Clinical Case Report

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A case report of Legionella and Mycoplasma pneumonia

Co-incidence or co-infection?

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Abstract

Rationale: Concurrent or sequential coinfections of *Legionella pneumophila* and *Mycoplasma pneumoniae* have been reported in the past though infrequently. Distinguishing a true co-infection from cross reactivity is often challenging as the diagnosis is mostly dependent on serological testing.

Patient concerns: A 77-year-old male presented with worsening dyspnea, cough with yellow sputum, diarrhea and fever of 2-days duration. Patient had history of chronic obstructive pulmonary disease (COPD) on home oxygen, bronchiectasis, rheumatoid arthritis (on methotrexate and leflunomide), treated pulmonary tuberculosis and 30-pack-year smoking. Chest X-ray showed bilateral interstitial changes with left lower lobe infiltrate. On day 5, his urine antigen for *L pneumophila* serogroup 1 was reported positive. The following day his serum *M pneumoniae* IgM antibody titers were reported elevated at 6647 U/mL. Patient was started on antibiotics and placed on non-invasive positive pressure ventilation.

Diagnosis: The patient was diagnosed with possible Legionella and Mycoplasma co-infection.

Outcomes: Sputum Mycoplasma polymerase chain reaction (PCR) and serum cold agglutinins were obtained on day 6 and later reported negative. He was treated with azithromycin for 10 days with clinical improvement.

Lessons: Serological testing alone is an indirect measure with poor sensitivity and specificity and has its own limitations. Urine antigen detection confirms *L pneumophila* serogroup 1 infection in a patient with suggestive symptoms. However, diagnosis of *M pneumonia* should be based on combination of tests including serology and PCR to confirm true co-infection.

Abbreviations: ED = emergency department, ELISA = enzyme-linked immunosorbent assay, PCR = polymerase chain reaction.

Keywords: atypical pneumonia, polymerase chain reaction, serology

1. Introduction

Isolation of 2 or more organisms as a cause of serious infection in a critically ill patient, either simultaneously or sequentially is well reported. However, the influence of co-infection of these organisms on clinical presentation and outcomes is less understood. Simultaneous detection of *Mycoplasma pneumoniae* and *Legionella pneumophila* in a patient with a diagnosis of pneumonia has been reported in the past.[1] These organisms are seldom isolated in the laboratory and diagnosis is based on serological testing. A high degree of serological cross-reactivity between the 2 organisms is seen and identification of true co-infection is challenging as the clinical presentation, disease course and to some extent management is similar.[1] We present a case of an elderly man who tested positive for both *L pneumophila* and *M pneumoniae* on serological testing and provide a detailed discussion of diagnostic work up required to confirming true co-infection.

2. Case presentation

A 77-year-old man presented to our emergency department (ED) with progressively worsening dyspnea, cough with yellow sputum, diarrhea and fever of 2-days duration. His medical history was significant for oxygen-dependent chronic obstructive pulmonary disease, bronchiectasis, rheumatoid arthritis and treated pulmonary tuberculosis. The patient underwent a wedge resection of left lung for interstitial lung disease 8 years before this admission and pathology revealed pleuroparenchymal fibroelastosis. He was on leflunomide and methotrexate for treatment of rheumatoid arthritis. He was a heavy smoker with more than 30-pack-year smoking history.

On arrival to the ED, patient was noted to be tachycardic, tachypneic with hypoxia. Physical examination revealed use of accessory muscles of respiration with decreased breath sounds

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An institutional review board approval was waived since this is a case report. Patient's anonymity has been maintained throughout the manuscript. Informed consent was obtained from the patient.

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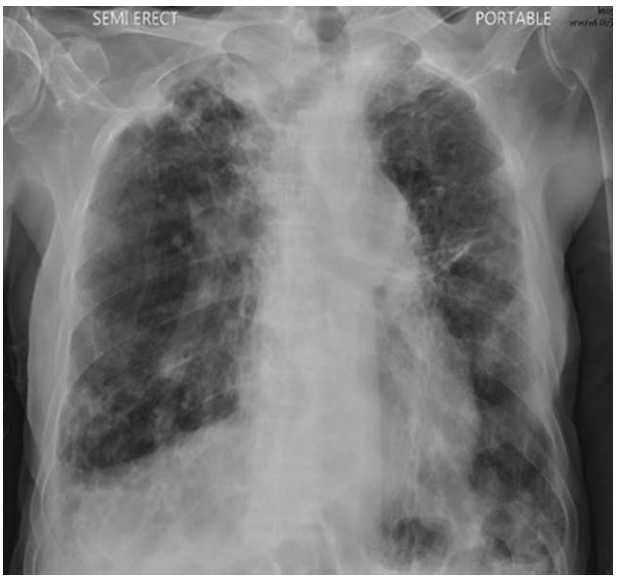

Figure 1. Chest X-ray: Bilateral chronic interstitial changes with superimposed left lower lobe infiltrate.

and bilateral crackles on auscultation. Laboratory studies were significant for acute on chronic respiratory acidosis, chronic anemia, and leukocytosis with neutrophil predominance. Non-invasive positive pressure ventilation was initiated. Chest X-ray revealed bilateral chronic interstitial changes with a left lower lobe infiltrate (Fig. 1). Broad-spectrum antibiotics including piperacillin-tazobactam, vancomycin, and azithromycin were initiated. Sputum and blood cultures, urine legionella antigen, and serum mycoplasma IgM antibody were sent and he was admitted to the intensive care unit.

His respiratory status gradually improved. On day 5, his urine antigen for *L pneumophila* serogroup 1 was reported positive. The following day his serum *M pneumoniae* IgM antibody titers were reported elevated at 6647 U/mL. Sputum Mycoplasma polymerase chain reaction (PCR) and serum cold agglutinins were obtained on day 6 and later reported negative. The patient was treated with azithromycin for 10 days with clinical improvement and was later discharged home.

3. Discussion

Grady and Gilfillan first reported cross-reactivity between *L pneumophila* and *M pneumoniae* in human sera in 1979. They found that 81% of patients with *L pneumophila* had seroreactivity with complement fixation tests against *M pneumoniae* and 29% of all cases seropositive for *M pneumoniae* were seropositive for *L pneumophila*.[1] Possible explanations for this association include reactivation of *M pneumoniae* infection, superinfection or secondary infection during hospitalization. Serological non-specificity and cross-reactivity between the 2 organisms may be a contributing factor as well.

Establishing a diagnosis based on serology is an indirect measure with poor sensitivity and specificity and has its own limitations. The preferred diagnostic tests for Legionnaires' disease are culture of lower respiratory secretions on selective media and the urinary antigen test. Despite the rapidity of the test and ease of performance, urine antigen testing has its limitations. This test most commonly uses monoclonal antibodies that specifically recognize most *L pneumophila* serogroup 1 lipopolysaccharide antigens that cause 50% to 80% of *L pneumophila*. Urine antigen testing has a sensitivity of 60% to 80% and a specificity of 99% in detecting this serogroup. However, urine antigen testing is not accurate in detecting other serogroups of *L pneumophila* or other species of Legionella which account for 20% to 50% of cases.[2–4] The diagnosis of other serotypes can only be done with culture and this remains the gold standard for diagnosis of Legionnaire's disease. Buffered charcoal yeast extract is specifically formulated for the isolation of Legionella and diagnosis by culture may require 3 to 14 days. Direct fluorescent antibody is a rapid test with sensitivity of 70% for detection of *L pneumophila* serogroup 1 and specificity approaching 99%.[5,6] PCR and in situ hybridization testing are rapid but require expertise and commercially-available kits have sensitivities ranging from 17% to 100% and specificities ranging from 95% to 100%[5,7,8]

Diagnostic work up for *M pneumoniae* is more challenging. Formation of cold agglutinins is the first humoral response to *M pneumoniae* and detection of cold agglutinins was considered an important tool in establishing a diagnosis of *M pneumoniae* in the past. The titers usually reach baseline values within 6 weeks of infection.[9] Testing is not always reliable as elevated levels can be seen only in 50% of patients and false positives can be associated with certain malignancies and infections caused by Epstein–Barr virus, Cytomegalovirus and Klebsiella.[10,11] Serological tests are readily available and collecting the specimen is relatively easy. Complement fixation testing and microparticle agglutination assay (MAG) were used extensively in the past. IgG response is usually absent in the first week of illness, slowly increases reaching peak titers at 5 weeks and may remain elevated for about 4 years.[12,13] The diagnosis of *M pneumoniae* infection can be established by a 4-fold rise of IgG titer in paired sera drawn 2 weeks apart. This will often delay diagnosis. Separate detection of IgM and IgA antibodies can help in establishing a rapid diagnosis. These assays are mostly based on enzyme-linked immunosorbent assay (ELISA) technique and recently a western immunoblot technique was developed. However, the development of antibodies in serum may sometimes take 2 weeks and in 1 retrospective study, IgM antibodies were found in about 40% of the sera sampled at 7 to 8 days after onset of symptoms but only occasionally in sera sampled earlier.[14] Multiple prior infections especially in the elderly may lead to a false negative result.[15] In a study assessing specific IgM response to MP infection, specific IgM appeared in the serum at approximately 7 days after the onset of symptoms, peaked between 10 and 30 days, and became undetectable by 12 to 26 weeks after onset of symptoms.[16] Therefore, detecting Mycoplasma IgM does not necessarily mean acute infection. Culture may take 5 weeks and has lesser sensitivity than serological assay. Difficulty in culturing this organism is likely due to the extensive nutrient requirements.

Direct antigen detecting tests using ELISA and hybridization assays have similar sensitivity and were used before PCR was widely available. Bernet et al first used PCR for diagnosis of *M pneumoniae* in 1989.[17] The role of PCR to diagnose *M pneumoniae* is difficult to establish due to various confounding factors including difficulty in obtaining samples, availability of different commercial kits and reference standards and variation of test results with timing of sample collection. False positive results occur with colonization or contamination and false negative results can be seen with PCR inhibitors in sample.[18] Despite these limitations, PCR is considered highly sensitive with genome detection level of 102 to 103 copies per milliliter of

sample for single-step PCR.[19] Nested PCR, which involves re-amplification of the PCR product, leads to a 102-fold increase in sensitivity.[20] Under optimal conditions, detection of *M pneumoniae* by PCR has a specificity of 100%.[21] Sputum sampling gives the highest rate of positive findings, followed by nasopharyngeal swabs. Throat swabs appear to be less efficient.[22]

Studies in the past showed a poor correlation between serological testing and PCR results in patients with *M pneumoniae*. In a community outbreak of *M pneumoniae* infection, only 21% of the confirmed cases had positive serology during the first week of illness, 56% during the second, and 100% during the third week. PCR was positive in all but 1 patient during the first week of illness.[23] In another study by Chang et al, using PCR as a gold standard, *M pneumoniae* IgM assay was found to have a sensitivity of 62.2%, a specificity of 85.5%, positive and negative predictive values of 52.3%, and 89.9%, respectively. Only 12.6% of patients who had both tests showed positive results at the same time.[24] Using PCR as gold standard, Beersma et al, evaluated 12 commercial enzyme immunoassay (EIA) assays by paired sera. The sensitivity of these assays varied from 35% to 77% and specificity was between 49% and 100%.[25] There are many reasons for the detection of *M pneumoniae* in respiratory secretions of patients without antibody response. Gnarpe et al found that 5.1% to 13.5% of healthy adults can have the organism in their throat as a colonizer.[26] Therefore, a combination of various tests is probably the most reliable approach in diagnosing *M pneumoniae* infection.

Clinicians very often encounter multiple pathogens that possibly explain the underlying infection. Therefore, it is important to distinguish true co-infection from laboratory cross-reactivity and past infection. This case brings forth an interesting association between *L pneumophila* and *M pneumoniae*. Though initially presumed to be a co-infection, further testing for *M pneumoniae* with a negative PCR confirmed serological non-specificity and possible cross-reactivity between the 2 organisms.

4. Conclusion

L pneumophila clinically resembles pneumonia secondary to *M pneumoniae* and other atypical pneumonia. Positive serology for the 2 organisms in a symptomatic patient does not imply co-infection due to poor sensitivity of serology. Urine antigen detection confirms *L pneumophila* serogroup 1 infection in a patient with suggestive symptoms. However, diagnosis of *M pneumonia* should be based on combination of tests including serology and PCR to confirm true co-infection.

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References

[1] Grady GF, Gilfillan RF. Relation of *Mycoplasma pneumoniae* seroreactivity, immunosuppression and chronic disease to Legionnaires' disease. Ann Intern Med 1979;90:607–10.
[2] Hicks LA, Garrison LE, Nelson GE, Hampton LM. Centers for Disease Control and PreventionLegionellosis—United States, 2000–2009. MMWR Morb Mortal Wkly Rep 2011;60:1083–6.
[3] Yzerman EP, den Boer JW, Lettinga KD, et al. Sensitivity of three urinary antigen tests associated with clinical severity in a large outbreak of Legionnaires' disease in The Netherlands. J Clin Microbiol 2002;40: 3232–6.
[4] Shimada T, Noguchi Y, Jackson JL, et al. Systematic review and meta-analysis urinary antigen tests for Legionellosis. Chest 2009;136: 1576–85.
[5] Hayden RT, Uhl JR, Qian X, et al. Direct detection of Legionella species from bronchoalveolar lavage and open lung biopsy specimens: comparison of light cycler PCR, in situ hybridization, direct fluorescence antigen detection, and culture. J Clin Microbiol 2001;39:2618–26.
[6] She RC, Billetdeaux E, Phansalkar AR, et al. Limited applicability of direct fluorescent-antibody testing for Bordetella sp. and Legionella sp. specimens for the clinical microbiology laboratory. J Clin Microbiol 2007;45:2212–4.
[7] Diederen BM, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice. J Clin Microbiol 2008;46:671–7.
[8] Benitez AJ, Winchell JM. Clinical application of a multiplex real-time PCR assay for simultaneous detection of Legionella species, Legionella pneumophila, and Legionella pneumophila serogroup 1. J Clin Microbiol 2013;51:348–51.
[9] Jacobs E. Serological diagnosis of Mycoplasma pneumoniae infections: a critical review of current procedures. Clin Infect Dis 1993;17:S79–82.
[10] Ali NJ, Sillis M, Andrews BE, et al. The clinical spectrum and diagnosis of Mycoplasma pneumoniae infection. Q J Med 1986;58:241–51.
[11] Daxboeck F, Krause R, Wenisch C. Laboratory diagnosis of Mycoplasma pneumoniae infection. Clin Microbio Infect 2003;9:263–73.
[12] Barker CE, Sillis F, Wreghitt TG. Evaluation of Serodia Myco II particle agglutination test for detection of *Mycoplasma pneumoniae* antibody: comparison with capture ELISA and indirect immunofluorescence. J Clin Pathol 1991;43:163–5.
[13] Jacobs E, Bennewitz A, Bredt W. Reaction pattern of human anti-*Mycoplasma pneumoniae* antibodies in enzyme-linked immunosorbent assays and immunoblotting. J Clin Microbiol 1986;23:517–22.
[14] Vikerfors T, Brodin G, Grandien M, et al. Detection of specific IgM antibodies for the diagnosis of *Mycoplasma pneumoniae* infections: a clinical evaluation. Scand J Infect Dis 1988;20:601–10.
[15] Sillis M. The limitations of IgM assays in the serological diagnosis of Mycoplasma pneumoniae infections. J Med Microbiol 1990;33:253–8.
[16] Moule JH, Caul EO, Wreghitt TG. The specific IgM response to *Mycoplasma pneumoniae* infection: interpretation and application to early diagnosis. Epidemiol Infect 1987;99:685–92.
[17] Bernet C, Garret M, De Barbeyrac B, et al. Detection of *Mycoplasma pneumoniae* by using the polymerase chain reaction. J Clin Microbiol 1989;27:2492–6.
[18] Zhang L, Zong ZY, Liu YB, et al. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. Indian J Med Res 2011;134:270–80.
[19] Skakni L, Sardet A, Just J, et al. Detection of *Mycoplasma pneumoniae* in clinical samples from pediatric patients by polymerase chain reaction. J Clin Microbiol 1992;30:2638–43.
[20] Abele-Horn M, Busch U, Nitschko H, et al. Molecular approaches to diagnosis of pulmonary disease due to *Mycoplasma pneumoniae*. J Clin Microbiol 1998;36:548–51.
[21] Ieven M, Goossens H. Relevance of nucleic acid amplification techniques for diagnosis of respiratory tract infections in the clinical laboratory. Clin Microbiol Rev 1997;10:242–56.
[22] Raty R, Ronkko E, Kleemola M. Sample type is crucial to the diagnosis of Mycoplasma pneumoniae pneumonia by PCR. J Med Microbiol 2005;54:287–91.
[23] Nilsson AC, Björkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiol 2008;8:93–193.
[24] Chang HY, Chang LY, Shao PL, et al. Comparison of real-time polymerase chain reaction and serological tests for the confirmation of Mycoplasma pneumoniae infection in children with clinical diagnosis of atypical pneumonia. J Microbiol Immunol Infect 2014; 47:137–44.
[25] Beersma MF, Dirven K, van Dam AP, et al. Evaluation of 12 commercial tests and the complement fixation test for Mycoplasma pneumoniae-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard". J Clin Microbiol 2005;43:2277–85.
[26] Gnarpe J, Lundbaeck A, Sundeloef B, et al. Evaluation of Prevalence of Mycoplasma pneumoniae in subjectively healthy individuals. Scand J Infect Dis 1992;24:161–4.