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

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Keywords:	Mucus plugs, refractory Mycoplasma Pneumoniae pneumonia, radiographic resolution

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1 **Title page**

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

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23 **Abstract**

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

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

32 **Results:** Of the 187 RMPP patients, Bronchial mucus plugs formation was detected in
33 73 (39.0%) patients. C-reaction protein (CRP) ≥ 50 mg/L, lactate dehydrogenase
34 (LDH) ≥ 480 U/L, Fever duration ≥ 10 days and presence of mucus plugs were
35 associated with a longer time to radiographic clearance (all $P < 0.01$). Compared with
36 children without mucus plugs, those with mucus plugs were significantly more likely
37 to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7;
38 $P < 0.01$).

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

42 **Strengths and limitations of this study:**

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

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4 45 ● Serial posteroanterior and lateral chest radiographs were obtained every 4
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6 46 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration
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8 47 on chest radiographs had resolved.

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11 48 ● Our study was a single-center based study, which might have potential biases
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13 49 and a multi-center study is needed in the future.

14
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16 50 **Key words:** Mucus plugs; refractory Mycoplasma Pneumoniae pneumoniae;
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18 51 radiographic resolution.

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68 Text

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

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

87 Methods**88 Cohort Description**

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4 89 Patients with CAP who hospitalized in Department of Respiratory Medicine in
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6 90 Children's Hospital of Soochow University from January 1, 2012 to December 31,
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8 91 2015, were evaluated prospectively for identifying patients who met criteria for RMPP.
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10 92 RMPP was considered when (1). Cough, fever or auscultatory findings together with
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12 93 pulmonary infiltrates on chest radiograph, (2). A significant rise in *M. pneumoniae*
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14 94 IgG or seroconversion in paired sera, together with *M. pneumoniae* DNA detected in
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16 95 nasopharyngeal aspirates, (3). Fever persisting ($>38.5^{\circ}\text{C}$) and radiological
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18 96 deterioration after the therapy of macrolide for 7 days or more. Bronchoscopy was
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20 97 indicated when lobar consolidation or atelectasis persisted on chest X-ray film after
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22 98 corticosteroid therapy for 1 week. Patients aged from 1 months to 14 years were
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24 99 eligible for participation. The following patients were excluded from the study: (1)
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26 100 patients with bronchopulmonary dysplasia, congenital heart diseases,
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28 101 immunodeficiency and heredity neurological disorders; (2) those have evidence of
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30 102 co-infection with other pathogens. The study was approved by the Institutional
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32 103 Review Board of Suzhou University, and informed consent was obtained for all
33
34 104 participants or their parents. Chest X rays films were followed up after discharge
35
36 105 every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration
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38 106 on chest radiographs had resolved.

107 ***Diagnostic tests for *M. pneumoniae****

108 Nasopharyngeal aspirates were obtained within 1 day after the patients admitted. As
109 described previously, specimens were tested to amplify the *M. pneumoniae* P1
110 adhesin gene by using PCR analysis⁶. A quantitative *M. pneumoniae* DNA diagnostic

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4 111 kit (DaAn Gene Co., Ltd. Guangzhou, China) was used. The target specific for M.
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6 112 pneumoniae genome is 16S rRNA gene.
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8 113 The paired serum samples were taken at admission and at least two weeks after the
9
10 114 first serum. The serum samples were tested for IgM and IgG antibodies against M.
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12 115 pneumoniae using a ELISA kit (Serion ELISA MP IgG/IgM, Institute Virion/Serion,
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14 116 Germany). The cut-off value was $0.5 \times$ mean optical density (OD) of control serum of
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16 117 the kit. As described previously, a significant rise in IgG titre was defined as a
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18 118 doubling of the OD value above the cut-off. A sero-conversion was defined as the first
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20 119 serum was negative, and the second serum had an OD at least twice the cut-off⁶.
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23 120 *Data Collection and Interpretation of Radiographs*

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28 121 Serial posteroanterior and lateral chest radiographs were obtained after discharge
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30 122 every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration
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32 123 on chest radiographs had resolved. All radiographs were evaluated independently by
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34 124 two radiologists (PP and WLG), who did not know the patients' clinical condition.
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36 125 Chest radiographs were reviewed by two radiologists in sequence with the prior films
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38 126 for comparison. If differences in interpretation of radiographs occurred, it would be
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40 127 resolved by joint consensus between the two radiologists. The radiographs were
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42 128 reviewed for the presence of consolidation, atelectasis and pleural disease (effusion or
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44 129 thickening). Consolidation, atelectasis and pleural disease were defined by standard
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46 130 radiographic criteria⁹.
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52 131 Clinical and laboratory data regarding gender, age, fever duration, length of
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54 132 hospital stay, White blood cell (WBC) count, percentage of neutrophils (%
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4 133 neutrophils), platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction
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6 134 protein (CRP) were collected.

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9 135 ***Statistical Analyses***

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11 136 For continuous variables, comparison of means was conducted by using the t test. For
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13 137 ordinally scaled data, the Wilcoxon rank sum test was used. For categorical variables,
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15 138 the chi-square or Fisher exact test were used. A univariate analysis for 8 influence
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17 139 factors (age, sex, WBC, CRP, LDH, number of involved lobes [unilobar vs multilobar
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19 140 involvement], presence of pleural effusion and presence of mucus plug) was
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21 141 performed. Multiple regression analysis was performed to select the variables
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23 142 associated with time to complete radiographic resolution. Probabilities of .05 or less
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25 143 were considered significant.

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29 144 **Results**

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31 145 A total of 223 RMPP patients were qualified for enrollment in the study. Twenty-one
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33 146 (9.4%) refused to participate, and 15 (6.7%) agreed to participate but did not return
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35 147 for their follow-up chest radiographs. Finally, 187 patients were recruited and
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37 148 received follow-up chest radiographs. These patients will be referred to as the study
38
39 149 group. The patients who were eligible but excluded (n = 36) demonstrated no
40
41 150 statistically significant difference in age and sex compared with the studied patients
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43 151 (n=187, both P>0.05). There was also no significant difference in presence of mucus
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45 152 plug in the study group and unenrolled group (P=0.44).

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47 153 The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2 y.
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49 154 Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar
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4 155 involvement, while 88 (47.1) had multilobar involvement. Forty- six (24.6%) had
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6 156 pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%)
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8 157 patients (Figure 1). The median fever duration was 10 (7, 13) days and the median
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11 158 length of hospital stay was 11 (9, 17) days.

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13 159 Approximately half of the patients had complete radiographic clearance by 4 weeks,
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15 160 and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and
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17 161 eighty-three (97.9%) had complete clearance at the end of the study period, and four
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19 162 (0.5%) had persistent abnormalities at 24 weeks (Table 2). The median time to
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21 163 radiographic clearance of all participants was 4 weeks (IQR:4–8 weeks).
22
23 164 Twenty-seven percent of the subjects had a time to radiographic clearance for >8
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25 165 weeks. In unadjusted analysis, time to radiographic clearance for >8 weeks was
26
27 166 associated with % neutrophils, CRP, LDH, pleural effusion, mucus plug, fever
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29 167 duration when compared with those with time to radiographic clearance ≤ 8 weeks (all
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31 168 $P < 0.01$; Table 3). Other variables (sex, age, lobar involvement, WBC) showed no
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33 169 difference.

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35 170 The multivariable logistic regression model for time to radiographic clearance
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37 171 for >8 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant
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39 172 predictors of a longer time to radiographic clearance were C-reaction protein ≥ 50
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41 173 mg/L, LDH ≥ 480 U/L, Fever duration ≥ 10 days and presence of mucus plugs. (all $P <$
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43 174 0.01). Compared with children without mucus plugs, those with mucus plugs were
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45 175 significantly more likely to have a longer time to radiographic clearance (adjusted OR:
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47 176 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

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177 **Discussion**

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

182 *M. pneumoniae* infection is a common respiratory disease in children¹⁰. In recent
183 years, an increasing number of RMPP patients are being reported, especially in the
184 Asian countries^{4, 11-14}. The role of mucus plug in refractory RMPP have been
185 extensively studied recently^{5, 6, 15}. Xu et al. identified age, fever duration, LDH and
186 CRP as independent risk factors for mucus plug formation⁵. Wang et al. found that in
187 the bronchoscopic imaging, the mucus plug served as a promising predictor for early
188 RMPP diagnosis for pediatric patients with large pulmonary lesions¹⁵. Our previous
189 study also found that RMPP patients with mucus plug were prone to be
190 corticosteroid-resistant, and had a longer fever duration and hospital stay⁶. Our study
191 further highlighted the role of mucus plugs in the time to radiographic clearance in
192 RMPP patients.

193 In our study, we found that presence of mucus plugs was associated with longer
194 time to radiographic clearance in RMPP patients. Liang and her colleagues found that
195 MPP with severe cilia abnormalities was associated with a longer time to radiographic
196 clearance⁸. In Liang's study, they mainly discussed cilia abnormalities, while they did
197 not focus on the mucus plugs in the bronchoscopic findings. Mucus plugs formation
198 was actually a manifestation of severe cilia abnormalities. Severe cilia abnormalities

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4 199 disrupt the mucociliary clearance and reduce the airway immune function, causing
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6 200 mucus plug that is responsible for the development of atelectasis and delayed
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8 201 radiographic resolution^{10, 16}. In RMPP patients, atelectasis is quite common
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11 202 radiographically. The persistent presence of atelectasis lead to a longer radiographic
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13 203 resolution time and even longstanding pulmonary sequelae such as bronchiectasis or
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15 204 bronchiolitis obliterans¹⁷⁻¹⁹. Thus, careful management and follow-up is needed for
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18 205 the patients with mucus plugs. Additional therapeutic interventions of bronchoscopy
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21 206 may be acquired to remove the mucous plug completely.

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23 207 The study has some limitations. First, our study was a single-center based study,
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25 208 which might have potential biases and a multi-center study is needed in the future.
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28 209 Second, there might be some patients who had a co-infection with other pathogens
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30 210 which could not be detected and might therefore lead to longer radiographic clearance.
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33 211 Third, the serums and nasopharyngeal samples were not collected on the same day
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35 212 after disease onset, which might produce bias.

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38 213 In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH
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40 214 and presence of mucus plugs to identify children at a longer time to radiographic
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42 215 clearance in patients with RMPP.

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49
50 218 **Contributors:** YDY conceived and designed the study. RZ and WJJ conducted the
51
52 219 study. LZH and XH analyzed the data and interpreted the data. LH provided guidance
53
54
55 220 on the data analysis. All authors drafted the manuscript and read, edited and approved

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4 221 the final version of the manuscript. All authors had full access to all of the data in the
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6 222 study and can take responsibility for the integrity of the data and the accuracy of the
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8 223 data analysis.
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30 232 Institutional Review Board of Suzhou University.
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35 234 **Data sharing statement:** No additional data are available.
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Figure legends

Figure 1 Bronchoscopic findings of the patient with mucus plug.

For peer review only

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340 **Tables**341 **Table 1 Descriptive Analysis of Demographic, Laboratory, Radiographic, and**342 **Bronchoscopic Findings of the Study Population**

Variables	
Male to female ratio	102/85
Age in years, mean \pm SD	6.1 \pm 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)

343

344 **Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae***
345 **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

346 *Patients remaining with abnormal radiographic findings.

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

350 to radiographic clearance

Characteristics	Time to radiographic	Time to radiographic	P
	clearance \leq 8wk (n=134)	clearance $>$ 8wk (n=53)	
Male/female	73/61	30/23	0.79
Age in years, mean \pm SD	5.7 \pm 2.5	6.3 \pm 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 $\geq 10d$, N%	26 (19.4)	23 (43.4)	<0.01

351

352 **Table 4 Multivariable predictors of time to radiographic clearance > 8 weeks**353 **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 $\geq 10d$	13.5 (7.8~41.4)	<0.01

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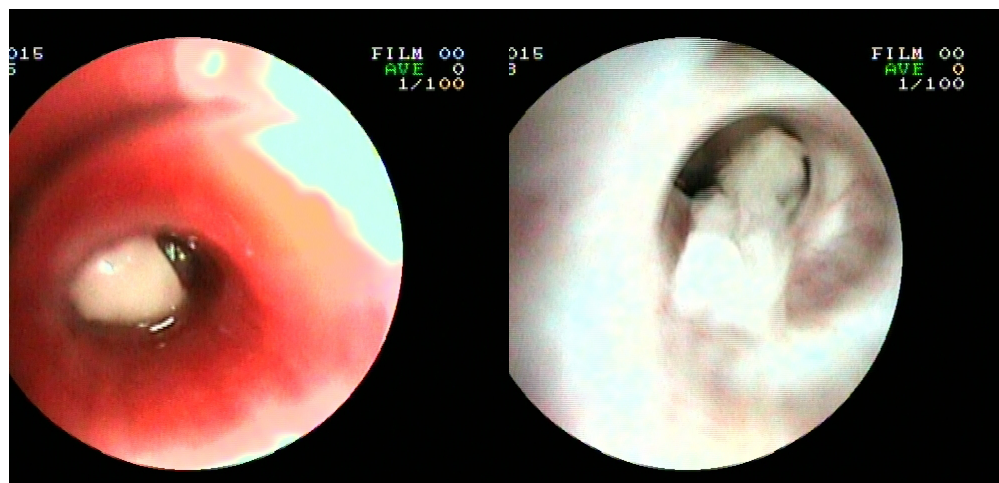


Figure1. Bronchoscopic findings of the patient with mucus plug.

352x169mm (72 x 72 DPI)

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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

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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Infectious diseases
Keywords:	Mucus plugs, refractory <i>Mycoplasma Pneumoniae</i> pneumonia, radiographic resolution

SCHOLARONE™
Manuscripts

1 **Title page**2 **Is presence of bronchial mucus plugs associated with longer radiographic**
3 **resolution in patients with refractory Mycoplasma Pneumoniae pneumonia? A**
4 **prospective cohort study**5 Lizhen Huang^{1,*}, Xia Huang^{2,*}, Wujun Jiang², Rong Zhang², Yongdong Yan², Li
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23 **Abstract**

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

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

34 **Results:** Of the 187 RMPP patients, Bronchial mucus plugs formation was detected in
35 73 (39.0%) patients. C-reaction protein (CRP) ≥ 50 mg/L, lactate dehydrogenase
36 (LDH) ≥ 480 U/L, Fever duration ≥ 10 days and presence of mucus plugs were
37 associated with a longer time to radiographic clearance (all $P < 0.01$). Compared with
38 children without mucus plugs, those with mucus plugs were significantly more likely
39 to have a longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7;
40 $P < 0.01$).

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

44 **Strengths and limitations of this study:**

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4 45 ● This is the first study to analyze prospectively the risk factors associated with
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6 46 longer time to radiographic clearance in patients with RMPP in China.
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8 47 ● A prospective follow-up of chest radiographs was obtained after discharge
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10 48 every 4 weeks up to a maximum of 24 weeks after diagnosis or until large
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12 49 infiltration on chest radiographs had resolved.
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14 50 ● There might be some patients who had a co-infection with other pathogens
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16 51 which could not be detected and might therefore lead to longer radiographic
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18 52 clearance.
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23 **Key words:** Mucus plugs; refractory Mycoplasma Pneumoniae pneumoniae;
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25 radiographic resolution.
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69 Introduction

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

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

88 Methods

89 ***Cohort Description***

90 Patients with CAP who hospitalized in Department of Respiratory Medicine in
91 Children's Hospital of Soochow University from January 1, 2012 to December 31,
92 2015, were evaluated prospectively for identifying patients who met criteria for RMPP.
93 RMPP was considered when (1). Cough, fever or auscultatory findings together with
94 pulmonary infiltrates on chest radiograph, (2). A significant rise in *M. pneumoniae*
95 IgG or seroconversion in paired sera, together with *M. pneumoniae* DNA detected in
96 nasopharyngeal aspirates, (3). Fever persisting (>38.5°C) and radiological
97 deterioration after the therapy of macrolide for 7 days or more. Bronchoscopy was
98 indicated when lobar consolidation or atelectasis persisted on chest X-ray film after
99 corticosteroid therapy for 1 week. The study was approved by the Institutional
100 Review Board of Suzhou University, and informed consent was obtained for all
101 participants or their parents. Chest X rays films were followed up after discharge
102 every 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration
103 on chest radiographs had resolved.

104 ***Patient and public involvement***

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

111 ***Diagnostic tests for M. pneumoniae***

112 Nasopharyngeal aspirates were obtained within 1 day after the patients admitted. As
113 described previously, specimens were tested to amplify the *M. pneumoniae* P1
114 adhesin gene by using PCR analysis⁶. A quantitative *M. pneumoniae* DNA diagnostic
115 kit (DaAn Gene Co., Ltd. Guangzhou, China) was used. The target specific for *M.*
116 *pneumoniae* genome is 16S rRNA gene.

117 The paired serum samples were taken at admission and at least two weeks after the
118 first serum. The serum samples were tested for IgM and IgG antibodies against *M.*
119 *pneumoniae* using an ELISA kit (Serion ELISA MP IgG/IgM, Institute Virion/Serion,
120 Germany). The cut-off value was 0.5×mean optical density (OD) of control serum of
121 the kit. As described previously, a significant rise in IgG titre was defined as a
122 doubling of the OD value above the cut-off. A sero-conversion was defined as the first
123 serum was negative, and the second serum had an OD at least twice the cut-off⁶.

124 ***Data Collection and Interpretation of Radiographs***

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

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4 133 thickening). Consolidation, atelectasis and pleural disease were defined by standard
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6 134 radiographic criteria⁹.

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8 135 Clinical and laboratory data regarding gender, age, fever duration, length of
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10 136 hospital stay, White blood cell (WBC) count, percentage of neutrophils (%
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12 137 neutrophils), platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction
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14 138 protein (CRP) were collected.

15 16 17 18 139 ***Statistical Analyses***

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20 140 For continuous variables, comparison of means was conducted by using the t test. For
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22 141 ordinally scaled data, the Wilcoxon rank sum test was used. For categorical variables,
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24 142 the chi-square or Fisher exact test were used. A univariate analysis for 8 influence
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26 143 factors (age, sex, WBC, CRP, LDH, number of involved lobes [unilobar vs multilobar
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28 144 involvement], presence of pleural effusion and presence of mucus plug) was
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30 145 performed. Multiple regression analysis was performed to select the variables
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32 146 associated with time to complete radiographic resolution. Probabilities of .05 or less
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34 147 were considered significant.

35 36 37 38 39 148 ***Patient and public involvement***

40
41 149 Patients with CAP who hospitalized in Department of Respiratory Medicine in
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43 150 Children's Hospital of Soochow University from January 1, 2012 to December 31,
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45 151 2015

46 47 48 49 152 **Results**

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51 153 There were totally 8482 patients were included in the four-year period. Among the
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53 154 8482 patients, 2124 (25.0%) were positive by PCR, 2374 (27.9%) had a significant

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4 155 antibody response. *M. pneumoniae* infection was finally diagnosed in 1721 (20.3%)
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6 156 patients. Of the patients with *M. pneumoniae* infection, 223 RMPP patients were
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8 157 qualified for enrollment in the study. Twenty-one (9.4%) refused to participate, and 15
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10 158 (6.7%) agreed to participate but did not return for their follow-up chest radiographs.
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13 159 Finally, 187 patients were recruited and received follow-up chest radiographs. These
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15 160 patients will be referred to as the study group. The patients who were eligible but
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17 161 excluded (n = 36) demonstrated no statistically significant difference in age and sex
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19 162 compared with the studied patients (n=187, both P>0.05). There was also no
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21 163 significant difference in presence of mucus plug in the study group and unenrolled
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23 164 group (P=0.44).

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28 165 The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2 y.
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30 166 Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar
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32 167 involvement, while 88 (47.1) had multilobar involvement. Forty- six (24.6%) had
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34 168 pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%)
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36 169 patients (Figure 1). The median fever duration was 10 (7, 13) days and the median
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38 170 length of hospital stay was 11 (9, 17) days.

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42 171 Approximately half of the patients had complete radiographic clearance by 4 weeks,
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44 172 and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and
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46 173 eighty-three (97.9%) had complete clearance at the end of the study period, and four
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48 174 (0.5%) had persistent abnormalities at 24 weeks (Table 2). The median time to
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50 175 radiographic clearance of all participants was 4 weeks (interquartile range:4–8 weeks).
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52 176 Twenty-seven percent of the subjects had a time to radiographic clearance for >8
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177 weeks. In unadjusted analysis, time to radiographic clearance for >8 weeks was
178 associated with % neutrophils, CRP, LDH, pleural effusion, mucus plug, fever
179 duration when compared with those with time to radiographic clearance ≤ 8 weeks (all
180 $P < 0.01$; Table 3). Other variables (sex, age, lobar involvement, WBC) showed no
181 difference.

182 The multivariable logistic regression model for time to radiographic clearance
183 for >8 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant
184 predictors of a longer time to radiographic clearance were C-reaction protein ≥ 50
185 mg/L, LDH ≥ 480 U/L, Fever duration ≥ 10 days and presence of mucus plugs. (all $P <$
186 0.01). Compared with children without mucus plugs, those with mucus plugs were
187 significantly more likely to have a longer time to radiographic clearance (adjusted OR:
188 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

189 Discussion

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

193 *M. pneumoniae* infection is a common respiratory disease in children¹⁰. In recent
194 years, an increasing number of RMPP patients are being reported, especially in the
195 Asian countries^{4, 11-14}. The role of mucus plug in refractory RMPP have been
196 extensively studied recently^{5, 6, 15}. Xu et al. identified age, fever duration, LDH and
197 CRP as independent risk factors for mucus plug formation⁵. Wang et al. found that in
198 the bronchoscopic imaging, the mucus plug served as a promising predictor for early

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3 199 RMPP diagnosis for pediatric patients with large pulmonary lesions¹⁵. Our previous
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6 200 study also found that RMPP patients with mucus plug were prone to be
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9 201 corticosteroid-resistant, and had a longer fever duration and hospital stay⁶. Our study
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11 202 further highlighted the role of mucus plugs in the time to radiographic clearance in
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13 203 RMPP patients.

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16 204 In our study, we found that presence of mucus plugs was associated with longer
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18 205 time to radiographic clearance in RMPP patients. Liang and her colleagues found that
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20 206 MPP with severe cilia abnormalities was associated with a longer time to radiographic
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22 207 clearance⁸. In Liang's study, they mainly discussed cilia abnormalities, while they did
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24 208 not focus on the mucus plugs in the bronchoscopic findings. Mucus plugs formation
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26 209 was actually a manifestation of severe cilia abnormalities. Severe cilia abnormalities
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28 210 disrupt the mucociliary clearance and reduce the airway immune function, causing
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30 211 mucus plug that is responsible for the development of atelectasis and delayed
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32 212 radiographic resolution^{10, 16}. In RMPP patients, atelectasis is quite common
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34 213 radiographically. The persistent presence of atelectasis lead to a longer radiographic
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36 214 resolution time and even longstanding pulmonary sequelae such as bronchiectasis or
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38 215 bronchiolitis obliterans¹⁷⁻¹⁹. Thus, careful management and follow-up is needed for
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40 216 the patients with mucus plugs.

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42 217 Currently, bronchoscopy is an important tool to perform therapeutic interventions
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44 218 in patients with lobar atelectasis^{7,20,21}. Zhang et al. investigated 35 pediatric subjects
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46 219 with RMPP and found that bronchoscopy was efficacious and well-tolerated for
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48 220 RMPP⁷. Abu-Hasan et al. suggested that bronchoscopy could be safe and effective in

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4 221 treating acute lung collapse and atelectasis that was refractory to conventional
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6 222 therapy²⁰. Kreider et al. also found that bronchoscopy was safe and effective in
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8 223 treating critically ill patients²¹. Our study also highlighted the importance of
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11 224 bronchoscopy, especially for patients with mucus plug.

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13 225 To investigate the risk factors for longer time to radiographic clearance, we also
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15 226 chose variables that are commonly examined in our hospital. Three independent
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17 227 factors of fever duration, CRP, LDH were identified. LDH and CRP were variables
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19 228 that are elevated in many pulmonary diseases and was reported to be associated with
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21 229 RMPP in several studies^{13,14,15,22}. Recently, Serum LDH 4 plus 5 was found to be a
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23 230 better biomarker than total LDH for RMPP in children²³. The precise mechanisms of
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25 231 RMPP remain unknown. Pathogen-related substances or other substances from
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27 232 injured host cells during host hyperactive immune reactions may be responsible for
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29 233 lung cell injury²⁴. Therefore, it is a natural concept that severe RMPP patients have
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31 234 more severe lung injury and higher laboratory parameter values such as CRP, LDH,
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33 235 requiring long-term recovery time. LDH level may be associated with true lung cell
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35 236 injury, and subsequent prolonged recovery period of tissue repair. It is reasonable to
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37 237 recommend that the early use of immune-modulators, without waiting for the
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39 238 antibiotic's effect, contributes to the effective reduction of immune-mediated lung
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41 239 injury in *M. pneumoniae* infection²⁵.

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43 240 The study has some limitations. First, our study was a single-center based study,
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45 241 which might have potential biases and a multi-center study is needed in the future.
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47 242 Second, there might be some patients who had a co-infection with other pathogens
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3 243 which could not be detected and might therefore lead to longer radiographic clearance.
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6 244 Third, the serums and nasopharyngeal samples were not collected on the same day
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8 245 after disease onset, which might produce bias.
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10 246 In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH
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12 247 and presence of mucus plugs to identify children at a longer time to radiographic
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14 248 clearance in patients with RMPP.
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22
23 251 **Contributors:** YDY conceived and designed the study. RZ and WJJ conducted the
24
25 252 study. LZH and XH analyzed the data and interpreted the data. LH provided guidance
26
27 253 on the data analysis. All authors drafted the manuscript and read, edited and approved
28
29 254 the final version of the manuscript. All authors had full access to all of the data in the
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31 255 study and can take responsibility for the integrity of the data and the accuracy of the
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33 256 data analysis.
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46 261 health and Family Planning Commission (grant numbers H201622).
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50 262 **Competing interests:** None declared.
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52 263 **Patient consent:** Parental/guardian consent obtained.
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55 264 **Ethics approval:** This research project was reviewed and approved by the
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4 265 Institutional Review Board of Suzhou University.

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6 266 **Provenance and peer review:** Not commissioned; externally peer reviewed.

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8 267 **Data sharing statement:** No additional data are available.

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30 **Figure legends**

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33 354 **Figure 1** Bronchoscopic findings of the patient with mucus plug.

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375 **Tables**376 **Table 1 Descriptive Analysis of Demographic, Laboratory, Radiographic, and**377 **Bronchoscopic Findings of the Study Population**

Variables	
Male to female ratio	102/85
Age in years, mean \pm SD	6.1 \pm 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)

378

379 **Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae***
380 **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

381 *Patients remaining with abnormal radiographic findings.

382

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

Characteristics	Time to radiographic	Time to radiographic	P
	clearance ≤ 8wk (n=134)	clearance > 8wk (n=53)	
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), ×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

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

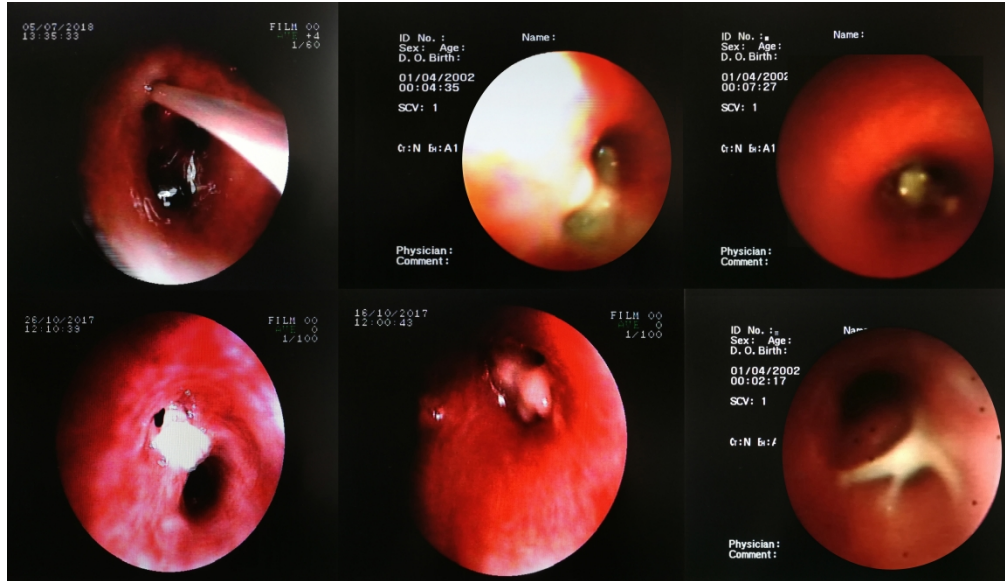
Characteristics	OR (95% CI)	P
C-reaction protein ≥50 mg/L	3.1 (1.7-5.2)	<0.01

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

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For peer review only

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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

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Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Infectious diseases
Keywords:	Mucus plugs, refractory <i>Mycoplasma Pneumoniae</i> pneumonia, radiographic resolution

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Manuscripts

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4 1 **Title page**

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6 2 **Independent predictors for longer radiographic resolution in patients with**
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9 3 **refractory *Mycoplasma pneumoniae* pneumonia: A prospective cohort study**

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11 4 Lizhen Huang¹, Xia Huang², Wujun Jiang², Rong Zhang², Yongdong Yan^{2*}, Li
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19 7 Wujiang District, Suzhou, China.

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4 23 **Abstract**

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6 24 **Objectives:** To examine prospectively the radiographic clearance of refractory
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9 25 *Mycoplasma pneumoniae* pneumonia (RMPP) in immunocompetent children, and to
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12 26 identify independent predictors of time to complete radiographic resolution in patients
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15 27 with RMPP.

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17 28 **Design:** A prospective cohort study.

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19 29 **Setting:** Children's Hospital of Soochow University, China.

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22 30 **Participants:** A total of 187 RMPP patients treated with bronchoscopy were
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25 31 prospectively enrolled in the study between Jan 2012 and Dec 2015.

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27 32 **Methods:** Serial chest radiographs were obtained after discharge every 4 weeks up to
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30 33 a maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs
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33 34 had resolved. Multivariate logistic regression was performed to identify independent
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35 35 predictors of time to complete radiographic resolution.

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37 36 **Results:** Of the 187 RMPP patients, bronchial mucus plugs formation was detected in
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40 37 73 (39.0%) patients. C-reaction protein (CRP) ≥ 50 mg/L, lactate dehydrogenase (LDH)
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43 38 ≥ 480 U/L, total fever duration ≥ 10 days and presence of mucus plugs were associated
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46 39 with a longer time to radiographic clearance (all $P < 0.01$). Compared with children
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49 40 without mucus plugs, those with mucus plugs were significantly more likely to have a
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52 41 longer time to radiographic clearance (adjusted OR: 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

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54 42 **Conclusions:** Clinicians might use the parameters of duration of fever, CRP, LDH and
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57 43 presence of mucus plugs to identify children at a longer time to radiographic clearance
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60 44 in patients with RMPP.

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4 45 **Strengths and limitations of this study:**

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6 46 ● This is the first study to analyze prospectively the risk factors associated with
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9 47 longer time to radiographic clearance in patients with RMPP in China.
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11 48 ● A prospective follow-up of chest radiographs was obtained after discharge every
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14 49 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration
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17 50 on chest radiographs had resolved.
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19 51 ● Our study was a single-center based study, which might have introduced a
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22 52 selection bias.
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24 53 ● There might be some patients who had a co-infection with other pathogens which
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27 54 could not be detected and might therefore lead to longer radiographic clearance.
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30 55 ● The serums and nasopharyngeal samples were not collected on the same day
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33 56 after disease onset, which might produce measurement bias.
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35 57 **Key words:** Mucus plugs; refractory *Mycoplasma pneumoniae* pneumonia;
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37 58 radiographic resolution.
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67 **Introduction**

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

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

85 **Methods**

86 *Cohort Description*

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

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4 89 were evaluated prospectively for identifying patients who met criteria for RMPP.
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6 90 RMPP was considered when (1) Cough, fever or auscultatory findings together with
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9 91 pulmonary infiltrates on chest radiograph; (2) a significant rise in *M. pneumoniae* IgG
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12 92 or seroconversion in paired sera, together with *M. pneumoniae* DNA detected in
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14 93 nasopharyngeal aspirates; (3) fever persisting (>38.5°C) and radiological deterioration
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17 94 after the therapy of macrolide for 7 days or more. Bronchoscopy was indicated when
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20 95 lobar consolidation or atelectasis persisted on chest X-ray film after corticosteroid
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23 96 therapy for 1 week. The study was approved by the Institutional Review Board of
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25 97 Suzhou University, and informed consent was obtained for all participants or their
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28 98 parents. Chest X rays films were followed up after discharge every 4 weeks up to a
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30 99 maximum of 24 weeks after diagnosis or until large infiltration on chest radiographs
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33 100 had resolved.

101 ***Patient and public involvement***

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

108 ***Diagnostic tests for *M. pneumoniae****

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

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4 111 by using PCR analysis⁶. A quantitative *M. pneumoniae* DNA diagnostic kit (DaAn
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6 112 Gene Co., Ltd. Guangzhou, China) was used. The target specific for *M. pneumoniae*
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9 113 genome is 16S rRNA gene.

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11 114 The paired serum samples were taken at admission and at least two weeks after the
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14 115 first serum sampling. The serum samples were tested for IgM and IgG antibodies
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17 116 against *M. pneumoniae* using an ELISA kit (Serion ELISA MP IgG/IgM, Institute
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19 117 Virion/Serion, Germany). The cut-off value was 0.5×mean optical density (OD) of
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22 118 control serum of the kit. As described previously, a significant rise in IgG titre was
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25 119 defined as a doubling of the OD value above the cut-off. A sero-conversion was defined
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27 120 as the first serum was negative, and the second serum had an OD at least twice the cut-
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30 121 off⁶.

31 32 122 ***Data Collection and Interpretation of Radiographs***

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35 123 Serial posteroanterior and lateral chest radiographs were obtained after discharge every
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38 124 4 weeks up to a maximum of 24 weeks after diagnosis or until large infiltration on chest
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41 125 radiographs had resolved. All radiographs were evaluated independently by two
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43 126 radiologists (PP and WLG), who did not know the patients' clinical condition. Chest
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46 127 radiographs were reviewed by two radiologists in sequence with the prior films for
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48 128 comparison. If differences in interpretation of radiographs occurred, it would be
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51 129 resolved by joint consensus between the two radiologists. The radiographs were
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54 130 reviewed for the presence of consolidation, atelectasis and pleural disease (effusion or
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56 131 thickening). Consolidation, atelectasis and pleural disease were defined by standard
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59 132 radiographic criteria⁹.

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4 133 Clinical and laboratory data regarding gender, age, total fever duration, length of
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6 134 hospital stay, white blood cell (WBC) count, percentage of neutrophils (% neutrophils),
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9 135 platelet (PLT) count, lactate dehydrogenase (LDH) and C-reaction protein (CRP) were
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12 136 collected.

137 ***Statistical Analyses***

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

151 **Results**

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

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4 155 the patients with *M. pneumoniae* infection, 223 RMPP patients qualified for enrollment
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6 156 in the study. Twenty-one (9.4%) refused to participate, and 15 (6.7%) agreed to
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9 157 participate but did not return for their follow-up chest radiographs. Finally, 187 patients
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12 158 were recruited and received follow-up chest radiographs. These patients were referred
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15 159 to as the study group. The patients who were eligible but excluded (n = 36)
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17 160 demonstrated no statistically significant difference in age and sex compared with the
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20 161 studied patients (n=187, both P>0.05). There was also no significant difference in
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22 162 presence of mucus plug in the study group and unenrolled group (P=0.44).

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25 163 The 187 RMPP patients (86 females and 101 males) had a mean age of 6.1 ± 2.2 y.
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27 164 Descriptive statistics are shown in Table 1. Ninety-nine (52.9%) had unilobar
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30 165 involvement, while 88 (47.1) had multilobar involvement. Forty-six (24.6%) had
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32 166 pleural effusion. Bronchial mucus plugs formation was detected in 73 (39.0%) patients
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35 167 (Figure 1). The median total fever duration was 10 (7, 13) days and the median length
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37 168 of hospital stay was 11 (9, 17) days.

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40 169 Approximately half of the patients had complete radiographic clearance by 4 weeks,
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42 170 and 72.7% demonstrated radiographic resolution by 8 weeks. One hundred and eighty-
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45 171 three (97.9%) had complete clearance at the end of the study period, and four (0.5%)
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48 172 had persistent abnormalities at 24 weeks (Table 2). The median time to radiographic
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51 173 clearance of all participants was 4 weeks (interquartile range: 4–8 weeks). Twenty-
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53 174 seven percent of the subjects had a time to radiographic clearance for >8 weeks. In
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56 175 unadjusted analysis, time to radiographic clearance for >8 weeks was associated with %
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58 176 neutrophils, CRP, LDH, pleural effusion, mucus plug, total fever duration when
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4 177 compared with those with time to radiographic clearance ≤ 8 weeks (all $P < 0.01$; Table
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6 178 3). Other variables (sex, age, lobar involvement, WBC) showed no difference.

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9 179 The multivariable logistic regression model for time to radiographic clearance for > 8
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11 180 weeks is shown in Table 4. Controlling for 6 clinical characteristics, significant
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13 181 predictors of a longer time to radiographic clearance were C-reaction protein ≥ 50 mg/L,
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15 182 LDH ≥ 480 U/L, total fever duration ≥ 10 days and presence of mucus plugs. (all $P <$
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17 183 0.01). Compared with children without mucus plugs, those with mucus plugs were
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19 184 significantly more likely to have a longer time to radiographic clearance (adjusted OR:
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21 185 11.5; 95% CI: 2.5–45.7; $P < 0.01$).

22 186 **Discussion**

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27 187 This is the first study, to our knowledge, that focus on the follow-up chest radiographic
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29 188 clearance in patients with RMPP. CRP, LDH, total fever duration and presence of
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31 189 mucus plugs were independently associated with longer time to radiographic clearance.

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37 190 *M. pneumoniae* infection is a common respiratory disease in children¹⁰. In recent
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39 191 years, an increasing number of RMPP patients are being reported, especially in the
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41 192 Asian countries^{4, 11-14}. The role of mucus plug in refractory RMPP have been
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43 193 extensively studied recently^{5, 6, 15}. Xu *et al.* identified age, total fever duration, LDH
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45 194 and CRP as independent risk factors for mucus plug formation⁵. Wang *et al.* found that
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47 195 in the bronchoscopic imaging, the mucus plug served as a promising predictor for early
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49 196 RMPP diagnosis for pediatric patients with large pulmonary lesions¹⁵. Our previous
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51 197 study also found that RMPP patients with mucus plug were prone to be corticosteroid-
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53 198 resistant, and had a longer total fever duration and hospital stay⁶. Our study further
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4 199 highlighted the role of mucus plugs in the time to radiographic clearance in RMPP
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6 200 patients.
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9 201 In our study, we found that presence of mucus plugs was associated with longer time
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11 202 to radiographic clearance in RMPP patients. Liang and her colleagues found that MPP
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13 203 with severe cilia abnormalities was associated with a longer time to radiographic
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15 204 clearance⁸, but they did not focus on analysis of the mucus plugs in the bronchoscopic
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17 205 findings. Mucus plugs formation was actually a manifestation of severe cilia
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19 206 abnormalities. Severe cilia abnormalities disrupt the mucociliary clearance, causing
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21 207 mucus plug that is responsible for the development of atelectasis and delayed
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23 208 radiographic resolution^{10, 16}. The persistent presence of atelectasis lead to a longer
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25 209 radiographic resolution time and longstanding pulmonary sequelae such as
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27 210 bronchiectasis or bronchiolitis obliterans¹⁷⁻¹⁹. Thus, careful management and follow-up
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29 211 is needed for the patients with mucus plugs.
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38 212 Currently, bronchoscopy is an important tool for therapeutic interventions in patients
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40 213 with lobar atelectasis^{7,20,21}. Zhang *et al.* investigated 35 pediatric subjects with RMPP
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42 214 and found that bronchoscopy was efficacious and well-tolerated⁷. Abu-Hasan *et al.*
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44 215 suggested that bronchoscopy could be safe and effective in treating acute lung collapse
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46 216 and atelectasis that was refractory to conventional therapy²⁰. Kreider *et al.* also found
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48 217 that bronchoscopy was safe and effective in treating critically ill patients²¹. Our study
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50 218 also highlighted the importance of bronchoscopy, especially for patients with mucus
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52 219 plug.
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58 220 To investigate the risk factors for longer time to radiographic clearance, we also
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4 221 chose variables that are commonly examined in our hospital. Three independent factors,
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6 222 including total fever duration, CRP, LDH were identified. LDH and CRP were variables
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9 223 that are elevated in many pulmonary diseases and was reported to be associated with
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11 224 RMPP in several studies^{13,14,15,22}. Recently, serum LDH 4 plus 5 were found to be better
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14 225 biomarkers than total LDH for RMPP in children²³. The precise mechanisms of RMPP
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17 226 remain unknown. Pathogen-related substances or other host factors during hyperactive
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20 227 immune reactions may be responsible for lung injury²⁴. Therefore, it may be logical to
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22 228 propose that severe RMPP patients have severe lung injury and higher clinical
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25 229 parameter values such as CRP, LDH, requiring long-term recovery time. LDH level
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28 230 may be associated with true lung injury, and subsequent prolonged recovery period of
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31 231 tissue repair. Therefore, it is reasonable to recommend that the early use of immune-
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33 232 modulators, without waiting for the antibiotic's effect, contributes to the effective
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35 233 reduction of immune-mediated lung injury in *M. pneumoniae* infection²⁵.

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38 234 The study has some limitations. Firstly, our study was a single-center based study,
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41 235 which might have introduced a selection bias. The results reported in our Soochow area
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44 236 cannot be extrapolated to other areas in China. Thus, a multi-center study is needed in
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47 237 the future. Secondly, there might be some patients who had a co-infection with other
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50 238 pathogens which could not be detected and might therefore lead to longer radiographic
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53 239 clearance. Thirdly, the serums and nasopharyngeal samples were not collected on the
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56 240 same day after disease onset, which might produce measurement bias.

56 241 In conclusion, clinicians might use the parameters of duration of fever, CRP, LDH
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59 242 and presence of mucus plugs to identify children at a longer time to radiographic
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4 243 clearance in patients with RMPP.
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14 247 study. LZH and XH analyzed the data and interpreted the data. LH provided guidance
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17 248 on the data analysis. All authors drafted the manuscript and read, edited and approved
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20 249 the final version of the manuscript. All authors had full access to all of the data in the
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23 250 study and can take responsibility for the integrity of the data and the accuracy of the
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25 251 data analysis.
26

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43 258 **Patient consent:** Parental/guardian consent obtained.
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45 259 **Ethics approval:** This research project was reviewed and approved by the Institutional
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48 260 Review Board of Suzhou University.
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53 262 **Data sharing statement:** No additional data are available.
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For peer review only

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4 342 **Figure legends**

5
6 343 **Figure 1** Bronchoscopic findings of refractory *Mycoplasma pneumoniae* pneumonia

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9 344 patients with mucus plug.

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364 **Tables**365 **Table 1 Descriptive analysis of demographic, laboratory, radiographic, and**
366 **bronchoscopic findings of the study population**

Variables	
Male to female ratio	102/85
Age in years, mean \pm SD	6.1 \pm 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)

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368 **Table 2 Radiographic resolution pattern in refractory *Mycoplasma pneumoniae***
369 **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

370 *Patients remaining with abnormal radiographic findings.

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

Characteristics	Time to radiographic clearance ≤ 8wk (n=134)	Time to radiographic clearance > 8wk (n=53)	P
	Male/female	73/61	
Age in years, mean±SD	5.7±2.5	6.3±2.9	0.47
White cell count, median (quartile), ×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 ≥ 10 d, N%	26 (19.4)	23 (43.4)	<0.01

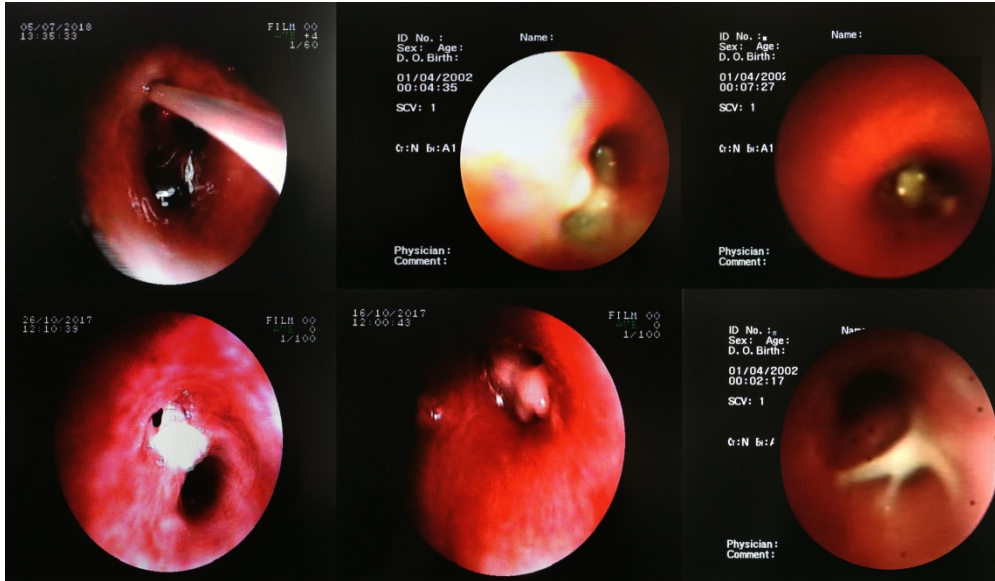
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376 **Table 4 Multivariable predictors of time to radiographic clearance > 8 weeks**
 377 **among children with refractory *Mycoplasma pneumoniae* pneumonia**

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 ≥ 10 d	13.5 (7.8~41.4)	<0.01
Bronchial mucus plugs formation	11.5 (2.5-45.7)	<0.01

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

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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>.