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CASE REPORT

A Case Report of the "Pathological Triad": Infection-Induced Cold Agglutination with Hemolytic Anemia

Jinling Xie, Shihao Chen, Yinzhong Chen, Weijian Zhang, Guoan Liu

Xinhui District People's Hospital, Jiangmen, China

SUMMARY

Background: **Although Mycoplasma pneumoniae is a recognized cause of community-acquired pneumonia, its hematologic complications are often overlooked in primary care settings. Here, we present a case that exemplifies the diagnostic challenges associated with Mycoplasma pneumoniae-induced cold agglutination and hemolytic anemia.**
Methods: **Complete blood count analysis was performed using an automated hematology analyzer, with discordant erythrocyte indices prompting thermal correction via 37°C water bath incubation. Serum biochemical markers, including Creatine Kinase-MB (CKMB), Lactate Dehydrogenase (LDH), Hydroxybutyrate Dehydrogenase (HBDH), Total Bilirubin (TBIL), and Direct Bilirubin (DBIL) were quantified using an automated biochemistry analyzer. Mycoplasma pneumoniae IgM and DNA were detected via immunochromatography and PCR, respectively. A direct anti-human globulin test (Coombs test) was conducted using standard reagents. Peripheral blood smears were examined microscopically for erythrocyte agglutination and morphology.**
Results: **Positive results for the Mycoplasma pneumoniae IgM antibody and DNA tests confirmed that the patient had active infectious pneumonia. Initial results of the automated blood cell analysis were as follows: RBC (1.6 x 10^{12}/L), HGB (111 g/L), MCH (69.4 pg), and MCHC (703 g/L). After a 37°C water bath, the results changed to: RBC: 3.73 x 10^{12}/L, HGB: 113 g/L, MCH: 30.1 pg, MCHC: 337 g/L. The RBC count, HGB, and HCT results were significantly lower than those recorded a week earlier, especially the HGB result, which decreased by 46 units. The Coombs test was positive. There were significantly elevated levels of biochemical markers (CKMB, LDH, and HBDH). Mild elevations were also noted in TBIL and DBIL. No comorbidities or hemolytic triggers were identified. Taken together, the evidence suggests that the patient experienced cold agglutination and progressive hemolysis following Mycoplasma pneumoniae infection.**
Conclusions: **This case establishes three evidence-based protocols for frontline practice. First, consider testing for Mycoplasma pneumoniae in patients with unexplained anemia following a respiratory infection. Second, apply thermal correction to blood samples when the MCHC results do not match the other erythrocyte parameters. Third, monitor for hemolysis for at least two weeks after Mycoplasma pneumoniae IgM is detected.**

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Correspondence:
Guoan Liu
Xinhui District People's Hospital
Jiangmen
Guangdong, 529100
China
Email: yjlpvxjl@yeah.net

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KEYWORDS

mycoplasma pneumoniae, infection, cold agglutination, hemolytic anemia, atypical, case report

INTRODUCTION

Mycoplasma pneumoniae is a major pathogen cause of community-acquired pneumonia and can lead to a diverse array of clinical conditions following infection.

While it primarily targets the respiratory system, it can also impact the neurological, cardiovascular, and hematological systems, resulting in multi-organ and systemic damage [1,2]. The extrapulmonary manifestations linked to this infection are frequently associated with an increased risk of adverse outcomes [2,3] Consequently, the early and precise identification of these complications is essential for effective clinical management.
This case discusses Mycoplasma pneumoniae infection and its related complications, particularly cold agglutination and hemolytic anemia. The hemolytic symptoms observed in this case were mild. This report highlights the association between Mycoplasma pneumoniae infection, cold agglutination, and hemolytic anemia based on a thorough evaluation of the clinical presentation, laboratory findings, and review of the literature. Furthermore, this case highlights the importance of a comprehensive hematologic analysis in the diagnosis of the condition. It also improves the understanding of the complications associated with Mycoplasma pneumoniae infection, with the goal of providing clinicians with better diagnostic methods that can refine treatment strategies and ultimately enhance patient outcomes.

CASE PRESENTATION

This case describes a 48-year-old male patient, ID number 569199, who was admitted to the Department of Respiratory and Critical Care Medicine of Xinhui District People's Hospital for pneumonia treatment on November 12, 2023. During a routine follow-up visit on November 19, 2023, the patient's complete blood count revealed significant in red blood cell parameters. Specifically, the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were inconsistent with the red blood cell (RBC) count, hemoglobin concentration (HGB), mean corpuscular volume (MCV), and hematocrit (HCT). These hematologic inconsistencies indicated potential underlying disease abnormalities, necessitating further diagnostic evaluation.
In the complete blood count program, the erythrocyte parameters include laboratory measurements and calculated values. The measured values include RBC count, HGB, MCV, and HCT. The calculated values, MCH and MCHC, are derived using the formulas: MCH = HGB/RBC count and MCHC = MCH/MCV = HGB/HCT. These parameters are essential for the diagnosis and classification of anemia. Typically, changes in these values are expected to align. However, in this particular patient, a low RBC count coupled with inconsistent HGB levels led to significant discrepancies between the calculated and measured values, resulting in a phenomenon referred to as “polarization” among these parameters, as shown in Table 1. This notable divergence highlights potential challenges in the accuracy and interpretation of hematologic results.
Further observation revealed a significant amount of fine, granular red cell aggregates in the sample tube, resembling sand settling in water. A smear test showed marked agglutination of red blood cells, suggesting that the sample might be a cold agglutination sample. To confirm this hypothesis, the sample was subjected to a warm water bath at 37°Cfor more than 20 minutes. Following this treatment, the consistencies between the erythrocyte parameters (RBC count, HGB, MCV, and HCT) returned to normal, confirming the presence of cold agglutinins. At this point, another problem was identified (as shown in Table 2): the results for RBC count, HGB, and HCT were significantly lower than those recorded a week earlier, especially HGB, which decreased by 46 units.
The patient's medical history revealed a spontaneous onset of cough, sputum production, shortness of breath, and fever ten days prior to hospitalization. Upon admission, a CT scan revealed extensive right lung inflammation and reactive lymph node enlargement, which had occurred one week before abnormal erythrocyte parameters were noted. Subsequent laboratory tests showed significantly elevated levels of biochemical markers, including Creatine Kinase-MB (CKMB), Lactate Dehydrogenase (LDH), and Hydroxybutyrate Dehydrogenase (HBDH). Mild elevations were also noted in total and direct bilirubin (TBIL and DBIL, respectively). Additionally, the positive results of Mycoplasma pneumoniae IgM antibody and Mycoplasma pneumoniae DNA confirmed that the patient was experiencing active infectious pneumonia. Of note, the patient had no pre-existing chronic diseases, nor any history of blood transfusions, trauma, or surgery.

DISCUSSION

The detection of Mycoplasma pneumoniae IgM antibody and DNA confirm Mycoplasma pneumoniae infection in the patient suggested that this pathogen may have caused or worsened his pneumonia. Mycoplasma pneumoniae is a common respiratory pathogen spread through droplets. It is a leading cause of community-acquired pneumonia and can present with various clinical symptoms. Beyond the usual pneumonia symptoms, this infection can lead to various complications, including diarrhea, myocarditis, cold agglutinin agglutinin syndrome and hemolytic anemia [2,4].
Cold agglutination is the phenomenon in which antibodies on the surface of RBCs react with antigens at low temperatures (4 - 20°C), causing blood samples agglutinate in cold environments, with complete dispersion of aggregates at room temperature or 37°C. This is a common phenomenon in laboratory testing and is usually associated with certain diseases, but is not a disease in itself. Healthy individuals typically harbor naturally occurring cold agglutinins at low titers (typically ≤ 1:32) that do not induce agglutination. However, several factors can lead to cold agglutinin syndrome, including infections like Mycoplasma pneumoniae, autoimmune

Table 1. Polarization of the detected and calculated values of the patient's RBC parameters.

	Projects	Values for the patient	Values for a healthy man	Reference ranges	Units
Detected values	RBC	1.6	4.81	4.3 - 5.8	10^{12}/L
	HGB	111	147	130 - 175	g/L
	HCT	15.8	42.4	40 - 50	%
	MCV	98.8	88.1	82 - 100	fL
Calculated values	MCH	69.4	30.6	27 - 34	Pg
	MCHC	703	347	316 - 354	g/L

Table 2. Changes in patient's RBC parameters (after water bath treatment vs. one week before).

	Projects	Values after water bath treatment	Values one week before	Reference ranges	Units
Calculated values	RBC	3.75	4.83	4.3 - 5.8	10^{12}/L
	HGB	113	159	130 - 175	g/L
	HCT	33.5	42.7	40 - 50	%
	MCV	89.3	88.4	82 - 100	fL
Measured values	MCH	30.1	32.9	27 - 34	Pg
	MCHC	337	372	316 - 354	g/L

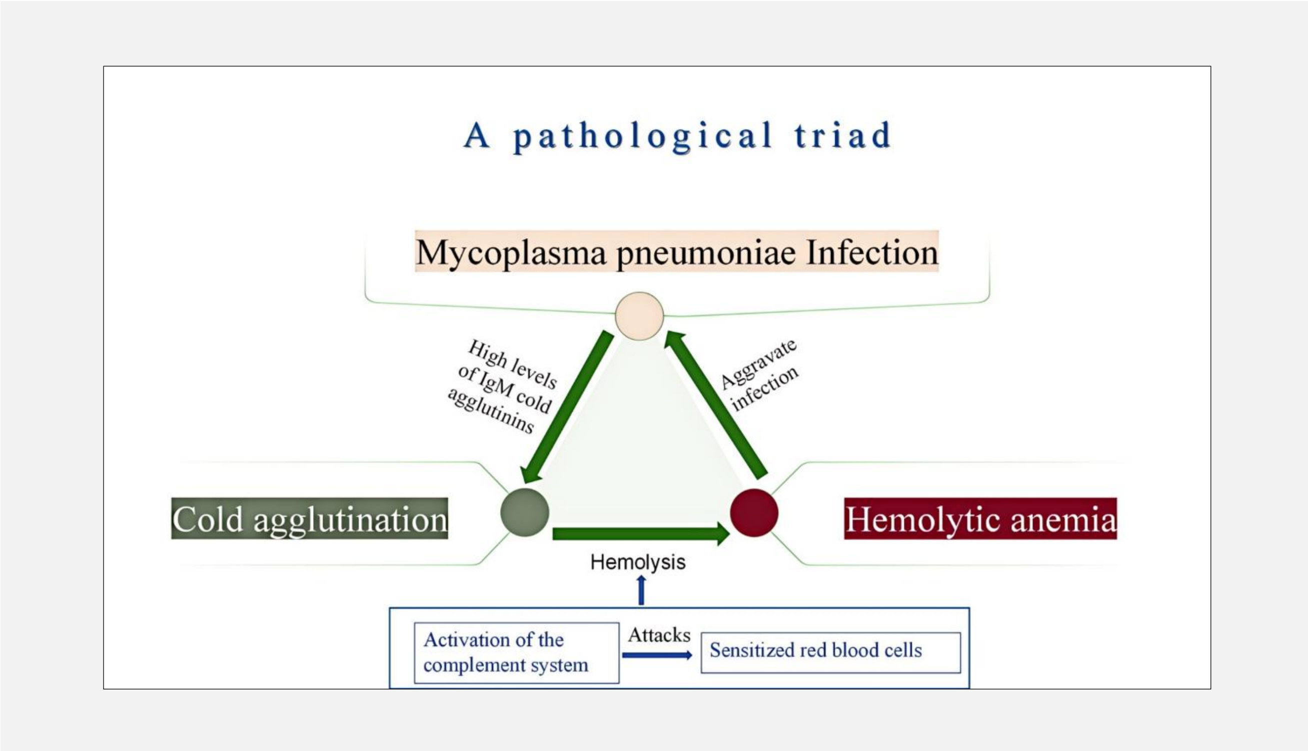

Figure 1. Link between Mycoplasma pneumoniae infection, cold agglutination and hemolytic anemia - “a pathological triad”.

diseases, and malignancies [5]. This case showed cold agglutination secondary to Mycoplasma pneumoniae infection, consistent with the previous report described [6, 7]. Cold agglutination interferes with hematologic testing, potentially resulting in falsely low RBC counts and elevated MCHC in complete blood counts [6]. During transfusion therapy, patients with cold agglutinins may develop transfusion reactions if blood products are not properly pre-warmed (37°C incubation recommended) prior to administration, as residual cold exposure triggers pathological RBC agglutination through thermal amplification of antigen-antibody interactions [8]. Consequently, clinical laboratories must accurately and timely recognize the cold agglutination during blood testing.

Hemolytic anemia is a rare but serious complication of Mycoplasma pneumoniae infection. It is most commonly observed in children [9], although it has also been occasionally reported in adults [10]. The infected individual in this case was an adult. This case was initially identified through the presence of cold agglutination. A thorough review of the patient's medical history and a review of the literature prompted further consideration. Finally, we concluded that the patient's condition, characterized by cold agglutination and hemolytic anemia, was due to Mycoplasma pneumoniae infection. This condition was consistent with previous case report, which described the phenomenon of cold agglutinin-induced hemolytic anemia [11].

The mechanism by which Mycoplasma pneumoniae infection leads to cold agglutinin disease and hemolytic anemia involves a complex immune response. While the exact details remain unclear, current research has highlighted important elements of this process. These include the formation of cold agglutinins and activation of the complement system, which ultimately leads to hemolysis [12,13]. Mycoplasma pneumoniae infection can induce the production of cold agglutinins, which lead to the agglutination of red blood cells at low temperatures. Additionally, high levels of cold agglutinins, along with the immune response induced by the infection, can activate the complement system. This activation can lead to the rupture of agglutinated red blood cells, resulting in hemolysis. This process can hinder the body's ability to combat the infection, worsening the condition and causing hemolytic anemia. As the infection advances, the interplay between Mycoplasma pneumoniae infection, cold agglutination, and hemolytic anemia forms a triadic closed-loop system, commonly referred to as the "Pathological triad" (as shown in Figure 1).

This case of infection presented another significant feature: despite a marked reduction in HGB, only mild hemolytic anemia was observed. The patient had mild elevations in TBIL and DBIL, and a marked increase in LDH, consistent with previously reported cases [10,13]. However, no other typical indicators of microangiopathic hemolytic anemia were observed. The subtle presentation of anemia symptoms, coupled with unremarkable laboratory markers, can easily lead to this condition being overlooked. This highlights the necessity of careful evaluation of hematologic parameters in patients with atypical infections and the importance to recognize immune-mediated complications. A study indicates that advanced diagnostic techniques, like quantitative immunoassays, could enhance the accuracy of diagnosing Mycoplasma pneumoniae infections and their complications, facilitating timely treatment [14]. Thus, the use of accurate diagnostic tools, the integration of clinical features with biomarkers such as LDH, TBIL and DBIL [12], and other hemolysis-related indicators in predictive models could be critical for early diagnosis and monitoring of disease progression.

Currently, the clinical significance of cold agglutinin disease caused by Mycoplasma pneumoniae is mainly understood through case reports [2,10,15] and small cohort studies [13,16]. The absence of large-scale, multicenter studies restricts how broadly the research findings can be applied. Therefore, more research is needed to clarify the incidence rate, diagnostic criteria, and management strategies for complications associated with Mycoplasma pneumoniae infection. Future research should focus on validating the observational results obtained thus far and delving deeper into the underlying immunologic mechanisms, which would enhance the clinical understanding of these interactions and improve treatment approaches. Additionally, prospective studies can investigate the long-term prognosis of patients with similar symptoms, offering valuable insights into managing this rare but potentially devastating disease.

CONCLUSION

This case shows that a case of cold agglutination and hemolytic anemia was induced by Mycoplasma pneumoniae infection. Cold agglutinin interfered with the blood test, resulting in abnormal erythrocyte parameters. These parameters returned to normal after water bath treatment, confirming the role of cold agglutinin. The patient's mild anemia, abnormal biochemical markers, and absence of gastrointestinal bleeding, hemoptysis, or other bleeding manifestations suggested hemolytic anemia. The patient was confirmed to have infectious pneumonia one week before the onset of hemolytic anemia, strongly suggesting that the hemolytic anemia was related to the infection. A comprehensive analysis of the patient's symptoms, laboratory results, and relevant literature revealed a pathologic association between infection, cold agglutination, and hemolytic anemia, which can be described as a "Pathological triad", as well as a diagnostic triad.

This case establishes a replicable model for preventing systemic complications of Mycoplasma pneumoniae through time-sensitive primary care coordination, using the IgM-to-hemolysis window to initiate targeted macrolide therapy when early hematologic red flags (discordant HGB/LDH trends, reticulocytosis) arise, coupled with community-adapted diagnostics (water bath CBC correction, rapid IgM assays) that reduce referral delays. By integrating these evidence-based protocols into routine respiratory infection management, primary care teams can achieve triple aim outcomes: reduction in hemolysis-related hospitalizations, cost savings over reactive care, and improved preventive care metrics under value-based reimbursement frameworks - ultimately redefining the frontline ability to intercept atypical infection trajectories before multi-organ damage occurs.

Data Availability Statement:
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement:
This study was approved by the Medical Research Ethics Committee of Jiangmen Xinhui District People's Hospital (Approval Number: XHEC-202409-K1). Written informed consent was obtained from the patient, ensuring his understanding of the study's purpose and procedures. All data collected were treated with strict confidentiality and anonymity to protect the participant's privacy.

Declaration of Interest:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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