Pneumoniae pneumonia? A prospective cohort study

Lizhen Huang[1,*], Xia Huang[2,*], Wujun Jiang[2], Rong Zhang[2], Yongdong Yan[2], Li Huang[2]

[1] Children's Hospital of Soochow University, Suzhou, China; First People's Hospital of Wujiang District, Suzhou, China.

[2] Children's Hospital of Nanjing Medical University, Nanjing, China

[3] Children's Hospital of Soochow University, Suzhou, China

*These authors contributed equally to this work.

Corresponding author: Yongdong Yan, E-mail: suzhoupulmonology@163.com, Tel +86 512 80698312, Fax +86 512 80698312, or Li Huang, E-mail: szdalv@163.com, Tel +86 512 80698312, Fax +86 512 80698312.

Abstract

Objectives: To examine prospectively the radiographic clearance of Refractory Mycoplasma Pneumoniae Pneumonia (RMPP) in immunocompetent children, and to identify independent predictors of time to complete radiographic resolution in patients with RMPP.

Methods: RMPP patients treated with bronchoscopy were prospectively enrolled in the study between Jan 2011 and Dec 2015. Serial chest radiographs were obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved. Multivariate logistic regression was performed to identify independent predictors of time to complete radiographic resolution.

Results: Of the 187 RMPP patients, Bronchial mucus plugs formation was detected in 73 (39.0%) patients. C-reaction protein (CRP) ≥50 mg/L, lactate dehydrogenase (LDH) ≥480 U/L, Fever duration ≥10 days and presence of mucus plugs were associated with a longer time to radiographic clearance (all $P < 0.01$). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

Conclusions: Clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic clearance in patients with RMPP.

Strengths and limitations of this study:

- This is the first study to analyze prospectively the risk factors associated with longer time to radiographic clearance in patients with RMPP in China.
- A prospective follow-up of chest radiographs was obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.
- There might be some patients who had a co-infection with other pathogens which could not be detected and might therefore lead to longer radiographic clearance.

Key words: Mucus plugs; refractory Mycoplasma Pneumoniae pneumonia; radiographic resolution.

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a common etiology of childhood community-acquired pneumonia (CAP) [1, 2]. *M. pneumoniae* infections are usually mild, while in recent decades, pediatricians are facing increasing numbers of refractory *Mycoplasma Pneumoniae* pneumonia (RMPP) patients. RMPP often show no improvement in clinical and radiological findings despite of appropriated macrolides treatment. Corticosteroids has been proven to be effective in treating RMPP [3, 4]. However, despite the use of corticosteroids, some patients with RMPP still have persisting fever and radiological deterioration. They required investigation using bronchoscopy[5, 6].

We encountered several cases of RMPP who had mucus plug formation under bronchoscopy. RMPP, especially RMPP with mucus plug may have a longer radiographic resolution time. Some patients may have longstanding pulmonary sequelae such as bronchiectasis[7, 8]. No investigations have been reported with careful statistical consideration given to the prognostic significance of factors in the radiographic resolution of RMPP. we sought to examine prospectively the radiographic clearance of RMPP in immunocompetent children. The risk factors associated with longer time to radiographic clearance in RMPP patients were analyzed.

Methods

Cohort Description

Patients with CAP who hospitalized in Department of Respiratory Medicine in Children's Hospital of Soochow University from January 1, 2012 to December 31, 2015, were evaluated prospectively for identifying patients who met criteria for RMPP. RMPP was considered when (1). Cough, fever or auscultatory findings together with pulmonary infiltrates on chest radiograph, (2). A significant rise in *M. pneumoniae* IgG or seroconversion in paired sera, together with *M. pneumoniae* DNA detected in nasopharyngeal aspirates, (3). Fever persisting (>38.5°C) and radiological deterioration after the therapy of macrolide for 7 days or more. Bronchoscopy was indicated when lobar consolidation or atelectasis persisted on chest X-ray film after corticosteroid therapy for 1 week. The study was approved by the Institutional Review Board of Suzhou University, and informed consent was obtained for all participants or their parents. Chest X rays films were followed up after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.

Patient and public involvement

From January 1, 2012 to December 31, 2015, the following patients were included in our study: (1) patients had cough, fever or auscultatory findings together with pulmonary infiltrates on chest radiograph; (2) age from 1 months to 14 years. The following patients were excluded from the study: (1) patients with bronchopulmonary dysplasia, congenital heart diseases, immunodeficiency and heredity neurological disorders; (2) those have evidence of co-infection with other pathogens.

Diagnostic tests for M. pneumoniae

Nasopharyngeal aspirates were obtained within 1 day after the patients admitted. As described previously, specimens were tested to amplify the *M. pneumoniae* P1 adhesin gene by using PCR analysis[6]. A quantitative *M. pneumoniae* DNA diagnostic kit (DaAn Gene Co., Ltd. Guangzhou, China) was used. The target specific for *M. pneumoniae* genome is 16S rRNA gene.

The paired serum samples were taken at admission and at least two weeks after the first serum. The serum samples were tested for IgM and IgG antibodies against *M. pneumoniae* using an ELISA kit (Serion ELISA MP IgG/IgM, Institute Virion/Serion, Germany). The cut-off value was 0.5×mean optical density (OD) of control serum of the kit. As described previously, a significant rise in IgG titre was defined as a doubling of the OD value above the cut-off. A sero-conversion was defined as the first serum was negative, and the second serum had an OD at least twice the cut-off [6].

Data Collection and Interpretation of Radiographs

Serial posteroanterior and lateral chest radiographs were obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved. All radiographs were evaluated independently by two radiologists (PP and WLG), who did not know the patients' clinical condition. Chest radiographs were reviewed by two radiologists in sequence with the prior films for comparison. If differences in interpretation of radiographs occurred, it would be resolved by joint consensus between the two radiologists. The radiographs were reviewed for the presence of consolidation, atelectasis and pleural disease (effusion or

thickening). Consolidation, atelectasis and pleural disease were defined by standard radiographic criteria[9].

Clinical and laboratory data regarding gender, age, fever duration, length of hospital stay, White blood cell (WBC) count, percentage of neutrophils (% neutrophils), platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction protein (CRP) were collected.

Statistical Analyses

For continuous variables, comparison of means was conducted by using the t test. For ordinally scaled data, the Wilcoxon rank sum test was used. For categorical variables, the chi-square or Fisher exact test were used. A univariate analysis for 8 influence factors (age, sex, WBC, CRP, LDH, number of involved lobes [unilobar vs multilobar involvement], presence of pleural effusion and presence of mucus plug) was performed. Multiple regression analysis was performed to select the variables associated with time to complete radiographic resolution. Probabilities of .05 or less were considered significant.

Patient and public involvement

Patients with CAP who hospitalized in Department of Respiratory Medicine in Children's Hospital of Soochow University from January 1, 2012 to December 31, 2015

Results

There were totally 8482 patients were included in the four-year period. Among the 8482 patients, 2124 (25.0%) were positive by PCR, 2374 (27.9%) had a significant

antibody response. *M. pneumoniae* infection was finally diagnosed in 1721 (20.3%) patients. Of the patients with *M. pneumoniae* infection, 223 RMPP patients were qualified for enrollment in the study. Twenty-one (9.4%) refused to participate, and 15 (6.7%) agreed to participate but did not return for their follow-up chest radiographs. Finally, 187 patients were recruited and received follow-up chest radiographs. These patients will be referred to as the study group. The patients who were eligible but excluded (n = 36) demonstrated no statistically significant difference in age and sex compared with the studied patients (n=187, both P>0.05). There was also no significant difference in presence of mucus plug in the study group and unenrolled group (P=0.44).

The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2y. Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar involvement, while 88 (47.1) had multilobar involvement. Forty- six (24.6%) had pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%) patients (Figure 1). The median fever duration was 10 (7, 13) days and the median length of hospital stay was 11 (9, 17) days.

Approximately half of the patients had complete radiographic clearance by 4 weeks, and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and eighty-three (97.9%) had complete clearance at the end of the study period, and four (0.5%) had persistent abnormalities at 24 weeks (Table 2). The median time to radiographic clearance of all participants was 4 weeks (interquartile range:4–8 weeks). Twenty-seven percent of the subjects had a time to radiographic clearance for >8

weeks. In unadjusted analysis, time to radiographic clearance for >8 weeks was associated with % neutrophils, CRP, LDH, pleural effusion, mucus plug, fever duration when compared with those with time to radiographic clearance ≤8 weeks (all P < 0.01; Table 3). Other variables (sex, age, lobar involvement, WBC) showed no difference.

The multivariable logistic regression model for time to radiographic clearance for >8 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant predictors of a longer time to radiographic clearance were C-reaction protein ≥50 mg/L, LDH ≥480 U/L, Fever duration ≥10 days and presence of mucus plugs. (all P < 0.01). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; P <0.01).

Discussion

This is the first study, to our knowledge, to focus on the follow-up chest radiographic clearance in patients with RMPP. CRP, LDH, fever duration and presence of mucus plugs were independently associated with longer time to radiographic clearance.

M. pneumoniae infection is a common respiratory disease in children[10]. In recent years, an increasing number of RMPP patients are being reported, especially in the Asian countries[4, 11-14]. The role of mucus plug in refractory RMPP have been extensively studied recently[5, 6, 15]. Xu et al. identified age, fever duration, LDH and CRP as independent risk factors for mucus plug formation[5]. Wang et al. found that in the bronchoscopic imaging, the mucus plug served as a promising predictor for early

RMPP diagnosis for pediatric patients with large pulmonary lesions[15]. Our previous study also found that RMPP patients with mucus plug were prone to be corticosteroid-resistant, and had a longer fever duration and hospital stay[6]. Our study further highlighted the role of mucus plugs in the time to radiographic clearance in RMPP patients.

In our study, we found that presence of mucus plugs was associated with longer time to radiographic clearance in RMPP patients. Liang and her colleagues found that MPP with severe cilia abnormalities was associated with a longer time to radiographic clearance[8]. In Liang's study, they mainly discussed cilia abnormalities, while they did not focus on the mucus plugs in the bronchoscopic findings. Mucus plugs formation was actually a manifestation of severe cilia abnormalities. Severe cilia abnormalities disrupt the mucociliary clearance and reduce the airway immune function, causing mucus plug that is responsible for the development of atelectasis and delayed radiographic resolution[10, 16]. In RMPP patients, atelectasis is quite common radiographically. The persistent presence of atelectasis lead to a longer radiographic resolution time and even longstanding pulmonary sequelae such as bronchiectasis or bronchiolitis obliterans[17-19]. Thus, careful management and follow-up is needed for the patients with mucus plugs.

Currently, bronchoscopy is an important tool to perform therapeutic interventions in patients with lobar atelectasis[7,20,21]. Zhang et al. investigated 35 pediatric subjects with RMPP and found that bronchoscopy was efficacious and well-tolerated for RMPP[7]. Abu-Hasan et al. suggested that bronchoscopy could be safe and effective in

treating acute lung collapse and atelectasis that was refractory to conventional therapy[20]. Kreider et al. also found that bronchoscopy was safe and effective in treating critically ill patients[21]. Our study also highlighted the importance of bronchoscopy, especially for patients with mucus plug.

To investigate the risk factors for longer time to radiographic clearance, we also chose variables that are commonly examined in our hospital. Three independent factors of fever duration, CRP, LDH were identified. LDH and CRP were variables that are elevated in many pulmonary diseases and was reported to be associated with RMPP in several studies[13,14,15,22]. Recently, Serum LDH 4 plus 5 was found to be a better biomarker than total LDH for RMPP in children[23]. The precise mechanisms of RMPP remain unknown. Pathogen-related substances or other substances from injured host cells during host hyperactive immune reactions may be responsible for lung cell injury[24]. Therefore, it is a natural concept that severe RMPP patients have more severe lung injury and higher laboratory parameter values such as CRP, LDH, requiring long-term recovery time. LDH level may be associated with true lung cell injury, and subsequent prolonged recovery period of tissue repair. It is reasonable to recommend that the early use of immune-modulators, without waiting for the antibiotic's effect, contributes to the effective reduction of immune-mediated lung injury in *M. pneumoniae* infection[25].

The study has some limitations. First, our study was a single-center based study, which might have potential biases and a multi-center study is needed in the future. Second, there might be some patients who had a co-infection with other pathogens

which could not be detected and might therefore lead to longer radiographic clearance. Third, the serums and nasopharyngeal samples were not collected on the same day after disease onset, which might produce bias.

In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic clearance in patients with RMPP.

Acknowledgements: We thank all participants of this study.

Contributors: YDY conceived and designed the study. RZ and WJJ conducted the study. LZH and XH analyzed the data and interpreted the data. LH provided guidance on the data analysis. All authors drafted the manuscript and read, edited and approved the final version of the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

This work was supported by the Science and Technology Program of Suzhou (grant numbers SYS201641 and SYS201558); Science and Technology Projects for the Youth of Suzhou (grant numbers KJXW2015013) and Research project of provincial health and Family Planning Commission (grant numbers H201622).

Competing interests: None declared.

Patient consent: Parental/guardian consent obtained.

Ethics approval: This research project was reviewed and approved by the

Institutional Review Board of Suzhou University.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

Reference

1. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. *FEMS Microbiol Rev*. 2008;32:956-973.

2. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev*. 2004;17:697-728, table of contents.

3. Lee KY, Lee HS, Hong JH, et al. Role of prednisolone treatment in severe Mycoplasma pneumoniae pneumonia in children. *Pediatr Pulmonol*. 2006;41:263-268.

4. You SY, Jwa HJ, Yang EA, Kil HR, Lee JH. Effects of Methylprednisolone Pulse Therapy on Refractory Mycoplasma pneumoniae Pneumonia in Children. *Allergy Asthma Immunol Res*. 2014;6:22-26.

5. Xu Q, Zhang L, Hao C, et al. Prediction of Bronchial Mucus Plugs Formation in Patients with Refractory Mycoplasma Pneumoniae Pneumonia. *J Trop Pediatr*. 2017;63:148-154.

6. Yan Y, Wei Y, Jiang W, Hao C. The clinical characteristics of corticosteroid-resistant refractory Mycoplasma Pneumoniae pneumonia in children. *Sci Rep*. 2016;6:39929.

7. Zhang Y, Chen Y, Chen Z, et al. Effects of bronchoalveolar lavage on refractory Mycoplasma pneumoniae pneumonia. *Respir Care*. 2014;59:1433-1439.

8. Liang H, Jiang W, Han Q, Liu F, Zhao D. Ciliary ultrastructural abnormalities in

Mycoplasma pneumoniae pneumonia in 22 pediatric patients. *Eur J Pediatr*. 2012;171:559-563.

9. Milne EN. Correlation of physiologic findings with chest roentgenology. *Radiol Clin North Am*. 1973;11:17-47.

10. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the Respiratory Tract and Beyond. *Clin Microbiol Rev*. 2017;30:747-809.

11. Wang M, Wang Y, Yan Y, et al. Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. *Int J Infect Dis*. 2014;29:18-23.

12. Yang M, Meng F, Wang K, et al. Interleukin 17A as a good predictor of the severity of Mycoplasma pneumoniae pneumonia in children. *Sci Rep*. 2017;7:12934.

13. Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The Clinical Characteristics and Predictors of Refractory Mycoplasma pneumoniae Pneumonia in Children. *PLoS One*. 2016;11:e0156465.

14. Lu A, Wang C, Zhang X, Wang L, Qian L. Lactate Dehydrogenase as a Biomarker for Prediction of Refractory Mycoplasma pneumoniae Pneumonia in Children. *Respir Care*. 2015;60:1469-1475.

15. Wang L, Lu S, Feng Z, et al. The early examination of combined serum and imaging data under flexible fiberoptic bronchoscopy as a novel predictor for refractory Mycoplasma pneumoniae pneumonia diagnosis. *Medicine (Baltimore)*. 2017;96:e9364.

16. Prince OA, Krunkosky TM, Sheppard ES, Krause DC. Modelling persistent Mycoplasma pneumoniae infection of human airway epithelium. *Cell Microbiol*. 2018;20.

17. Zhao C, Liu J, Yang H, Xiang L, Zhao S. Mycoplasma pneumoniae-Associated Bronchiolitis Obliterans Following Acute Bronchiolitis. *Sci Rep*. 2017;7:8478.

18. Metaxas EI, Balis E, Papaparaskevas J, Spanakis N, Tatsis G, Tsakris A. Bronchiectasis exacerbations: The role of atypical bacteria, respiratory syncytial virus and pulmonary function tests. *Can Respir J*. 2015;22:163-166.

19. Kim CK, Chung CY, Kim JS, Kim WS, Park Y, Koh YY. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. *Pediatrics*. 2000;105:372-378.

20. Abu-Hasan MN, Chesrown SE, Jantz MA. Successful use of bronchoscopic lung insufflation to treat left lung atelectasis. *Pediatr Pulmonol*. 2013;48:306-309.

21. Kreider ME, Lipson DA. Bronchoscopy for atelectasis in the ICU: a case report and review of the literature. *Chest*. 2003;124:344-350.

22. Izumikawa K. Clinical Features of Severe or Fatal Mycoplasma pneumoniae Pneumonia. *Front Microbiol*. 2016;7:800.

23. Liu TY, Lee WJ, Tsai CM, et al. Serum lactate dehydrogenase isoenzymes 4 plus 5 is a better biomarker than total lactate dehydrogenase for refractory Mycoplasma pneumoniae pneumonia in children. *Pediatr Neonatol*. 2017;17:S1875-9572.

24. Lee KY. A common immunopathogenesis mechanism for infectious diseases: the protein-homeostasis-system hypothesis. *Infect Chemother*. 2015;47:12-26

25. Lee KY. Pneumonia, Acute Respiratory Distress Syndrome, and Early Immune-Modulator Therapy. *Int J Mol Sci*. 2017;18: E388.

Figure legends

Figure 1 Bronchoscopic findings of the patient with mucus plug.

Tables

Table 1 Descriptive Analysis of Demographic, Laboratory, Radiographic, and Bronchoscopic Findings of the Study Population

Variables	
Male to female ratio	102/85
Age in years, mean ± SD	6.1 ± 2.2
Unilobar disease	99 (52.9)
Multilobar disease	88 (47.1)
Pleural effusion, n (%)	46 (24.6)
White cell count, median (quartile), $\times 10^9$/L	7.8 (6.1, 11.0)

% neutrophils, median (quartile)	75.5 (61.1, 82.4)
C-reaction protein, median (quartile), mg/L	32.9 (12.4, 59.7)
Lactic dehydrogenase, median (quartile), U/L	669.5 (486.5, 789.1)
Bronchial mucus plugs formation, N%	73 (39.0)
Fever duration, median (quartile), days	10 (7, 13)
Length of hospital stay, median (quartile), days	11 (9, 17)

Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae* pneumonia

Period (week)	Remaining patients* (n=187)	Mucus plug group (n=73)	Non-mucus plug group (n=114)
0	187	73	114
4	91	48	43
8	53	28	25
12	18	13	5
16	6	5	1
20	4	4	0
24	4	4	0

*Patients remaining with abnormal radiographic findings.

Table 3 Demographic, laboratory, radiographic, and bronchoscopic findings of children with refractory Mycoplasma pneumoniae pneumonia, according to time to radiographic clearance

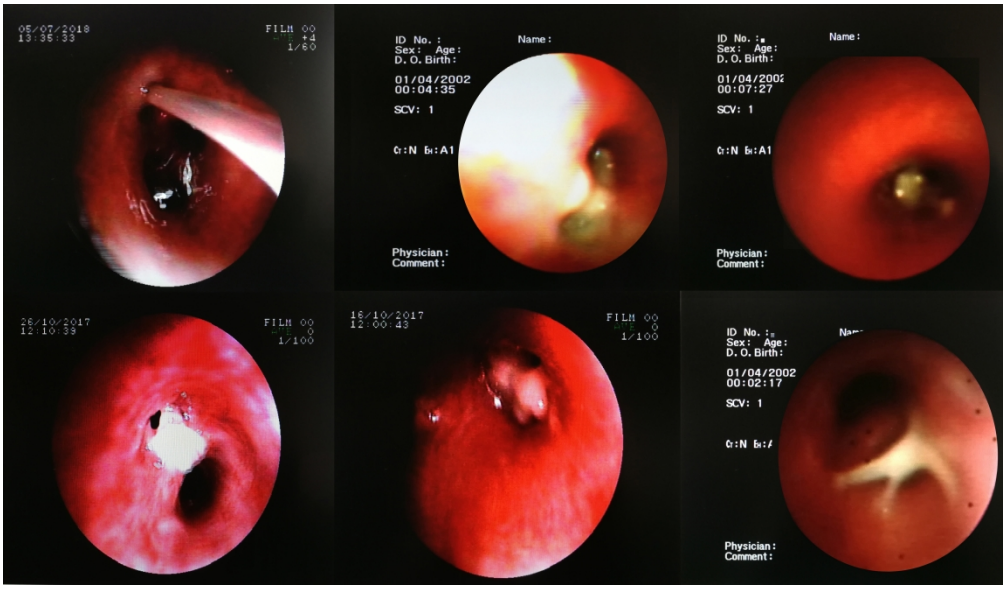
Characteristics	Time to radiographic clearance≤ 8wk (n=134)	Time to radiographic clearance> 8wk (n=53)	P
Male/female	73/61	30/23	0.79
Age in years, mean±SD	5.7±2.5	6.3±2.9	0.47
White cell count, median (quartile), $\times 10^9$/L	8.4 (6.3,11.2)	8.54 (6,11.8)	0.91
% neutrophils, median (quartile)	60.3 (21.5, 66.4)	67.4 (22.1, 72.5)	<0.01
C-reaction protein, median (quartile), mg/L	11.8 (4.9,28.7)	22.9 (9.7,66.4)	<0.01
Lactic dehydrogenase, median (quartile), U/L	396 (326.6, 506.2)	515.9 (329.5,688.9)	<0.01
Multilobar disease, N%	11 (8.2)	6 (11.3)	0.51
Pleural effusion, N%	24 (17.9)	17 (32.0)	<0.01
Bronchial mucus plugs formation, N%	13 (9.7)	21 (39.6)	<0.01
Fever duration ≥10d, N%	26 (19.4)	23 (43.4)	<0.01

Table 4 Multivariable predictors of time to radiographic clearance > 8 weeks among 402 children with refractory Mycoplasma pneumoniae pneumonia

Characteristics	OR (95% CI)	P
C-reaction protein ≥50 mg/L	3.1 (1.7-5.2)	<0.01

Lactic dehydrogenase ≥480 U/L	2.8 (1.5-4.5)	<0.01
Fever duration ≥10d	13.5 (7.8~41.4)	<0.01
Bronchial mucus plugs formation	11.5 (2.5-45.7)	<0.01

389



Bronchoscopic findings of the patient with mucus plug.

651x377mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	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	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5 , 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5 , 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4 , 5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6 , 7
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	*6 , 7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	7
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	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7

		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7
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	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
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	8
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	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
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	10

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.

BMJ Open

Independent predictors for longer radiographic resolution in patients with refractory Mycoplasma pneumoniae pneumonia: A prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023719.R2
Article Type:	Research
Date Submitted by the Author:	15-Oct-2018
Complete List of Authors:	Huang, Lizhen ; Children's Hospital of Soochow University, Department of Respiratory Medicine; First People's Hospital of Wujiang District Huang, Xia ; Children's Hospital of Nanjing Medical University Jiang, Wujiang; Children's Hospital of Soochow University, Department of Respiratory Medicine Zhang, Rong; Children's Hospital of Soochow University, Department of Respiratory Medicine Yan, Yongdong; Children's Hospital of Soochow University, ; Huang, Li ; Children's Hospital of Soochow University, Department of Respiratory Medicine
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Infectious diseases
Keywords:	Mucus plugs, refractory Mycoplasma Pneumoniae pneumonia, radiographic resolution

Title page

Independent predictors for longer radiographic resolution in patients with refractory *Mycoplasma pneumoniae* pneumonia: A prospective cohort study

Lizhen Huang[1,], Xia Huang[2,], Wujun Jiang[2], Rong Zhang[2], Yongdong Yan[2*], Li Huang[2*]

[1] Children's Hospital of Soochow University, Suzhou, China; First People's Hospital of Wujiang District, Suzhou, China.

[2] Children's Hospital of Nanjing Medical University, Nanjing, China

[3] Children's Hospital of Soochow University, Suzhou, China

*These authors contributed equally to this work.

Corresponding author: Yongdong Yan, E-mail: suzhoupulmonology@163.com, Tel +86 512 80698312, Fax +86 512 80698312, or Li Huang, E-mail: szdalv@163.com, Tel +86 512 80698312, Fax +86 512 80698312.

Abstract

Objectives: To examine prospectively the radiographic clearance of refractory *Mycoplasma pneumoniae* pneumonia (RMPP) in immunocompetent children, and to identify independent predictors of time to complete radiographic resolution in patients with RMPP.

Design: A prospective cohort study.

Setting: Children's Hospital of Soochow University, China.

Participants: A total of 187 RMPP patients treated with bronchoscopy were prospectively enrolled in the study between Jan 2012 and Dec 2015.

Methods: Serial chest radiographs were obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved. Multivariate logistic regression was performed to identify independent predictors of time to complete radiographic resolution.

Results: Of the 187 RMPP patients, bronchial mucus plugs formation was detected in 73 (39.0%) patients. C-reaction protein (CRP) ≥50 mg/L, lactate dehydrogenase (LDH) ≥480 U/L, total fever duration ≥10 days and presence of mucus plugs were associated with a longer time to radiographic clearance (all $P < 0.01$). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

Conclusions: Clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic clearance in patients with RMPP.

Strengths and limitations of this study:

- This is the first study to analyze prospectively the risk factors associated with longer time to radiographic clearance in patients with RMPP in China.
- A prospective follow-up of chest radiographs was obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.
- Our study was a single-center based study, which might have introduced a selection bias.
- There might be some patients who had a co-infection with other pathogens which could not be detected and might therefore lead to longer radiographic clearance.
- The serums and nasopharyngeal samples were not collected on the same day after disease onset, which might produce measurement bias.

Key words: Mucus plugs; refractory *Mycoplasma pneumoniae* pneumonia; radiographic resolution.

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a common etiology of childhood community-acquired pneumonia (CAP) [1, 2]. *M. pneumoniae* infections are usually mild, while in recent decades, pediatricians are facing increasing numbers of refractory *Mycoplasma pneumoniae* pneumonia (RMPP) patients. RMPP often show no improvement in clinical and radiological findings despite of appropriated macrolides treatment. Corticosteroids has been proven to be effective in treating RMPP [3, 4]. However, despite the use of corticosteroids, some patients with RMPP still have persisting fever and radiological deterioration. They required investigation using bronchoscopy[5, 6].

We encountered several cases of RMPP who had mucus plug formation under bronchoscopy. RMPP, especially those with mucus plug may have a longer radiographic resolution time. Some patients may have longstanding pulmonary sequelae such as bronchiectasis[7, 8]. No investigations have been reported with careful statistical consideration given to the prognostic significance of factors in the radiographic resolution of RMPP. We sought to examine prospectively the radiographic clearance of RMPP in immunocompetent children. The risk factors associated with longer time to radiographic clearance in RMPP patients were analyzed.

Methods

Cohort Description

Patients with CAP who hospitalized in Department of Respiratory Medicine in Children's Hospital of Soochow University from January 1, 2012 to December 31, 2015,

were evaluated prospectively for identifying patients who met criteria for RMPP. RMPP was considered when (1) Cough, fever or auscultatory findings together with pulmonary infiltrates on chest radiograph; (2) a significant rise in *M. pneumoniae* IgG or seroconversion in paired sera, together with *M. pneumoniae* DNA detected in nasopharyngeal aspirates; (3) fever persisting (>38.5°C) and radiological deterioration after the therapy of macrolide for 7 days or more. Bronchoscopy was indicated when lobar consolidation or atelectasis persisted on chest X-ray film after corticosteroid therapy for 1 week. The study was approved by the Institutional Review Board of Suzhou University, and informed consent was obtained for all participants or their parents. Chest X rays films were followed up after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved.

Patient and public involvement

From January 1, 2012 to December 31, 2015, the following patients were included in our study: (1) patients had cough, fever or auscultatory findings together with pulmonary infiltrates on chest radiograph; (2) age from 1 months to 14 years. The following patients were excluded from the study: (1) patients with bronchopulmonary dysplasia, congenital heart diseases, immunodeficiency and heredity neurological disorders; (2) those had evidence of co-infection with other pathogens.

Diagnostic tests for M. pneumoniae

Nasopharyngeal aspirates were obtained within 1 day after the patients admitted. As described previously, specimens were tested to amplify fragment of P1 adhesin gene

by using PCR analysis[6]. A quantitative *M. pneumoniae* DNA diagnostic kit (DaAn Gene Co., Ltd. Guangzhou, China) was used. The target specific for *M. pneumoniae* genome is 16S rRNA gene.

The paired serum samples were taken at admission and at least two weeks after the first serum sampling. The serum samples were tested for IgM and IgG antibodies against *M. pneumoniae* using an ELISA kit (Serion ELISA MP IgG/IgM, Institute Virion/Serion, Germany). The cut-off value was 0.5×mean optical density (OD) of control serum of the kit. As described previously, a significant rise in IgG titre was defined as a doubling of the OD value above the cut-off. A sero-conversion was defined as the first serum was negative, and the second serum had an OD at least twice the cut-off [6].

Data Collection and Interpretation of Radiographs

Serial posteroanterior and lateral chest radiographs were obtained after discharge every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs had resolved. All radiographs were evaluated independently by two radiologists (PP and WLG), who did not know the patients' clinical condition. Chest radiographs were reviewed by two radiologists in sequence with the prior films for comparison. If differences in interpretation of radiographs occurred, it would be resolved by joint consensus between the two radiologists. The radiographs were reviewed for the presence of consolidation, atelectasis and pleural disease (effusion or thickening). Consolidation, atelectasis and pleural disease were defined by standard radiographic criteria[9].

Clinical and laboratory data regarding gender, age, total fever duration, length of hospital stay, white blood cell (WBC) count, percentage of neutrophils (% neutrophils), platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction protein (CRP) were collected.

Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS). For continuous variables, comparison of means was conducted by using the t test. For ordinally scaled data, the Wilcoxon rank sum test was used. For categorical variables, the chi-square or Fisher exact test were used. A univariate analysis for 8 influence factors (age, sex, WBC, CRP, LDH, number of involved lobes [unilobar vs multilobar involvement], presence of pleural effusion and presence of mucus plug) was performed. Multiple regression analysis was performed to select the variables associated with time to complete radiographic resolution. Probabilities of .05 or less were considered significant. A sample size estimation was calculated using Power Analysis and Sample Size (PASS) software. Based on a likely sample proportion of interest variable having the tested trait (P) of 45%[5], with 95% confidence ($\alpha = 0.05$) and a 10% margin of error of the estimate, the minimum required sample size was n=132.

Results

There were totally 8482 patients included in the four-year period. Among the 8482 patients, 2124 (25.0%) were positive by PCR, 2374 (27.9%) had a significant antibody response. *M. pneumoniae* infection was finally diagnosed in 1721 (20.3%) patients. Of

the patients with *M. pneumoniae* infection, 223 RMPP patients qualified for enrollment in the study. Twenty-one (9.4%) refused to participate, and 15 (6.7%) agreed to participate but did not return for their follow-up chest radiographs. Finally, 187 patients were recruited and received follow-up chest radiographs. These patients were referred to as the study group. The patients who were eligible but excluded (n = 36) demonstrated no statistically significant difference in age and sex compared with the studied patients (n=187, both P>0.05). There was also no significant difference in presence of mucus plug in the study group and unenrolled group (P=0.44).

The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2y. Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar involvement, while 88 (47.1) had multilobar involvement. Forty-six (24.6%) had pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%) patients (Figure 1). The median total fever duration was 10 (7, 13) days and the median length of hospital stay was 11 (9, 17) days.

Approximately half of the patients had complete radiographic clearance by 4 weeks, and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and eighty-three (97.9%) had complete clearance at the end of the study period, and four (0.5%) had persistent abnormalities at 24 weeks (Table 2). The median time to radiographic clearance of all participants was 4 weeks (interquartile range: 4–8 weeks). Twenty-seven percent of the subjects had a time to radiographic clearance for >8 weeks. In unadjusted analysis, time to radiographic clearance for >8 weeks was associated with % neutrophils, CRP, LDH, pleural effusion, mucus plug, total fever duration when

compared with those with time to radiographic clearance ≤8 weeks (all P < 0.01; Table 3). Other variables (sex, age, lobar involvement, WBC) showed no difference.

The multivariable logistic regression model for time to radiographic clearance for >8 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant predictors of a longer time to radiographic clearance were C-reaction protein ≥50 mg/L, LDH ≥480 U/L, total fever duration ≥10 days and presence of mucus plugs. (all P < 0.01). Compared with children without mucus plugs, those with mucus plugs were significantly more likely to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; P <0.01).

Discussion

This is the first study, to our knowledge, that focus on the follow-up chest radiographic clearance in patients with RMPP. CRP, LDH, total fever duration and presence of mucus plugs were independently associated with longer time to radiographic clearance.

M. pneumoniae infection is a common respiratory disease in children[10]. In recent years, an increasing number of RMPP patients are being reported, especially in the Asian countries[4, 11-14]. The role of mucus plug in refractory RMPP have been extensively studied recently[5, 6, 15]. Xu *et al*. identified age, total fever duration, LDH and CRP as independent risk factors for mucus plug formation[5]. Wang *et al*. found that in the bronchoscopic imaging, the mucus plug served as a promising predictor for early RMPP diagnosis for pediatric patients with large pulmonary lesions[15]. Our previous study also found that RMPP patients with mucus plug were prone to be corticosteroid-resistant, and had a longer total fever duration and hospital stay[6]. Our study further

highlighted the role of mucus plugs in the time to radiographic clearance in RMPP patients.

In our study, we found that presence of mucus plugs was associated with longer time to radiographic clearance in RMPP patients. Liang and her colleagues found that MPP with severe cilia abnormalities was associated with a longer time to radiographic clearance[8], but they did not focus on analysis of the mucus plugs in the bronchoscopic findings. Mucus plugs formation was actually a manifestation of severe cilia abnormalities. Severe cilia abnormalities disrupt the mucociliary clearance, causing mucus plug that is responsible for the development of atelectasis and delayed radiographic resolution[10, 16]. The persistent presence of atelectasis lead to a longer radiographic resolution time and longstanding pulmonary sequelae such as bronchiectasis or bronchiolitis obliterans[17-19]. Thus, careful management and follow-up is needed for the patients with mucus plugs.

Currently, bronchoscopy is an important tool for therapeutic interventions in patients with lobar atelectasis[7,20,21]. Zhang *et al.* investigated 35 pediatric subjects with RMPP and found that bronchoscopy was efficacious and well-tolerated[7]. Abu-Hasan *et al.* suggested that bronchoscopy could be safe and effective in treating acute lung collapse and atelectasis that was refractory to conventional therapy[20]. Kreider *et al.* also found that bronchoscopy was safe and effective in treating critically ill patients[21]. Our study also highlighted the importance of bronchoscopy, especially for patients with mucus plug.

To investigate the risk factors for longer time to radiographic clearance, we also

chose variables that are commonly examined in our hospital. Three independent factors, including total fever duration, CRP, LDH were identified. LDH and CRP were variables that are elevated in many pulmonary diseases and was reported to be associated with RMPP in several studies[13,14,15,22]. Recently, serum LDH 4 plus 5 were found to be better biomarkers than total LDH for RMPP in children[23]. The precise mechanisms of RMPP remain unknown. Pathogen-related substances or other host factors during hyperactive immune reactions may be responsible for lung injury[24]. Therefore, it may be logical to propose that severe RMPP patients have severe lung injury and higher clinical parameter values such as CRP, LDH, requiring long-term recovery time. LDH level may be associated with true lung injury, and subsequent prolonged recovery period of tissue repair. Therefore, it is reasonable to recommend that the early use of immune-modulators, without waiting for the antibiotic's effect, contributes to the effective reduction of immune-mediated lung injury in *M. pneumoniae* infection[25].

The study has some limitations. Firstly, our study was a single-center based study, which might have introduced a selection bias. The results reported in our Soochow area cannot be extrapolated to other areas in China. Thus, a multi-center study is needed in the future. Secondly, there might be some patients who had a co-infection with other pathogens which could not be detected and might therefore lead to longer radiographic clearance. Thirdly, the serums and nasopharyngeal samples were not collected on the same day after disease onset, which might produce measurement bias.

In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH and presence of mucus plugs to identify children at a longer time to radiographic

clearance in patients with RMPP.

Acknowledgements: We thank all participants of this study.

Contributors: YDY conceived and designed the study. RZ and WJJ conducted the study. LZH and XH analyzed the data and interpreted the data. LH provided guidance on the data analysis. All authors drafted the manuscript and read, edited and approved the final version of the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

This work was supported by the Science and Technology Program of Suzhou (grant numbers SYS201641 and SYS201558); Science and Technology Projects for the Youth of Suzhou (grant numbers KJXW2015013) and Research project of provincial health and Family Planning Commission (grant numbers H201622).

Competing interests: None declared.

Patient consent: Parental/guardian consent obtained.

Ethics approval: This research project was reviewed and approved by the Institutional Review Board of Suzhou University.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

Reference

1. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. *FEMS Microbiol Rev*. 2008;32:956-973.

2. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev*. 2004;17:697-728, table of contents.

3. Lee KY, Lee HS, Hong JH, et al. Role of prednisolone treatment in severe Mycoplasma pneumoniae pneumonia in children. *Pediatr Pulmonol*. 2006;41:263-268.

4. You SY, Jwa HJ, Yang EA, Kil HR, Lee JH. Effects of Methylprednisolone Pulse Therapy on Refractory Mycoplasma pneumoniae Pneumonia in Children. *Allergy Asthma Immunol Res*. 2014;6:22-26.

5. Xu Q, Zhang L, Hao C, et al. Prediction of Bronchial Mucus Plugs Formation in Patients with Refractory Mycoplasma Pneumoniae Pneumonia. *J Trop Pediatr*. 2017;63:148-154.

6. Yan Y, Wei Y, Jiang W, Hao C. The clinical characteristics of corticosteroid-resistant refractory Mycoplasma Pneumoniae pneumonia in children. *Sci Rep*. 2016;6:39929.

7. Zhang Y, Chen Y, Chen Z, et al. Effects of bronchoalveolar lavage on refractory Mycoplasma pneumoniae pneumonia. *Respir Care*. 2014;59:1433-1439.

8. Liang H, Jiang W, Han Q, Liu F, Zhao D. Ciliary ultrastructural abnormalities in Mycoplasma pneumoniae pneumonia in 22 pediatric patients. *Eur J Pediatr*. 2012;171:559-563.

9. Milne EN. Correlation of physiologic findings with chest roentgenology. *Radiol Clin North Am*. 1973;11:17-47.

10. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the Respiratory Tract and Beyond. *Clin Microbiol Rev*. 2017;30:747-809.

11. Wang M, Wang Y, Yan Y, et al. Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. *Int J Infect Dis*. 2014;29:18-23.

12. Yang M, Meng F, Wang K, et al. Interleukin 17A as a good predictor of the severity of Mycoplasma pneumoniae pneumonia in children. *Sci Rep*. 2017;7:12934.

13. Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The Clinical Characteristics and Predictors of Refractory Mycoplasma pneumoniae Pneumonia in Children. *PLoS One*. 2016;11:e0156465.

14. Lu A, Wang C, Zhang X, Wang L, Qian L. Lactate Dehydrogenase as a Biomarker for Prediction of Refractory Mycoplasma pneumoniae Pneumonia in Children. *Respir Care*. 2015;60:1469-1475.

15. Wang L, Lu S, Feng Z, et al. The early examination of combined serum and imaging data under flexible fiberoptic bronchoscopy as a novel predictor for refractory Mycoplasma pneumoniae pneumonia diagnosis. *Medicine (Baltimore)*. 2017;96:e9364.

16. Prince OA, Krunkosky TM, Sheppard ES, Krause DC. Modelling persistent Mycoplasma pneumoniae infection of human airway epithelium. *Cell Microbiol*. 2018;20.

17. Zhao C, Liu J, Yang H, Xiang L, Zhao S. Mycoplasma pneumoniae-Associated Bronchiolitis Obliterans Following Acute Bronchiolitis. *Sci Rep*. 2017;7:8478.

18. Metaxas EI, Balis E, Papaparaskevas J, Spanakis N, Tatsis G, Tsakris A. Bronchiectasis exacerbations: The role of atypical bacteria, respiratory syncytial virus and pulmonary function tests. *Can Respir J*. 2015;22:163-166.

19. Kim CK, Chung CY, Kim JS, Kim WS, Park Y, Koh YY. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. *Pediatrics*. 2000;105:372-378.

20. Abu-Hasan MN, Chesrown SE, Jantz MA. Successful use of bronchoscopic lung insufflation to treat left lung atelectasis. *Pediatr Pulmonol*. 2013;48:306-309.

21. Kreider ME, Lipson DA. Bronchoscopy for atelectasis in the ICU: a case report and review of the literature. *Chest*. 2003;124:344-350.

22. Izumikawa K. Clinical Features of Severe or Fatal Mycoplasma pneumoniae Pneumonia. *Front Microbiol*. 2016;7:800.

23. Liu TY, Lee WJ, Tsai CM, et al. Serum lactate dehydrogenase isoenzymes 4 plus 5 is a better biomarker than total lactate dehydrogenase for refractory Mycoplasma pneumoniae pneumonia in children. *Pediatr Neonatol*. 2017;17:S1875-9572.

24. Lee KY. A common immunopathogenesis mechanism for infectious diseases: the protein-homeostasis-system hypothesis. *Infect Chemother*. 2015;47:12-26

25. Lee KY. Pneumonia, Acute Respiratory Distress Syndrome, and Early Immune-Modulator Therapy. *Int J Mol Sci*. 2017;18: E388.

Figure legends

Figure 1 Bronchoscopic findings of refractory *Mycoplasma pneumoniae* pneumonia patients with mucus plug.

Tables

Table 1 Descriptive analysis of demographic, laboratory, radiographic, and bronchoscopic findings of the study population

Variables	
Male to female ratio	102/85
Age in years, mean ± SD	6.1 ± 2.2
Unilobar disease	99 (52.9)
Multilobar disease	88 (47.1)
Pleural effusion, n (%)	46 (24.6)
White cell count, median (quartile), $\times 10^9$/L	7.8 (6.1, 11.0)
% neutrophils, median (quartile)	75.5 (61.1, 82.4)
C-reaction protein, median (quartile), mg/L	32.9 (12.4, 59.7)
Lactic dehydrogenase, median (quartile), U/L	669.5 (486.5, 789.1)
Bronchial mucus plugs formation, N%	73 (39.0)
Total fever duration, median (quartile), days	10 (7, 13)
Length of hospital stay, median (quartile), days	11 (9, 17)

Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae* pneumonia

Period (week)	Remaining patients* (n=187)	Mucus plug group (n=73)	Non-mucus plug group (n=114)
0	187	73	114
4	91	48	43
8	53	28	25
12	18	13	5
16	6	5	1
20	4	4	0
24	4	4	0

*Patients remaining with abnormal radiographic findings.

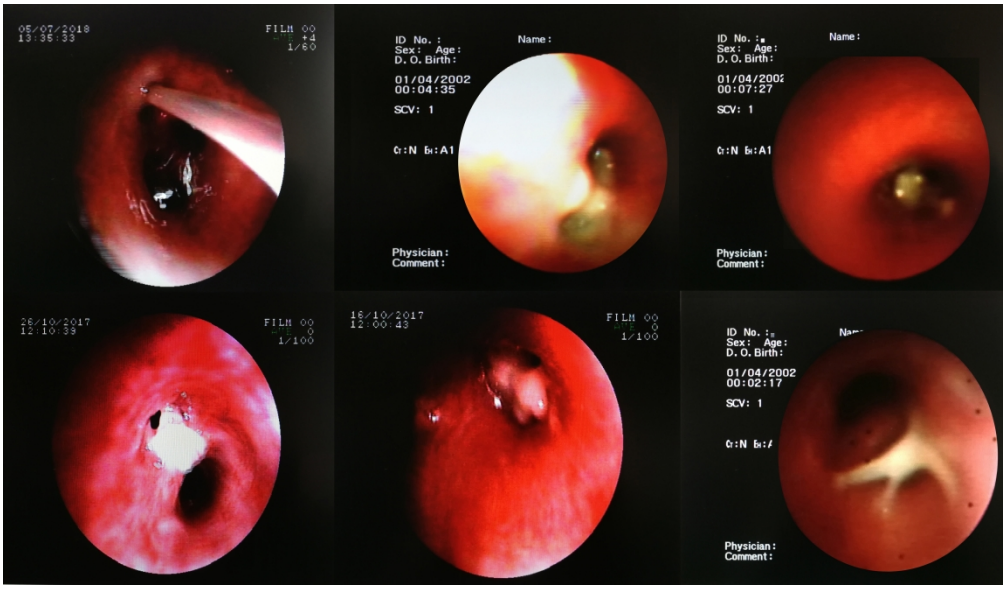
Table 3 Demographic, laboratory, radiographic, and bronchoscopic findings of children with refractory *Mycoplasma pneumoniae* pneumonia, according to time to radiographic clearance

Characteristics	Time to radiographic clearance≤ 8wk (n=134)	Time to radiographic clearance> 8wk (n=53)	P
Male/female	73/61	30/23	0.79
Age in years, mean±SD	5.7±2.5	6.3±2.9	0.47
White cell count, median (quartile), $\times 10^9$/L	8.4 (6.3,11.2)	8.54 (6,11.8)	0.91
% neutrophils, median (quartile)	60.3 (21.5, 66.4)	67.4 (22.1, 72.5)	<0.01
C-reaction protein, median (quartile), mg/L	11.8 (4.9,28.7)	22.9 (9.7,66.4)	<0.01
Lactic dehydrogenase, median (quartile),	396 (326.6, 506.2)	515.9 (329.5,688.9)	<0.01

U/L			
Multilobar disease, N%	11 (8.2)	6 (11.3)	0.51
Pleural effusion, N%	24 (17.9)	17 (32.0)	<0.01
Bronchial mucus plugs formation, N%	13 (9.7)	21 (39.6)	<0.01
Total fever duration ≥10d, N%	26 (19.4)	23 (43.4)	<0.01

Table 4 Multivariable predictors of time to radiographic clearance > 8 weeks among children with refractory *Mycoplasma pneumoniae* pneumonia

Characteristics	OR (95% CI)	P
C-reaction protein ≥50 mg/L	3.1 (1.7-5.2)	<0.01
Lactic dehydrogenase ≥480 U/L	2.8 (1.5-4.5)	<0.01
Total fever duration ≥10d	13.5 (7.8~41.4)	<0.01
Bronchial mucus plugs formation	11.5 (2.5-45.7)	<0.01



Bronchoscopic findings of refractory Mycoplasma pneumoniae pneumonia patients with mucus plug.

651x377mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	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	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5 , 6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5 , 6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6 , 7
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	*6 , 7*
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	7
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	7,8
		(b) Give reasons for non-participation at each stage	7,8
		(c) Consider use of a flow diagram	7,8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential	7,8

		confounders	
		(b) Indicate number of participants with missing data for each variable of interest	7,8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
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	9
		(*b*) Report category boundaries when continuous variables were categorized	9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
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	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	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	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	12

*Give information separately for exposed and unexposed groups.

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 http://www.strobe-statement.org.