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Review Article

Rational stepwise approach for *Mycoplasma pneumoniae* pneumonia in children

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Abstract *Mycoplasma pneumoniae* is a common pathogen that causes community-acquired pneumonia. In the past, *M. pneumoniae* was sensitive to macrolide antibiotics, and *M. pneumoniae* pneumonia (MPP) was usually a benign and self-limiting disease. However, despite use of the appropriate antibiotics, persistent fever and clinical deterioration may occur, leading to severe disease. Two major complicated conditions that may be clinically encountered are macrolide-resistant MPP and refractory MPP. Regarding the epidemics in Taiwan, before 2017, the mean rate of macrolide resistance was below 30%. Notably, since 2018, the prevalence of macrolide-resistant MPP in Taiwan has increased rapidly. Macrolide-resistant MPP shows persistent fever and/or no radiological regression to macrolide antibiotics and may even progress to severe and complicated pneumonia. Tetracyclines (doxycycline or minocycline) or fluoroquinolones are alternative treatments for macrolide-resistant MPP. Refractory MPP is characterized by an excessive immune response against the pathogen. In this context, corticosteroids have been suggested as an immunomodulator for downregulating the overactive host immune reaction. Overuse of macrolides may contribute to macrolide resistance, and thereafter, an increase in macrolide-resistant MPP. Delayed effective antimicrobial treatment is associated with prolonged and/or more severe disease. Thus, the appropriate prescription of antibiotics, as well as the rapid and accurate diagnosis of MPP, is important. The exact starting point, dose, and duration of the immunomodulator are yet to be established. We discuss these important issues in this review.

Abbreviations: MPP, *M. pneumoniae* pneumonia; CAP, community acquired pneumonia; MR, macrolide-resistant; MS, macrolide-sensitive; RMPP, *refractory* mycoplasma pneumoniae pneumonia.

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Introduction to *Mycoplasma pneumoniae* pneumonia (MPP)

Mycoplasma pneumoniae (M. pneumoniae) is an important infectious pathogen of the lower respiratory tract that is often transmitted via respiratory droplets following close contact with a symptomatic patient. *M. pneumoniae* causes infections in people of any age and is an important pathogen of community-acquired pneumonia (CAP) in school-aged children and young adults. The percentage of *M. pneumonia*-causing CAP in children is 15–37% in Taiwan,[1–3] and this ratio is higher in children aged 5-10 years-old than in other age groups.[1]

The symptoms of *M. pneumoniae* are variable and include fever, cough (dry or productive), sore throat, coryza, and occasionally, headache.[4] *M. pneumoniae* has also been linked to acute exacerbation of asthma. Some severe respiratory illnesses caused by *M. pneumoniae* infection, such as lung abscesses, bronchiolitis obliterans, bronchiectasis, pleural effusion, pulmonary embolism, and respiratory distress syndrome, have also been reported.[4] For extrapulmonary manifestations, nearly every organ can be affected. These severe or extrapulmonary presentations may result from an excessive immune response or abundant bacterial load.[4,5] Macrolide-resistant *M. pneumoniae* infection and refractory MPP are two conditions that may complicate the clinical treatment of MPP.

Diagnostic tests for *Mycoplasma pneumoniae* infection

Culture

Although the culture is of 100% specific to the pathogen, it is often not clinically feasible because *in vitro* growth of *M. pneumoniae* requires a specific broth or agar. Culture methods have been the gold standard for diagnosis but are too insensitive producing a result after several days or even several weeks and are therefore not relevant for the management of acute illness.[4]

Nucleic acid amplification

Polymerase chain reaction (PCR) testing can provide rapid diagnosis, and oropharyngeal/nasopharyngeal specimens are mostly used for PCR-based detection in children. Owing to its commercial availability, sensitivity, and specificity, PCR testing has replaced culture as a diagnostic tool widely used in clinic.[4] However, diagnosis of mycoplasma infections by PCR should be based on the clinical condition, since *M. pneumoniae* may colonize the respiratory tract for several weeks after infection, even with proper antimicrobial treatment.[6]

Serological tests

M. pneumoniae-specific IgM antibodies may be undetectable for the first 7 days following the onset of symptoms and can persist in the serum for several months post-infection.[7] Therefore, a single test for positive or negative antibody titer in an acute phase serum sample is not always an accurate diagnostic for acute *M. pneumoniae* infection. Our previous study showed that the initial positive test rate for *M. pneumoniae* IgM upon hospital admission was 63.6%, and the cumulative positive test rate for *M. pneumoniae* IgM increased to 97.5% one week after admission.[8] Paired sera demonstrating a seroconversion from negative to positive or an increase in antibody titers (i.e., a two-fold increase in *M. pneumoniae* IgM or a four-fold increase in *M. pneumoniae* IgG) between acute and convalescent phases allow for highly accurate diagnosis.[4,9,10] Nevertheless, for patients with impaired immunity, including infants, the immune response maybe too low to be detected.[4]

Detecting *M. pneumoniae* IgM-secreting cells has been suggested as a better diagnostic method for acute infection because these cells appear and disappear earlier than antibodies.[11] However, this technique requires fresh blood samples and is time consuming. Further studies are required to validate the clinical values of this proposed diagnostic method.

Rapid screening test

Some commercialized immunoassays for the detection of *M. pneumoniae* antigens or specific IgM provide rapid and practical information for *M. pneumoniae* infection diagnosis. In terms of the rapid antigen test, a throat or nasopharyngeal swab is used to obtain a sample of the *M. pneumoniae* antigen from the patient's pharynx. The sensitivity and specificity of these rapid tests was found to be 60–80% and 90–100%, respectively by using PCR as standard.[12–14] Thus, a positive rapid antigen test result is highly suggestive of an active *M. pneumoniae* infection. However, a negative rapid antigen test result does not exclude the possibility of *M. pneumoniae* infection. "Capilia™ Mycoplasma" is one of the commercialized rapid antigen tests available in Taiwan.

Another type of rapid test detects mycoplasma IgM by using lateral flow immunoassay. This immunoassay is performed using capillary blood from a fingerstick sample, and one of the commercialized products that is available in Taiwan is "Biocard™ *Mycoplasma* IgM rapid test". When using *Mycoplasma* IgM ELISA as the standard, the sensitivity and specificity of this rapid test is 62.2% and 100%, respectively.[15] Similar to mycoplasma IgM determination, this test may be negative in the initial stage of infection and positive for a relatively long period post-infection.[9]

Recommended diagnostic approach

MPP may be difficult to diagnose solely based on clinical symptoms such as cough, fever, and auscultatory findings, thereby necessitating diagnostic laboratory tests, though lack of standard methods. In the early phase of infection, a positive PCR test may accompany a negative serological test, as it takes time for IgM antibodies to develop. In the convalescent phase, a positive serological test may accompany a negative PCR test.[4] Thus, there is no single

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reliable test for accurate diagnosis of *M. pneumoniae* infection. For patients with suspected clinical features, the combination of PCR and IgM tests may be the most optimal approach for early diagnosis, especially among pediatric patients.[16] Patients with suspected clinical features and one of the following three laboratory results may be diagnosed as having acute mycoplasma infection:

1) Seroconversion (specific *M. pneumoniae* IgM from negative converse to positive).
2) An increase in specific antibody (two-fold increase in *M. pneumoniae* IgM or four-fold increase in specific IgG by titer or dilution test) within a two-week interval.
3) Positive PCR results.

Macrolide-resistant *Mycoplasma pneumoniae* pneumonia

Prevalence and characteristics of macrolide-resistant *Mycoplasma pneumoniae* pneumonia

Recently, macrolide-resistant *M. pneumoniae* has become increasingly prevalent worldwide, partly owing to the broad use of macrolides.[17] The reported macrolide resistance rate of *M. pneumoniae* in Taiwan between 2010 and 2017 was 15–30%,[1,3,18] and unpublished data from our group and others show that this rate has been increasing from 2016 to 2019. The resistance rate was about 60–90% in neighboring countries, including China, Japan, and Korea, while it was <30% in America and Europe from 2008 to 2013.[4]

Macrolides inhibit protein synthesis by binding to domains II and/or V of the 23S rRNA in the 50S bacterial ribosomal subunit.[4] Mutations in domain V of the 23S rRNA of *M. pneumoniae* is the major mechanism of macrolide resistance.[19] To clarify whether the causative organism is sensitive or resistant to macrolides, the causative organism is isolated and tested for drug susceptibility or indicated of a point mutation in the 23S ribosomal RNA domain V by PCR.[4,9] A2063 G/C, A2064 G/C, and C2617 G/A have been shown to be the mutation sites for macrolide resistance. Moreover, A2063G accounts for over 80% of point mutations in Taiwan from 2010 to 2017.[18,20]

Clinical presentations

The clinical presentation of children with macrolide-resistant MPP is similar to that of children with macrolide-sensitive MPP. Usually, patients with macrolide-resistant MPP experience long febrile days owing to delayed effective antibiotic therapy,[17,18,21] but whether resistant strains cause more severe disease remains controversial. Some studies have suggested that, during hospitalization, the severity of inflammatory markers and radiological findings of patients with macrolide-resistance MPP were not higher than those with macrolide-sensitive MPP.[17,22] However, Zhou et al. reported that patients with macrolide-resistant MPP have more extrapulmonary complications and more severe radiological findings than patients with macrolide-sensitive MPP.[23] Although PCR detection of macrolide-resistance *M. pneumoniae* can aid the effective prescription of antibiotics, this test is not routinely used in the clinic because it is costly and the symptoms of some patients with macrolide-resistant MPP can still improve with macrolide treatment alone.[22,24]

Treatment of *Mycoplasma pneumoniae* pneumonia

Although *M. pneumoniae* infection is self-limited in most cases,[3,21] complications may occur if an ineffective antimicrobial agent is used.[1,23] Persistent fever and/or more severe or extrapulmonary manifestations may occur because of delayed treatment.[18] Thus, early detection and proper treatment are helpful for preventing deterioration. The common empirical antibiotics for CAP, such as beta-lactams, are ineffective because *M. pneumoniae lacks a cell wall*. The main drugs used for the treatment of *M. pneumoniae* infection are macrolides, tetracyclines, and fluoroquinolones.[4]

First line antibiotic: macrolides

Macrolides inhibit protein synthesis by binding to bacterial ribosomes. Macrolides have both direct antimicrobial activities and anti-inflammatory effects on cytokine production, including interleukin (IL)-8, in the treatment of *M. pneumoniae* infection.[21] In general, macrolides are the most potent antibiotics against macrolide-sensitive *M. pneumoniae* because of their low minimal inhibitory concentrations (MICs).[4] Furthermore, pathogen elimination following treatment with macrolides is higher than with other antibiotics.[25] For macrolide-sensitive *M. pneumoniae*, the MIC of azithromycin is less than 0.0005 μg/mL, which is much lower than doxycycline (MIC $\leq$ 0.25 μg/mL) and levofloxacin (MIC $\leq$ 0.5 μg/mL).[26] Therefore, macrolide is recommended as the first-line choice for patients with MPP who have not yet received the appropriate antibiotics.

The recommended duration and dose of macrolide treatment for *M. pneumoniae* infection is 14 days for erythromycin (25–50 mg/kg/day, 4–6 doses/day, orally), 10 days for clarithromycin (10–15 mg/kg/day, 2–3 doses/day, orally), and 3 days for azithromycin (10 mg/kg/day, once daily, orally).[9] The most common adverse event following macrolide treatment is gastrointestinal upset, with azithromycin having the lowest adverse effect rate and longest half-life.[25]

Effects of macrolide on macrolide-sensitive *Mycoplasma pneumoniae* pneumonia

Previous studies have shown that 71–88% of patients with macrolide-sensitive MPP defervesce within 48 h of the starting their macrolide treatment.[22,27,28] In contrast, in macrolide-resistant MPP cases, fever remains in 52–73% of patients and 30% of patients for more than 48 h and 72 h, respectively, after initiating macrolide treatment.[22,27–29] Thus, the response to macrolides can be assessed by defervescence within 72 h or not after initiation of treatment.[9,30] If fever persists or clinical

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Table 1 Immunosuppressant therapy regimens and outcome for children with *Mycoplasma pneumoniae* pneumonia.

Study	Study year	Study site	Criteria for immunosuppressant therapy	Case numbers	Antimicrobials	Immunosuppressant regimens	Outcome
Oishi et al.[47]	August 2006 to February 2008	Japan	High level of IL-18 (>1000 pg/ml)	2 (age: 5 and 8 years old)	Azithromycin or minocycline	Methylprednisolone (1 mg/kg/dose, tid)	Clinical conditions improved
Liu et al.[10]	October 2015 to March 2017	Taiwan	Refractory MPP	16 (age: 2—18 years old)	Azithromycin	Methylprednisolone (1 mg/kg/dose, tid for 3 days)	Clinical conditions improved
Shan et al.[41]	May 2013 to May 2015	China	Refractory MPP	151 (age: 2—14 years old)	Azithromycin	Methylprednisolone (2 mg/kg/day, for 3 days), or IVIG (400 mg/kg/day, for 3 days)	Clinical condition improved faster with immunosuppressant therapy
Yan et al.[50]	January 2012 to December 2014	China	Refractory MPP	183 (mean age: 6.20 ± 2.59 years old)	Azithromycin	Methylprednisolone (2 mg/kg/day)	Clinical conditions improved, and 80.3% cases defervesced within 72 h after steroid therapy
Inamura et al.[37]	April 2010 to November 2012	Japan	Refractory MPP	5 (age: 1—14 years old)	Minocycline or tosufloxacin	Methylprednisolone (1 mg/kg/day, for 5—8 days), or methylprednisolone (30 mg/kg/day, for 3 days)	Clinical conditions improved
Tamura et al.[43]	January 1998 to December 2006	Japan	Refractory MPP	6 (age: 3—9 years old)	Erythromycin, clindamycin, or azithromycin with or without minocycline	Methylprednisolone (30 mg/kg, qd for 3 days)	Clinical conditions improved, and all cases defervesced within 14 h after steroid therapy
You et al.[42]	January 2011 to December 2011	Korea	Refractory MPP	12 (age: 3—13 years old)	Clarithromycin or roxithromycin	Methylprednisolone (30 mg/kg, qd for 3 days)	Clinical conditions improved, and all cases defervesced within 2 h after steroid therapy
Sun et al.[48]	Published year 2015—2019	China	Severe pneumonia with poor general or respiratory conditions, or radiological findings	1049 (age: 9 month—12 years old)	(not mentioned)	Methylprednisolone (1—2 mg/kg, qd), or methylprednisolone (10—30 mg/kg, qd)	High-dose methylprednisolone is safe and more effective
Luo et al.[38]	May 2007 to May 2010	China	Refractory MPP	58 (mean age: 7.6 ± 4.5 years old)	Azithromycin	Oral prednisolone (1 mg/kg/dose, bid for 5 days)	Clinical condition improved faster with steroid therapy, and all cases defervesced within 48 h after steroid therapy

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Lee et al.[49]	1993 to 2003	Korea	Severe progressive pneumonia 6.0 (±1.5) days after symptoms present	15 (age: 3–14 years old)	Erythromycin, miokamycin, roxithromycin, or clarithromycin	Oral prednisolone (1 mg/kg/day, for 3–7 days, then weaned over 1 week)	Clinical conditions improved, and 93.3% cases defervesced within 24 h after steroid therapy

MPP, *Mycoplasma pneumoniae* pneumonia; tid, thrice daily; qd, once daily; bid, twice daily.

manifestations do not improve 72 h after initiation of macrolide treatment, physicians should first exclude other possible causes of pneumonia or infection, and a second-line antibiotic for *M. pneumoniae* may be prescribed for suspected macrolide-resistant *M. pneumoniae* infection.

Second-line antibiotics for *Mycoplasma pneumoniae* pneumonia: tetracyclines and fluoroquinolones

The use of tetracyclines (doxycycline or minocycline) and fluoroquinolones is considered for patients with *M. pneumoniae* infection who do not respond to macrolides. MICs for tetracyclines and fluoroquinolones in macrolide-resistant *M. pneumoniae* strains are comparable to those in susceptible strains, but the MICs of azithromycin often exceed 64 μg/mL.[21] A previous study has shown that fever subsides within 72 h of switching to tetracyclines (doxycycline or minocycline) or fluoroquinolones in nearly all patients with macrolide-resistant MPP.[3,29] Although fluoroquinolones and tetracyclines are not usually recommended for children, they are the only realistic options until new effective drugs against macrolide-resistant strains become available.

Tetracyclines

Tetracyclines reversibly inhibit bacterial protein synthesis by binding to the ribosomal complex.[31] Tetracyclines are contraindicated in children younger than 8 years of age, because they can cause permanent dental discoloration.[9] Tetracyclines may also cause enamel hypoplasia and reversible, depression of bone growth. Doxycycline, a type of tetracycline antibiotic, has not been shown to cause permanent staining of teeth at the recommended dose and duration of treatment.[32] The recommended dose of doxycycline for MPP is 4 mg/kg/day administered twice daily. For safety and efficiency reasons, we advise that doxycycline be used as the first alternative antibiotic for macrolide-resistant MPP in Taiwan.[27,29] However, before doxycycline is prescribed, detailed evaluation for the possibility of infection with other pathogens or mixed infections is essential. Common bacteria that co-infect with *M. pneumoniae* include *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*, while *common co-infecting viruses include* human bocavirus, human rhinovirus, *influenza*, and respiratory syncytial virus.[1,3,18]

Fluoroquinolones

Fluoroquinolones inhibit DNA replication, and their use in children under the age of 18 years is relatively contraindicated. Cartilage damage in juvenile animals following fluoroquinolone treatment has been noted, and risk of tendon rupture is also shown to increase in people with concomitant exposure to corticosteroids.[4] Although not as effective as other fluoroquinolones such as moxifloxacin and levofloxacin,[33] ciprofloxacin is the only fluoroquinolone used in children with reimbursement by National Health Insurance in Taiwan, and its cartilage-toxicity effect is now considered the lowest among the other fluoroquinolones.

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Moreover, a systematic review on ciprofloxacin use in severe neonatal infections showed that no serious adverse events, including joint toxicity, were observed.[34]

Refractory *Mycoplasma pneumoniae* pneumonia

Definition

In addition to macrolide-resistant MPP, refractory MPP is another condition that may complicate the clinical treatment of MPP. Refractory MPP is defined as persistent fever and/or deterioration of clinical or radiological findings after administration of the appropriate antibiotic treatment for 7 days or more.[5,10,35–43] Patients with refractory MPP have a significantly longer duration of fever, length of hospitalization, and higher incidence of extra-pulmonary complications than those with non-refractory MPP.[36]

Because of a lack of evidence, the clinical relevance of resistant strains in refractory MPP is debatable. Macrolide-resistant strains may not be associated with refractory MPP because some studies have shown that the resistance rate of refractory MPP is similar to that of general mycoplasma pneumonia.[18,23,27,28,37,39] The association between refractory MPP and an increase in macrolide-resistant strains requires further investigation.

Immunopathogenesis of refractory *Mycoplasma pneumoniae* pneumonia

The pathogenesis of *M. pneumoniae* infection is related to the stimulation of macrophages via toll-like receptors and release of inflammatory cytokines and chemokines, such as IL-18 and IL-8,[4] which may cause an inflammatory reaction resulting in pneumonia.[44] In certain conditions, the host immune response may become altered, leading to serious inflammatory processes despite treatment with effective antimicrobial agents.[9] Pulmonary injuries associated with refractory MPP or severe *MPP* occur owing to excessive host immune responses rather than direct microbial damage.[45,46] Lactate dehydrogenase (LDH) is generally considered to be a reliable biomarker of refractory MPP because it is upregulated in this disease and it is commercial available. The cut-off level of LDH for considering refractory MPP treatment ranges from 379 to 480 IU/L according to different report.[9,10,35,40,47] Some other inflammatory biomarkers, including CRP,[40] ESR,[35] α-hydroxybutyrate dehydrogenase (HBDH),[35] IL-10,[36] IL-6,[40] IFN-γ,[5,36] and TNF-α[5] are also used to predict refractory MPP, but LDH is still the most reliable biomarker now. In short, refractory MPP results from immune response hyperactivity, and anti-inflammation drug plays a role in treatment.

Treatment of refractory *Mycoplasma pneumoniae* pneumonia: steroid or intravenous immunoglobulin (IVIG)

Corticosteroids are used to decrease inflammatory responses. Systemic administration of corticosteroids has been shown to be an effective treatment for patients with refractory MPP.[9,47] Moreover, the elevated LDH levels in refractory MPP are also downregulated following steroid treatment.[39]

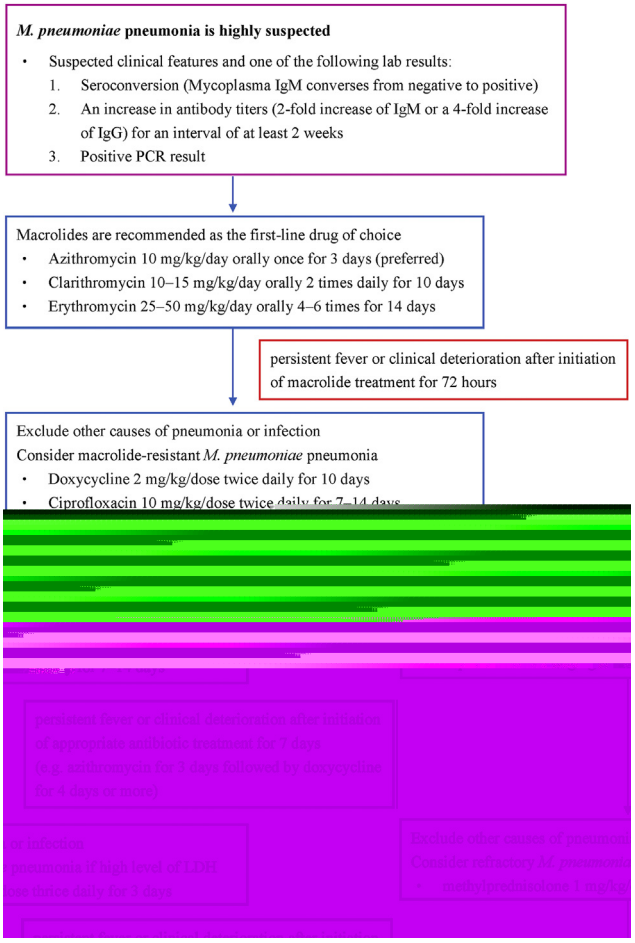

Figure 1. Rational stepwise approach for treating *Mycoplasma pneumoniae* pneumonia in children.

Most patients with refractory MPP show clinical improvements after treatment with corticosteroids, but the optimal regimen is still undetermined. Most of the proposed doses, frequencies, and durations of steroid treatment are summarized in Table 1. All regimens result in positive outcomes. Recently, a meta-analysis comparing the effectiveness and safety of high- and low-dose methylprednisolone in treating severe MPP showed that high-dose methylprednisolone was safer and more effective than a low-dose regimen.[48] However, this study was limited to Chinese children and additional data are required to arrive at a solid conclusion.

IVIG treatment, 400 mg/kg/day for 3 days,[41] may be an alternative approach for refractory MPP treatment because of its anti-inflammatory effects, especially considering the safety of corticosteroids.[41] There were no adverse reactions reported during or after corticosteroid or IVIG administration.[37,41] Furthermore, the clinical presentations and radiological findings did not worsen after discontinuation of steroid treatment, and patients did not show any additional complications due to infection.[38,49]

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Steroid-resistant refractory *Mycoplasma pneumoniae* pneumonia

Most patients with refractory MPP achieved defervescence within 48 h of starting steroid treatment.[38,49] A fever may persist for more than 3 days following steroid treatment in approximately 20% of children with refractory MPP.[50] A study on this condition defined corticosteroid-resistant refractory MPP as a persistent or relapsed fever for >72 h after intravenous methylprednisolone.[50] In this case, increasing the dose of steroids or administering IVIG could be considered. Intravenously administered methylprednisolone at 4 mg/kg/day, followed by an increase to 6 mg/kg/day if fever persists or 400 mg/kg/day of IVIG for two consecutive days, has also been reported.[50] However, additional cases studies are required to derive concrete conclusions.

Suggestion of a stepwise approach for *Mycoplasma pneumoniae* pneumonia treatment(Fig. 1)

We recommend diagnosing *M. pneumoniae* using PCR and/or paired serum tests, though lack of standard methods. A rapid antigen or antibody test can be used for MPP diagnosis but requires careful interpretation. When *M. pneumoniae* infection is diagnosed or highly suspected, azithromycin (10 mg/kg/day for 3 days) should be prescribed as the first-line treatment, although the prevalence of macrolide-resistant strains has recently been shown not low. We advise this course of action because macrolides have shown improved bacterial eradication and immunomodulatory effects, and some patients may benefit from treatment with macrolides even in the presence of a macrolide-resistant strain. Alternatives to azithromycin are clarithromycin or erythromycin. If fever persists, clinical manifestations do not improve, and/or radiological images progress after 72 h of initiating macrolide treatment, macrolide-resistant MPP should be considered and the second-line antibiotic for *M. pneumoniae* can be prescribed after excluding other possible causes of pneumonia or infection. Doxycycline is the preferred first choice of macrolide-resistant MPP treatment at a suggested dose of 2 mg/kg/dose twice daily for 10 days. Refractory MPP is usually diagnosed when persistent fever and/or deterioration of clinical and radiological findings have occurred for 7 days or more after the administration of the appropriate antibiotics (azithromycin for 3 days followed with doxycycline for 4 days or more). We suggest careful reevaluation to exclude the possibility of infection with other pathogens or mixed infection and testing to determine concentrations of LDH before making a diagnosis of refractory MPP. High concentrations of LDH are suggestive of refractory MPP, and systemic corticosteroid could be considered for treatment. There is no consensus for the optimized dosage and duration of steroid treatment so far. In our experience, patients with refractory MPP receiving methylprednisolone (1 mg/kg/dose, thrice daily for 3 days) recovered smoothly without any obvious side effects.[10] In the case of persistent or recrudescent fever of over 72 h after methylprednisolone, an increase in the steroid dose or IVIG can be considered.

Declaration of competing interest

The authors declare that there are no competing financial interests related to this work.

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