

Mycoplasma pneumoniae Pneumonia Associated With Methemoglobinemia and Anemia: An Overlooked Association?

Tawfik Khoury,¹ Ayman Abu Rmeileh,¹ Jonathan David Kornspan,³ Roy Abel,¹ Meir Mizrahi,¹ and Ran Nir-Paz²

Departments of ¹Internal Medicine, ²Clinical Microbiology and Infectious Diseases, Hadassah-Hebrew University Medical Center, and ³Department of Microbiology and Molecular Genetics, Hebrew University-Hadassah Medical School, Jerusalem, Israel

We report a case of acute methemoglobinemia and anemia in a patient with *Mycoplasma pneumoniae* pneumonia. We suggest that *M. pneumoniae* secretes a putative protein that can induce methemoglobin in red blood cells. Thus, *Mycoplasma pneumoniae* may induce methemoglobinemia in patients who have low oxygen saturation and anemia.

Keywords. hemolytic anemia; methemoglobinemia; *Mycoplasma pneumoniae*.

Mycoplasma pneumoniae (MP) is one of the leading pathogens causing respiratory tract infections in both children and adults, and it is suggested to cause 10%–40% of cases of community-acquired pneumonia [1]. It is estimated that extrapulmonary manifestations can occur in approximately 25% of the cases. The most common are seen in the skin and the central nervous system, but cardiovascular, gastrointestinal, and hematological systems have been reported as well [1]. The mechanism for the extrapulmonary complications may be mediated through autoimmune reactions, but some suggest that they are caused by direct invasion by the microorganisms [1].

Methemoglobin (MetHb) is an oxidized state of the iron molecule from its ferrous (Fe^{2+}) state to its ferric (Fe^{3+}) state.

Methemoglobin is formed naturally and normally accounts for less than 1% of total body hemoglobin. In the ferric form, the iron in the heme moiety cannot bind oxygen, causing a left-shifted, oxygen-hemoglobin dissociation curve and secondary tissue ischemia [2]. Medications are the most common cause of MetHb (including inhaled nitric oxide, benzocaine, and dapsone). In a large case series [3], dapsone was responsible for approximately half the cases acquired MetHb and is considered the most common cause of the disease. Moreover, hereditary forms of MetHb also exist [2].

In this study, we report a case of MetHb and hemolytic anemia secondary to MP pneumonia infection in a 79-year-old female.

METHODS

Mycoplasma Growth Conditions

Mycoplasma pneumoniae strain M129 (obtained from our strain collection) was used in this study. *Mycoplasma pneumoniae* was cultured for 48–72 h at 37°C on 75-cm² tissue culture flasks (Nunc, Roskilde, Denmark) in modified Hayflick's medium [4] containing 20% horse serum. Bacterial growth was monitored by measuring the absorbance at 595 nm and by recording pH changes in the growth medium. Culture supernatants were collected and centrifuged at 14 000 × g for 5 min to remove MP cells.

Spectrophotometric Assessment of Hemoxidation

Hemoxidation was determined spectrophotometrically using sheep blood samples (Novamed, Jerusalem, Israel) as described previously [5]. In brief, blood samples were washed twice in phosphate-buffered saline and diluted to a final concentration of 1% packed cells. Samples of 100 μL MP culture supernatant were incubated with 1% packed red blood cells (RBCs) in a total volume of 1 mL (referred to as the test mixture) for 18 h at 37°C in a rotary shaker (30 rpm). To measure hemoxidation, defined as the oxidation of hemoglobin to MetHb, we added 0.5 mL of the test mixture to 1.5 mL of distilled water to lyse RBCs. Released MetHb was determined by measuring supernatant absorbance at spectra of 500–700 nm. Heat-inactivated samples (100°C for 10 min) of MP supernatant were used as baseline control because heat inactivation abolished hemoxidative activity. Hydrogen peroxide (3% mass/volume) was purchased from VITAMED LTD (Binyamina, Israel).

Case Report

A 79-year-old female was admitted due to dry cough and room air oxygen saturation (SaO_2) of 65% measured by pulse oximetry,

Received 17 November 2014; accepted 12 February 2015.

Correspondence: Ran Nir-Paz, MD, Department of Clinical Microbiology and Infectious Diseases, Hadassah Hebrew University Medical Center, Ein Kerem, Jerusalem 91120, Israel (ran.nir-paz@mail.huji.ac.il).

Open Forum Infectious Diseases

© The Author 2015. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com. DOI: 10.1093/ofid/ofv022

Table 1. Laboratory Values on Admission

Laboratory Tests	Results
Hemoglobin	8.4 g/dL (12–16)
Leukocytes	22 200 per cubic millimeter (4–10)
Platelets	300 10E9/L (140–400)
PCO ₂	30.7 mmHg (35–45)
PO ₂	161 mmHg (>80)
HCO ₃	20.6 mmol/L (18–24)
PH	7.44 (7.38–7.42)
SaPO ₂	65%
Methemoglobin	8.5% (0–1.5%)
Clinical variables	
Breath sounds	24 breath/min
Heart beats	100 beats/min
Body temperature	36.9 C
Blood pressure	148/63 mmHg

without other symptoms. She had mild upper and lower motor neuron disease for 20 years, but she was able to maintain her own daily activities. In addition, she had hypertension, diabetes mellitus, and hyperlipidemia. Her medications included 850 mg of metformin twice a day, 6 units of insulin glargine once a day, 3 units of regular insulin 3 times a day, 20 mg of enalapril twice a day, 20 mg of simvastatin once a day, 25 mg of hydrochlorothiazide once a day, and 30 mL of lactulose once a day. On admission, the patient was dyspneic and tachypneic with 26 breath per minute, blood pressure was 148/63 mmHg, pulse rate was 100 beats/minute, and temperature was 36.9°C. Her physical examination was unremarkable except for cyanosis of her lips. Blood tests (Table 1) showed leukocytosis, elevated C-reactive protein (CRP), and a new fall in her hemoglobin level to 8.4 g/dL. Five days before the current admission, her hemoglobin was 13 g/dL. A computerized tomographic angiography of the lungs showed bilateral ground glass opacities, and no pulmonary embolism was found. The patient had a reticulocyte count of 21.5% (normal: 1%–4%), lactate dehydrogenase of 2646 unit/L (normal: 240–480 unit/L), and total bilirubin of 25 µmol/L (normal: 0–17). In addition, a direct antiglobulin test (Coomb’s test) was negative, peripheral blood smear showed rouleaux without evidence of schistocytes or bite cells,

and a serum MetHb concentration was 8.5% (normal: 0%–1.5%). These results suggest that hemolysis was possibly induced by either cold agglutinins or methemoglobinemia. A presumptive diagnosis of MP pneumonia was made, and intravenous (IV) treatment with azithromycin was initiated. In addition, methylene blue (MB) was given for the treatment of MetHb. The diagnosis of MP infection was confirmed by throat swab polymerase chain reaction [6]. Macrolide antibiotics were continued for 1 week with clinical improvement, hemoglobin reached 11 g/dL, whereas the leukocyte count and CRP levels declined to near normal values and MetHb normalized (Table 2). The patient was discharged after 10 days of hospitalization.

RESULTS AND DISCUSSION

Mycoplasma pneumoniae causes upper and lower respiratory tract infection and it occurs in all age groups. Many extrapulmonary manifestations were reported [1]; however, we did not find any reports of MP infections associated with MetHb.

Methemoglobin should be suspected when pulse oximetry shows significantly lower SaO₂ than the saturation calculated from the arterial blood gases analysis (saturation gap) [3]. Methemoglobin can have a wide spectrum of manifestations ranging from asymptomatic cyanosis to death, depending on the degree of MetHb [7]. The mainstay of treatment is stopping the triggering agent, adding oxygen support, and IV treatment with MB, which activates the NADPH pathway, thus accelerating the reduction process of MetHb. The MB treatment is indicated when a patient’s MetHb exceeds 20% or when the patient exhibits symptoms of oxygen deficiency, such as dyspnea and alteration of consciousness [7]. Methemoglobin was considered in this case due to the patient’s low SaO₂ despite 100% oxygen support and the high oxygen partial pressure on arterial blood gases. Therefore, we initiated MB treatment even though MetHb was only 8.5%. There were no other predisposing factors associated with MetHb in our patient.

It was intriguing that the co-occurrence of MetHb and acute MP infection was not reported previously, thus we were prompted to attempt to elucidate a possible mechanism. A few *Mycoplasma* species were previously suggested to induce

Table 2. Progress of Blood Gases Analysis and Methemoglobin Concentration

Laboratory Findings	Initial	1 Day After	2 Days After	3 Days After	4 Days After
PH	7.44	7.45	7.46	7.42	7.41
PCO ₂ (mmHg)	30.7	31.5	40.7	44.7	40
PO ₂ (mmHg)	161	102	140	122	101
HCO ₃ (mmol/L)	20.6	21.3	30.6	29.4	27.8
SaO ₂ saturation	65%	75%	90%	100%	100%
Methemoglobin	8.5%	5.4%	2.9%	1%	0.6%

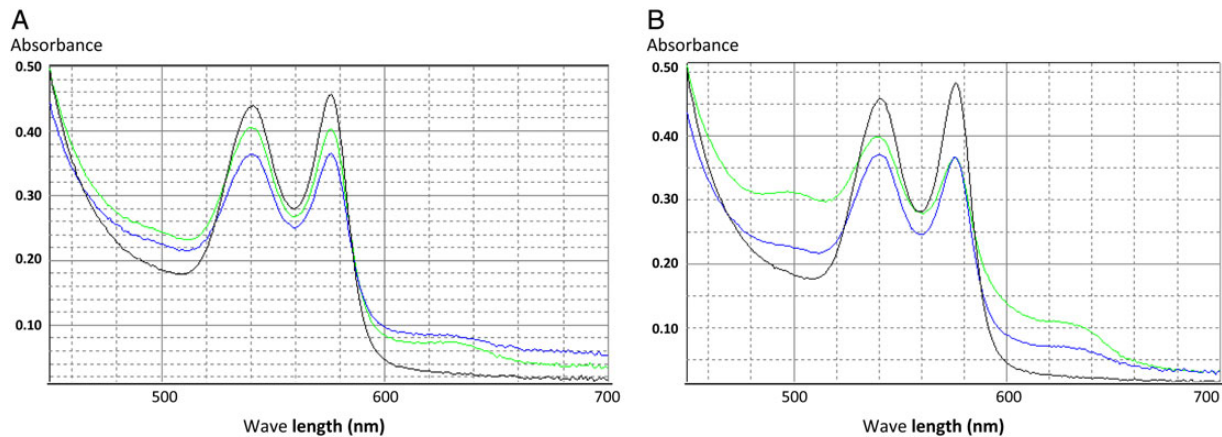


Figure 1. Spectral changes induced in sheep red blood cells (RBCs) hemoglobin by *Mycoplasma pneumoniae* (MP) supernatant and H₂O₂. (a) Sheep RBCs were treated with either 100 µL of heat-inactivated supernatant (black line) or 100 µL of MP culture supernatant (green and blue lines). After incubation for 18 h, RBCs were lysed with water. Increases at 630 nm and decreases at 540 and 575 nm indicate the conversion of hemoglobin to methemoglobin (MetHb). Results presented here are representative of 3 independent experiments. (b) Sheep RBCs were treated with various concentrations of H₂O₂ and incubated at room temperature for 5 min. Red blood cells were then lysed with water. Increases at 630 nm and decreases at 540 and 575 nm indicate the conversion of hemoglobin to MetHb. Black line, untreated RBCs; blue line, RBCs treated with 5 mM H₂O₂; green line, RBCs treated with 18 mM H₂O₂.

MetHb, including *Mycoplasma gallisepticum* (chicken pathogen [8]), *Mycoplasma penetrans* (human pathogen [5]), and *Mycoplasma hyorhinitis* (swine pathogen; J. D. Kornspan and S. Rotem, unpublished data). In addition, for almost half a century, *M. pneumoniae* has been known as a producer of H₂O₂ [9]. One of the mechanisms suggested is specifically related to the accumulation of intracellular H₂O₂ [10]. However, no one before has suggested that MP can cause MetHb. Thus, to prove this mechanism, we adopted the methodology proposed by Kannan and Baseman [5]. As seen in Figure 1a, MP supernatant has the distinct ability to create MetHb while incubated with sheep RBCs. As expected, incubation of exogenous H₂O₂ (5–40 mM for 5 min at room temperature) with sheep RBCs resulted in a color change from red to brown, and the absorption spectra of H₂O₂-treated RBCs exhibited an absorbance peak at 630 nm (Figure 1b), which was similar to the spectrum observed in the MP supernatant-treated RBCs. In addition, heat inactivation of MP supernatant abolished this activity, suggesting that MP secrete a protein that is responsible for this pathogenic phenomenon (Figure 1a). Therefore, we may assume that MP or related MP molecules mediate the oxidation of hemoglobin to MetHb, and we can also generalize that our observation may not be unique to our patient, and these observations may have been overlooked before. However, this in vitro experiment does not fully clarify the entire pathogenic mechanism in our patient. Further investigations in an animal model are warranted, including isolation of the putative protein or change in H₂O₂ concentrations, that will reveal the actual mechanism and conditions responsible for MetHb occurrence in humans as well as in animals.

CONCLUSIONS

In conclusion, MP can cause severe presentation in some cases. Methemoglobin can be a rare complication of MP infection that may be attributed to a secreted protein. The fact that MetHb can be a possible complication of MP should encourage clinicians to correctly evaluate the severity of MP infection and possibly treat MetHb if needed. Further prospective studies are warranted to estimate the actual number of patients with MP-associated MetHb.

Acknowledgments

We thank Deborah Rund (Department of Hematology, Hadassah-Hebrew University Medical Center) for critical review of the manuscript and for valuable comments.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. Clin Microbiol Rev **2004**; 17:697–728.
2. Haymond S, Cariappa R, Eby CS, et al. Laboratory assessment of oxygenation in methemoglobinemia. Clin Chem **2005**; 51:434–44.
3. Ash-Bernal R, Wise R, Wright SM. Acquired methemoglobinemia: a retrospective series of 138 cases at 2 teaching hospitals. Medicine (Baltimore) **2004**; 83:265–73.
4. Hayflick L, Stinebring WR. Intracellular growth of pleuropneumonia-like organisms (PPLO) in tissue culture and in ovo. Ann N Y Acad Sci **1960**; 79:433–49.
5. Kannan TR, Baseman JB. Hemolytic and hemoxidative activities in *Mycoplasma penetrans*. Infect Immun **2000**; 68:6419–22.
6. Averbuch D, Hidalgo-Grass C, Moses AE, et al. Macrolide resistance in *Mycoplasma pneumoniae*, Israel, 2010. Emerg Infect Dis **2011**; 17:1079–82.

7. Wright RO, Lewander WJ, Woolf AD. Methemoglobinemia: etiology, pharmacology, and clinical management. *Ann Emerg Med* **1999**; 34: 646–56.
8. Thomas L, Bitensky MW. Methaemoglobin formation by *Mycoplasma gallisepticum*: the role of hydrogen peroxide. *Nature* **1966**; 210:963–4.
9. Cohen G, Somerson NL. *Mycoplasma pneumoniae*: hydrogen peroxide secretion and its possible role in virulence. *Ann N Y Acad Sci* **1967**; 143:85–7.
10. Almagor M, Kahane I, Gilon C, et al. Protective effects of the glutathione redox cycle and vitamin E on cultured fibroblasts infected by *Mycoplasma pneumoniae*. *Infect Immun* **1986**; 52:240–4.