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Case reports in pediatric infectious diseases

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Edited by
Hans Van Rostenberghe, Kazumichi Fujioka and Dimitri Van der Linden

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Case reports in pediatric infectious diseases 2022

Topic editors

Hans Van Rostenberghe — University of Science Malaysia (USM), Malaysia

Kazumichi Fujioka — Kobe University, Japan

Dimitri Van der Linden — Cliniques Universitaires Saint-Luc, Belgium

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EDITED AND REVIEWED BY
Rolando Ulloa-Gutierrez,
Hospital Nacional de Niños "Dr. Carlos Sáenz Herrera", Costa Rica

*CORRESPONDENCE
Hans Van Rostenberghe
hansvro@usm.my
hansvro@gmail.com

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Editorial: Case reports in pediatric infectious diseases 2022

Hans Van Rostenberghe[1,2]*, Kazumichi Fujioka[3] and Dimitri Van der Linden[4]

[1]Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia, [2]Department of Paediatrics, Hospital USM, Kelantan, Malaysia, [3]Department of Paediatrics, Kobe University, Kobe, Japan, [4]Cliniques Universitaire Saint-Luc, Woluwe-Saint-Lambert, Belgium

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Editorial on the Research Topic
Case reports in pediatric infectious diseases 2022

Introduction

Case reports of patients with unusual findings have been a source of inspiration for researchers and have contributed to improved clinical practice in the past (1). Especially in the field of infectious diseases in children, it is essential to have a broad knowledge of even the rare and unusual complications of common diseases and the common presentations of rare diseases. On top of that, the evolution in diagnostic methods has been fast and complex and reports of cases diagnosed with advanced laboratory techniques can contribute to improved diagnostic processes.

The editors of this special issue feel that in general, the scientific community, and more specifically, boards of universities deciding on promotion of staff tend to undervalue the contribution that case reports make to the advancement of new knowledge and to science as such. Pioneers in medicine were great supporters of documenting each unusual presentation of patients and sharing with a wide range of physicians, the findings and outcomes of such cases (2).

Writing a good case report is not easy though. Authors may get lost in less relevant details or present a textbook chapter in the introduction of the report, discouraging the target audience from finishing the reading of the report. A concise focused summary of literature can highlight why the particular case is so special and needs to be shared with as many doctors seeing similar patients as possible.

Another problem encountered is the lack of proper documentation of proper findings to exclude alternative diagnoses or contributing factors to the outcome of the patients. In order to write a good case report, the clinician should plan ahead and get all relevant investigations properly documented, of course with the permission of the patients or their guardian.

Editors of other journals have suggested that it may be a good idea to incorporate more training for physicians, not only during the undergraduate years but also in continuing medical education to ensure the skills of writing a good report are readily available in most doctors seeing a wide variety of cases.

Guidelines for reporting cases reports are available in literature (3), even though they are less well known and less applied than common guidelines for other types of scientific reports (e.g., consort statement for publication of randomised controlled trials).

It has been the great pleasure of the editors of this special issue to select and compile nine very relevant case reports in paediatric infectious diseases. In 4 reports, comprehensive pathogen analysis was useful for rapid diagnosis in pediatric infectious diseases, and it is desirable to examine its utilization in this area in the future. Other reports describe rare complications or unexpected diagnoses in patients with infectious diseases.

Below a concise critical review of each of the articles is presented.

Summaries of case reports

Huang et al. present a case of a 10-year-old immunocompetent boy presenting with a 5-day history of intermittent, left-sided chest pain, having a left lung nodule caused by *Streptococcus intermedius*. The nodule was identified by chest radiography. The cultures of aspirates of the nodule were negative but the germ was identified by metagenomic next-generation sequencing. He was given proper antibiotics and did not require a surgical intervention. The authors highlight the rarity of the case and the importance of metagenomic next-generation sequencing in the diagnostic process.

Zhuang et al. reported a case of Congenital tuberculosis in a neonate following *in vitro* fertilization-embryo transfer. They performed pathogenic microorganism metagenomic analysis and isolated the *Mycobacterium tuberculosis* complex in a preterm infant poorly responding to treatment. Subsequently, maternal pelvic tuberculosis was confirmed. Since, *in vitro* fertilization (IVF) treatment and pregnancy can exacerbate latent tuberculosis, identifying maternal tuberculosis during IVF treatment using metagenomic approach can be a useful option.

Rodriguez et al. reported a case of an unusual pneumonia pathogen, detected by plasma cell free next-generation sequencing in an immunocompetent adolescent with acute respiratory distress syndrome. This case details a rapid diagnosis of legionella pneumonia causing severe acute respiratory distress syndrome (ARDS) in an otherwise healthy adolescent through plasma microbial cell-free DNA next generation sequencing (mcfDNA-NGS). Diagnosis by mcfDNA-NGS of this unexpected pathogen led to narrowing of antimicrobials and this novel technology can be a useful tool for paediatric infectious disease diagnosis.

Liao et al. reported a case of Respiratory tract infection of fatal severe human bocavirus 1 in a 13-month-old child. They reported the case of a 13-month-old boy who presented with a cough, shortness of breath, and wheezing, and who eventually died of severe pneumonia and acute respiratory distress syndrome (ARDS). They confirmed that HBoV1 was the only detected pathogen by Metagenomics next-generation sequencing (mNGS).

Zhang and Yu reported a rare case of severe and fulminant paediatric *Mycoplasma pneumoniae* in an immuno-competent child. There were severe lung lesions associated with pleural effusion, coagulopathy, diffuse alveolar haemorrhage and severe respiratory distress, requiring ventilator and intravenous extracorporeal membrane oxygenation (VV-ECMO) support. The authors want to highlight that early recognition and prompt institution of advanced life support measures the treatment can be successful.

Zhang et al. reported another rare complication of *Mycoplasma pneumoniae*: multiple cardiac thrombi and pulmonary embolism. They successfully treated the case with thrombolytic and anticoagulant therapy. While other thromboembolic complications of *Mycoplasma pneumoniae* have been reported, this may be the first report of cardiac thrombi as a complication of infection by this organism.

Han et al. reported a case of pleural empyema and necrotizing pneumonia related to methicillin resistant *Staphylococcus aureus* (MRSA) secondary infection in a teenager who initially presented with Influenza A virus infection. They describe the clinical symptoms and treatment and they highlight the importance of early recognition and application of thoracoscopy for this potentially fatal pleural empyema caused by MRSA and influenza A co-infection. The authors hope that this article will raise awareness regarding rarely occurring severe respiratory infections by MRSA in a child with normal immune function after influenza A virus infection.

Feussner et al. reported a lethal case of mastoiditis and cerebral herniation as a complication of otitis media in a 13 year old patient with Arnold Chiari Malformation. This case demonstrates a very rare lethal complication of acute otitis media on the basis of a cerebral malformation and emphasizes the need to stay alert when patients complain of symptoms after assumed resolution.

Chang et al. reported an adolescent immune-compromised patient with histoplasmosis, coming from a non-endemic area. The patient presented with nonspecific symptoms and was successfully treated with amphotericin B and itraconazole. The authors encourage a high index of suspicion for this pathogen in immune-compromised patients, even in non-endemic areas.

Conclusion

In conclusion, the cases reported in this issue are likely to contribute to a better clinical practice and it is hoped that they are a source of inspiration for clinicians to keep sharing their special cases in excellent case reports.

Author contributions

HR wrote the introduction and summarized articles. KF reviewed the introduction and summarized articles. DL was a co-editor of the research topic. All authors contributed to the article and approved the submitted version.

Conflict 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.

Publisher's note

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CASE REPORT
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Case Report: Cardiac Multiple Thrombus and Pulmonary Embolism Associated With Mycoplasma Pneumonia Infection in a Child

Tongqiang Zhang[1†], Jiafeng Zheng[1†], Hongbo Wang[1†], Yongsheng Xu[1], Jing Ning[1*] and Chunquan Cai[2*]*

[1] Department of Respiratory, Tianjin Children's Hospital (Children's Hospital of Tianjin University), Tianjin, China, [2] Tianjin Institute of Pediatrics, Tianjin Key Laboratory of Birth Defects for Prevention and Treatment, Tianjin Children's Hospital (Children's Hospital of Tianjin University), Tianjin, China

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Edited by:
Kazumichi Fujioka,
Kobe University, Japan

Reviewed by:
Feng Liu,
Nanjing Children's Hospital, China
Ruike Zhao,
Shanghai Children's Medical Center, China

***Correspondence:**
Jing Ning
tianjinnj@126.com
Yongsheng Xu
xxyyss@126.com
Chunquan Cai
15122656313@126.com

[†] These authors have contributed equally to this work

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Mycoplasma pneumoniae (MP) is a common pathogen of lower respiratory tract infection in children and adolescents. Some patients with MP infection are self-limiting, while with the increase of severe or refractory Mycoplasma pneumoniae pneumonia (MPP) in recent years, there is a great increase in reports of thromboembolism in multiple organs, including lung, brain, spleen, and peripheral arteries. Cardiac multiple thrombi and pulmonary embolism associated with MP infection have not been reported. The most effective treatment option for cardiac thrombus was surgical resection for fear of thrombus detachment and causing new thromboembolism. Herein, we present a patient with cardiac multiple thrombi and pulmonary embolism in MPP for the first time. In our case, the child recovered after conservative medical treatment, which provides a therapeutic option for children with cardiac multiple thrombi.

Keywords: Mycoplasma pneumoniae, cardiac thrombus, pulmonary embolism, children, infection

INTRODUCTION

Mycoplasma pneumoniae (MP) is a common pathogen of lower respiratory tract infection in children and adolescents and has been known to cause various kinds of extrapulmonary manifestations including rash, vasculitis, liver function damage, nervous system sequelae, hemolytic anemia, pericarditis, and thrombosis (1–3). With the increasing cases of children with severe Mycoplasma pneumoniae pneumonia (MPP) in recent years, thromboembolism in various organs including lung, brain, spleen, and peripheral arteries has been reported (1, 4), while multiple intracardiac thrombosis was very rare. We report a previously healthy 8-year-old girl with severe pneumonia developed multiple cardiac thrombus and pulmonary thromboembolism, and etiological examination confirmed MP infection. After thrombolysis, anticoagulation, systemic application of corticosteroids and antibiotics, the prognosis was good. The aim of this study was to provide clinical experience for the diagnosis and treatment of such diseases.

CASE DESCRIPTION

A previously healthy 8-year-old girl was referred to the Department of Respiratory at Tianjin Children's Hospital (China) for 5 days of high fever and 2 days of violent coughing. The patient

had no related histories of congenital metabolic disease, congenital heart disease, cardiovascular surgery, family thrombus, antiphospholipid antibody syndrome, systemic lupus erythematosus, dilated cardiomyopathy, nephrotic syndrome, inflammatory bowel disease, etc. During the course of the disease, there was no hemoptysis, fatigue, and syncope in the patient. A lung computed tomographic (CT) scan revealed a massive consolidation shadow in the right lung lower lobe and pleural effusion. Despite intravenous administration of ceftriaxone (80 mg/kg/day) and azithromycin (10 mg/kg/day) for 3 days at the local hospital, fever and respiratory status continued to deteriorate. Therefore, she was referred to our hospital on 30 July 2019. Evaluation of her cold agglutinin titer of MP-IgM revealed 1:160 while C-reactive protein level was >200 mg/L. She was administered intravenous latamoxef (80 mg/kg/day) combined with azithromycin (10 mg/kg/day) as anti-infection medications and methylprednisolone (2 mg/kg/day) as an anti-inflammatory medication. On day 2, elevated inflammatory marker and liver function levels were detected (neutrophil ratio 90%, ferritin 653 ng/L, lactic dehydrogenase 653 U/L, alanine aminotransferase 109 U/L, and aspartate aminotransferase 29 U/L). MP-DNA concentration in bronchoalveolar lavage fluid was 5.0×10^7 copies/ml. There were no bacteria, fungus, or viruses (adenovirus, respiratory syncytial virus, influenza virus, rhinovirus, human metapneumovirus, Rhinovirus, and Epstein Barr virus) found in sputum, bronchoalveolar lavage, and blood. On the third day, she developed a sudden pain on the right side of her neck and dyspnea. Echocardiography (**Figure 1C**) revealed multiple mass echos in the right ventricle (4 × 3 mm, 13 × 5 mm, and 9 × 5 mm). There was no enlargement of the right ventricle and right atrium. Echocardiography showed ejection fraction was 77%, left ventricular short axis shortening was 45%, with normal ventricular wall motion and systolic function. The children had normal blood pressure, without enlargement of the liver and enlarged jugular vein. There was no galloping rhythm in heart auscultation. The myocardial enzymes and myocardial injury markers in the patient were also normal. Chest CT angiography showed a filling defect in the left lower pulmonary artery and right ventricle (**Figures 1A,B**). Coagulation analysis revealed elevated D-dimer levels (10 mg/L; normal reference range, 0–0.55 mg/L) and fibrinogen (FIB) (6.683 g/L; normal reference values, 2–4 g/L). Based on these findings, cardiac multiple thrombus and pulmonary thromboembolism was suspected (**Table 1**). This disease is life-threatening and usually treated by surgical intervention for fear of thrombus detachment and caused new thromboembolism. However, the patients' parents opted for conservative medical treatment. Therefore, she was administered with intravenous urokinase (initial 4,400 IU/kg, 10 min, later 4,400 IU/kg/h, q12h, total 5 days) for thrombolysis, low molecular weight heparin calcium (200 IU/kg/d, q12h) for anticoagulation and aspirin (4 mg/kg/day) to inhibit platelet aggregation. The symptoms improved after thrombolysis for 9 h, and only two massive echoes (9 × 5 mm and 13 × 5 mm) were found in the echocardiogram after 11 h. We also explored the thrombophilia screen. Plasma protein C activity 152.3% (normal reference values, 70–140%), plasma protein S activity 46.85% (normal reference values, 70–123%), Antithrombin III activity increased by 132.2% (normal reference values,

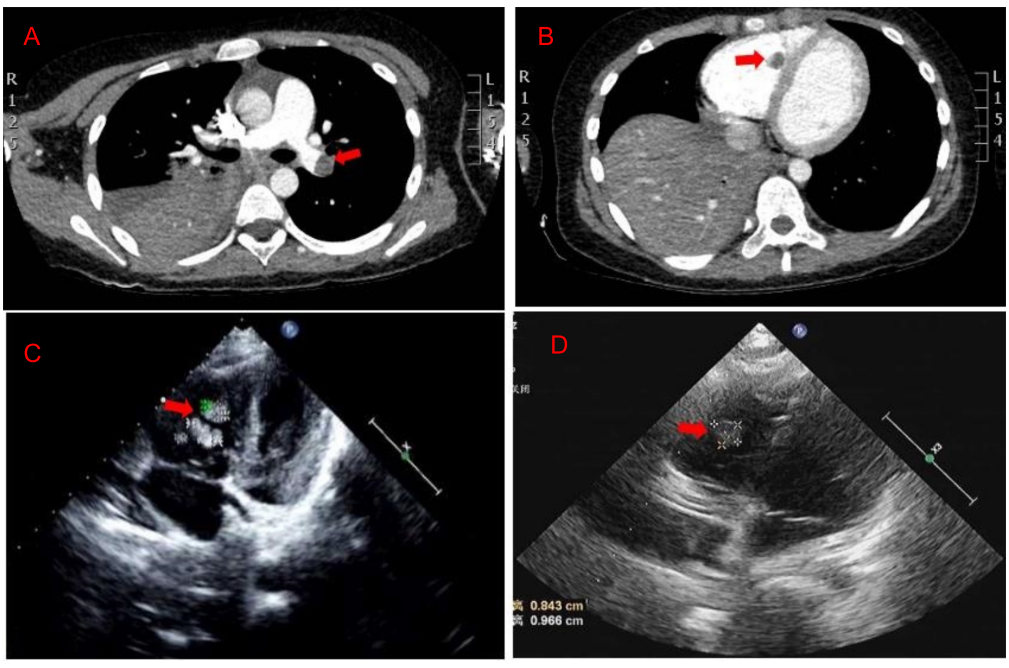

FIGURE 1 | (A,B) The chest CT angiography showed a filling defect in the left lower pulmonary artery and right ventricle. **(C)** Echocardiography revealed multiple mass echos in the right ventricle (4 × 3 mm, 13 × 5 mm, and 9 × 5 mm) on day 3. **(D)** Echocardiography revealed mass echos in the right ventricle (8 × 13 mm) on day 12.

TABLE 1 | General information of MPP children.

The basic characteristics	Case
Age (year)	8
Gender	Female
Embolism position	Right ventricle, left Pulmonary arterial
Outside the lung damage	Liver damage
Hypoxemia	Yes
Thoracic puncture	Yes
Fiberoptic bronchoscopy	Yes
Length of hospital stay	50 days
Days of fever	9 days
Days of thrombus from onset	Hospital day 3
Days of thrombus disappear	3 months after leaving the hospital
Antibiotics before admission	Ceftriaxone sodium and azithromycin for 2 days
Antibiotics after admission	Cephalosporin,
Anti-inflammatory therapy	Methylprednisolone 6 mg/kg/d
Human immunoglobulin	
Thrombolytic therapy	Urokinase
Anticoagulant therapy	Sobilin, rivaroxaban, aspirin
Plasma protein C activity	152.3%(normal reference values, 70–140%)
plasma protein S activity	46.85%(normal reference values, 70–123%)
Anti-thrombin III activity increased	132.2%(normal reference values, 75–125%)
karyotype: Golgi type plus granulation; anticardiolipin antibody negative, anti-β 2-glycoprotein antibody	(-)

75–125%). Anti-nuclear antibodies (ANAs) were positive, titer 1:100, karyotype: Golgi and granular type. Anticardiolipin (aCL) antibodies and anti-β 2-glycoprotein antibodies were both negative, while lupus anticoagulant (LA) was positive. There was no definite pathogenic mutation in the screening of thrombotic disease-related single gene genetic diseases.

After 10 days of hospitalization, her condition gradually stabilized and the filling defect of the left lower pulmonary artery was decreased by CTA in the lung. Administration of low molecular weight heparin calcium was changed to rivaroxaban (15 mg/qd) as the anticoagulation therapy. However, on day 12, the patient suddenly complained of pain in the right shoulder and intercostal region and fidgety. Echocardiography (**Figure 1D**) showed a right ventricular mass echo (8 mm × 13 mm). One of the right ventricular thrombus had disintegrated and caused a new pulmonary embolism. She was treated with urokinase (4,400 IU/kg/h, q12) for 3 days again (**Figure 2**), and rivaroxaban anticoagulation therapy was continued. On day 13, she did not complain of pain in the shoulder or intercostal region. Hospitalization was continued for 30 days and the echocardiography still showed a mass echo (about 6 mm × 11 mm) in the right ventricle. After which she was discharged and oral rivaroxaban administration was continued. After 2 and 3 months of follow-up, there was no cardiac thrombosis and pulmonary embolism respectively. In 2 years of follow-up, we found no recurrence of thrombosis in the heart and lungs.

DISCUSSION

In this study, we first reported a child who initially suffered from respiratory tract infection, pulmonary CT showed massive inflammatory changes, accompanied by pleural effusion. There was a significantly increased in the levels of blood inflammatory markers, liver function, and D-dimer. The child was previously healthy and had no history of cardiovascular disease, immune system disease, blood disease, external injury, or specific drug history. Multiple cardiac thrombosis and pulmonary thromboembolism were confirmed by chest CTA and echocardiography, and only MP infection was confirmed by etiological examination. After thrombolysis, anticoagulation, systemic application of corticosteroids and antibiotics, the prognosis was good.

In recent years,reports about thrombosis or thromboembolism in vessels related to MPP have gradually increased in the Chinese Mainland and abroad (4, 7, 8). However, cases of cardiac thrombosis in children with MP infection are rare. In 2006, Bakshi et al. first reported left atrial thrombosis in a 4-year-old child with MP infection (9). Li et al. found 2 cases of 6-year-old MPP with intracardiac thrombosis (10): 1 case with right atrial thrombosis 8 days after onset, and another case with right ventricular thrombosis 11 days later. There was also a 9-year-old patient with right ventricular thrombus 8 days after the onset of MPP symptoms (5). This study reports for the first time that children with severe MPP are complicated with multiple cardiac thrombi and pulmonary embolism 8 days after the onset of fever. Although the incidence of thrombosis varies, thrombus occurs within 8 days to 3 weeks after fever or cough (average 12 days) (5). The clinical manifestation of cardiac thrombus was not typical except for the manifestation of lung disease, and it was discovered by accident during the examination of cardiac ultrasound (10, 11). In this study, the children suddenly suffered neck pain, chest pain, dyspnea, and other clinical manifestations, which cannot be determined as the unique manifestation of cardiac thrombus, as may be the manifestation of pulmonary embolism. In this case, it was found that imaging examinations of respiratory and circulatory systems, such as lung CT and cardiac ultrasound, should be actively applied in case of severe MPP in school-age children. Among the reported cases, one of the 43 children with MPP complicated with thrombus had a history of allergic purpura, and the others did not mention a history of underlying diseases (4). The patient in our report also had no related histories of congenital metabolic disease, heart disease, cardiovascular surgery, family thrombus, nephrotic syndrome, etc. Therefore, children with severe MPP without underlying diseases should be alert to the occurrence of thrombosis.

The pathogenesis of MP infection complicated with thrombosis or embolism is complicated, including direct invasion, immune-mediated injury reaction, hypercoagulable or thrombotic state, vasculitis, and toxin injury. The common monitoring indexes of the hypercoagulable states include D-dimer and FIB. D-dimer is a specific degradation product of cross-linked fibrin, which reflects blood hypercoagulable state, intravascular thrombosis, and secondary fibrinolysis.

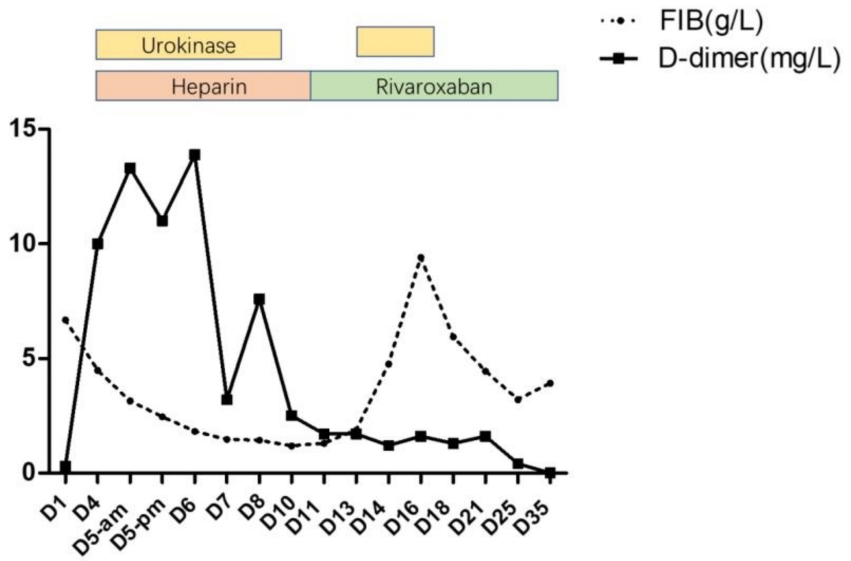

FIGURE 2 | Changes of FIB, D-dimer before and after application of thrombolytic drugs.

Several reports of MPP complicated with pulmonary embolism in children showed that D-dimer was significantly higher than normal (12–14). According to a research on 43 children with MPP complicated with thrombosis, it was found that FIB and D-dimer reached the peak within 6–15 days after the onset of the disease, with concentrations of 4.5 ± 2.2 g/L and 11.1 ± 12.4 mg, respectively. Patients with >5.0 mg/L accounted for 58.1% and with >2.0 mg/L accounted for 93.0% (4). This case reported that the change of D-dimer was consistent with the above research. The D-dimer was 0.3 mg/L on admission. When multiple cardiac thrombi and pulmonary embolism occurred on the eighth day, the D-dimer increased significantly (10 mg/L). After thrombolytic therapy, the level of D-dimer temporarily increased to the peak (13.9 mg/L) and decreased gradually. Therefore, dynamic monitoring the levels of D-dimer is of great significance to the pediatrician. In addition, we also revealed the dynamic changes of fibrinogen. In our case report, the level of FIB was 6.683 mg/L on admission and decreased gradually after thrombolytic therapy. Surprisingly, the levels of FIB increased again on the 13th day (after the second thrombolysis for 1 day), reached the peak (9.402 g/L) on the 16th day, and then decreased gradually. Throughout the whole course of the disease, abnormal blood coagulation indexes may still occur even after active anticoagulation treatment, reminding pediatricians of the importance of dynamic monitoring of blood coagulation function to avoid the possibility of systemic bleeding due to excessive anticoagulation. At the same time, avoid anticoagulation not timely which leads to blood re-entering the hypercoagulable state.

Protein C, protein S, and Antithrombin III are important factors causing intravascular agglutination. When the activity of protein C and protein S decreases, it is easy to form a blood hypercoagulable state and promote thrombosis (15). In this case, the activity of protein S also decreased temporarily and returned to normal after recheck after 2 months. Graw-Panzer et al. reported a case of MP infection complicated with pulmonary and popliteal vein embolism and detected a temporary decrease in its protein S activity, suggesting that the blood hypercoagulable state may be promoted by affecting the decrease of protein S activity in children with MPP (13).

It has been reported that autoimmune inflammation caused by antiphospholipid antibodies (aCL, LA, and anti-β 2 glycoproteins) and ANAs play an important role in the process of thrombosis (16, 17). Liu et al. (4) analyzed 43 children with MPP complicated with thrombus in Beijing Children's Hospital and found that the positive was 50% in ANAs, 60% in aCL antibodies, 64% in β2 glycoprotein-IgM, and 42.1% in LA. In this case, ANAs and LA were positive, which was consistent with the previous report. It is suggested that the transient hypercoagulable state caused by antiphospholipid antibodies and ANAs induced by MP infection may play an important role in the pathogenesis of Mycoplasma-associated thrombosis.

Echocardiography and CTA play an important role in the diagnosis and monitoring of cardiac thrombosis and pulmonary thromboembolism. Echocardiography can directly detect cardiac thrombosis and was widely used in the clinic because of its simple operation, with no trauma and radiation for the patients (18). However, if lack of vigilance for Mycoplasma infection-related cardiac thrombosis, it is easy to be misdiagnosed as a tumor or other diseases. It was reported that a 9-year-old child with MP infection showed right ventricular mass shadow by echocardiography. Because it was difficult to determine whether the mass shadow in the cardiac was a tumor, surgically was conducted for diagnosis and treatment of diseases. Pathological examination confirmed that the mass shadow was a relatively new fibrin thrombus with less white blood cells (5). Therefore, it should be combined with clinical manifestations, laboratory index, and other imaging findings to avoid missed diagnosis and

misdiagnosis. The examination of CTA can quickly determine the location and degree of cardiac thrombus and pulmonary embolism (19), which is widely used in clinical practice. This child was diagnosed with pulmonary thromboembolism by chest CTA. In addition, there are three cases in which pleural effusion was detected in cardiac thrombosis patients after MP infection (10, 11). Our study also found pleural effusion in this case, therefore, more attention should be paid to children with severe MPP complicated with pleural effusion.

The treatment of MPP complicated by cardiac thrombus is rare and there is no unified standard. At present, it mainly includes anticoagulation therapy, thrombolytic therapy, and surgical thrombectomy. The choice of treatment depends on the patient's clinical manifestation, embolus size, number, location, and hemodynamics. There was a 9-year-old patient with MPP who showed a right ventricular mass partially detached and almost floating in the cardiac by echocardiography, the patient underwent surgical thrombectomy (5). In another case complicated with right ventricular thrombus (17.0 mm × 9.3 mm) after anticoagulant therapy for 12 days, reexamination of echocardiography showed that there was no significant change in the thrombus, and surgical thrombectomy was performed (10). Our team recommended surgical intervention for fear of thrombus detachment and causing new thromboembolism. However, the patients' parents refused and opted for conservative medical treatment.

Thrombolytic therapy is the best treatment for acute thromboembolism in adults; however, its application in children is still rarely reported. The main thrombolytic drugs were urokinase, streptokinase, and recombinant tissue plasminogen activator (20). There are few reports on urokinase treatment of MPP complicated with a thrombus in children. A 5-year-old boy with MPP complicated by popliteal artery embolism was reported in Korea. The popliteal artery blood flow recovered and the clinical symptoms improved after intra-arterial infusion of urokinase (21). There was also a report that a 5-year-old patient with a thrombus of the left atrium and right middle cerebral artery was treated with low molecular weight heparin and aspirin after 24 h of urokinase treatment, and the cardiac thrombus disappeared 9 days later (11). In this case, we performed urokinase thrombolysis twice, and one cardiac embolus fell off 11 h after the first thrombolysis. Heparin drugs (unfractionated heparin or low molecular weight heparin) and Vitamin K antagonists were used for anticoagulation treatment. Li et al. (10) reported that a case of 6-year-old MPP complicated with right atrial thrombus (7.5 mm × 4.0 mm) was initially treated with low molecular weight heparin for 7 days, followed by sequential oral warfarin for 2 months, and the intracardiac thrombus disappeared. In our report, the patient was admitted with low molecular weight heparin for 7 days, followed by oral Rivaroxaban for 3 months, cardiac thrombus and pulmonary embolism gradually disappeared, and the prognosis was good. A phase 3 clinical trial of Rivaroxaban in the treatment of acute venous thromboembolism in children reported that, compared with standard anticoagulants (low molecular weight heparin or Vitamin K antagonist), Rivaroxaban can reduce the probability of the thrombus recurrence without increasing the risk of bleeding (22). Ma et al. (23) reported a case of the thrombotic storm in children controlled by Rivaroxaban and there was no thrombotic recurrence and bleeding during follow-up. Rivaroxaban was also applied to tumor-associated venous thrombosis in a child for one month, and the left upper limb brachial vein thrombosis disappeared (24). The experience of using Rivaroxaban in the treatment of MPP complicated with cardiac thrombosis and pulmonary embolism has not been reported. This case provides thrombolysis and anticoagulation experience for the treatment of multiple cardiac thrombosis and pulmonary thromboembolism.

CONCLUSION

We provide a rare case of MP infection complicated with cardiac multiple thrombi and pulmonary embolism in children. The aim of this study was to provide clinical experience for the diagnosis and treatment of such diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JN and CC: conception and design. YX: administrative support. TZ: provision of study patient. HW: collection and assembly of data. JZ: search literatures. All authors wrote the manuscript, contributed to the intellectual content of this manuscript, and approved the final manuscript as submitted.

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EDITED BY
Kazumichi Fujioka,
Kobe University, Japan

REVIEWED BY
Rajesh Kulkarni,
Post Graduate Institute Yashwantrao Chavan Memorial Hospital, India
Clemax Sant'Anna,
Federal University of Rio de Janeiro, Brazil

*CORRESPONDENCE
Weiqi Liu
lwq_8103@163.com

†These authors have contributed equally to this work

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Congenital tuberculosis in a neonate following *in vitro* fertilization-embryo transfer: A case report

Guiying Zhuang[1†], Linzhi Yang[1†], Liuhong Qu[1], Weiqi Liu[2*] and Huanjin Zhu[2]

[1]Division of Neonatology, The Maternal and Children Health Care Hospital (Huzhong Hospital) of Huadu, Guangzhou, China, [2]Department of Clinical Laboratory, The Maternal and Children Health Care Hospital (Huzhong Hospital) of Huadu, Guangzhou, China

Background: Congenital tuberculosis is becoming increasingly common, but congenital tuberculosis infection in neonates following *in vitro* fertilization and embryo transfer (IVF-ET) has been rarely reported; a diagnosis of congenital tuberculosis is often delayed due to the non-specificity of maternal IVF treatments and clinical manifestations during pregnancy—particularly in low-birth-weight preterm infants.

Case presentation: We herein report a case of congenital tuberculosis. The infant was born at 27+5 weeks of gestation and was admitted to the hospital due to hypopnea after birth. Due to a poor response to treatment, we conducted pathogenic microorganism metagenomic analysis to assess the nucleotide sequences within the *Mycobacterium tuberculosis* complex. After collecting sputum, the strains from the tuberculosis analysis were isolated and confirmed. From a detailed examination of the mother and in accordance with the child's congenital tuberculosis, we confirmed the diagnosis of pelvic tuberculosis.

Conclusion: IVF treatment and pregnancy can exacerbate latent tuberculosis, especially in women from a family with a history of tuberculosis infections. We posit that the optimal way to prevent neonatal congenital tuberculosis in IVF-ET is to procure a detailed maternal medical or family history and to identify and treat maternal tuberculosis during IVF treatment.

KEYWORDS

congenital tuberculosis, *in vitro* fertilization-embryo transfer, infection, *Mycobacterium tuberculosis*, neonate

Introduction

Tuberculosis is a disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) infection, with an estimated 1.7 billion people (23%) infected worldwide; moreover, there are over 10 million new cases annually (1). Tuberculosis has now surpassed human immunodeficiency virus/acquired immunodeficiency syndrome as the major cause of death caused by infectious pathogens (2). Congenital tuberculosis may be

caused by inhalation or ingestion of *M. tuberculosis* by the mother and then passing the bacterium through the placenta into the fetus, or by the fetus passing through the birth canal. Peng et al. (3) studied 170 children with congenital tuberculosis and found that most had no specific manifestations for the first 2–3 weeks and that the majority of mothers were diagnosed following the child's diagnosis. Such an early clinical-stage diagnosis thus engenders greater demands: due to the atypical early clinical manifestations, the mortality rate was reported to be as high as 44% (4). Although the development of *in vitro* fertilization (IVF) and other assisted reproductive technologies (ARTs) over recent decades has allowed an increasing number of infertile women to conceive healthy babies, physical examination of the mother's own health is often ignored during the IVF treatment process, and this may conceal disease in the offspring. Among the existing publications on congenital tuberculosis, there are few reports of congenital tuberculosis infection in infants born from IVF. In this report, we describe a case of congenital tuberculosis in a premature infant born from IVF/ART and confirmed by macromicro (DNA) testing and positive acid-fast bacilli in sputum smears.

Case report

The neonate

A Chinese mother delivered a male baby by cesarean section at 27+5 weeks after IVF-embryo transfer (IVF-ET). The neonate's birthweight was 1,060 g, and his Apgar scores were 9-10-10. After birth, the boy was admitted to the NICU due to shallow breathing. Physical examination upon admission disclosed a body temperature of 36°C, a pulse rate of 144 beats/min, respiratory rate of 42 beats/min; and blood pressure of 66/44 mmHg. The child exhibited shallow breathing, grunting, and low breath sounds in both lungs; cardiac and abdominal examination, however, showed no abnormal signs. Initial examination at the time of hospitalization showed the following: blood test results disclosed white blood cells (WBCs) at 17.58 × 10^9/L, neutrophils (NEU) at 42.50%, hemoglobin (HGB) at 155 g/L, platelets (Plt) at 208 × 10^9/L; a C-reactive protein (CRP) level < 0.5 mg/L; and a procalcitonin (PCT) of 0.41 ng/mL. Chest X-ray displayed lung changes and neonatal pneumonia in the premature infant, but plain abdominal X-ray revealed no abnormalities (Figure 1A). After admission to the hospital, the child was mechanically ventilated and treated with pulmonary surfactant (PS) and intravenous nutrition.

Mechanical ventilation was changed on the second day after hospitalization to non-invasive assisted ventilation, and we noted no apnea or periodic breathing under continuous non-invasive assisted ventilation over the next 14 days; transcutaneous oxygen saturation ($TcSO_2$) was maintained at 90–94%. During this period the child was breastfed and occasionally experienced abdominal distension, vomiting, and feeding intolerance. With a maximal feeding amount of 30 ml/Kg, body weight increased to 1,200 g, and the child's growth curve was at the 25th percentile.

However, the patient's condition changed 15 days after birth. In the non-invasive assisted ventilation (nCPAP) mode, the child manifested clinical symptoms such as dyspnea, obvious abdominal distension, and weakened bowel sounds. Thus, the boy's condition changed 2 weeks after being born prematurely; and although nosocomial infection was considered, the infection-related examination showed obvious abnormalities in his complete blood count (WBCs, 12.11 × 10^9/L; neutrophils, 72.9%; HGB, 106 g/L; PLTs, 247 × 10^9/L; a CRP of 2.7 mg/L; and a PCT of 0.54 ng/ml). However, blood cultures from two sites as well as sputum, stool, and urine culture did not divulge any bacterial growth. Chest X-ray displayed a thickening of lung markings and small flakes of fuzzy shadows, supporting a diagnosis of neonatal pneumonia. The abdominal intestine was also slightly distended, and intestinal dysfunction was considered (Figure 1B). We therefore administered mechanical ventilation and advised fasting, gastrointestinal decompression, and anti-infective and other treatments.

On day 18 after birth and under continuous mechanical ventilation, the boy's airway secretions increased, his sputum became thick, and erythematous, papulomacular, coalescent rash with indistinct borders (not consistent with common beningn neonatal skin rashes or drug rashes). The blood test results showed 15.63 × 10^9/L WBCs, 81.3% NEUs, an HGB of 111 g/L, Plt of 144 × 10^9/L, CRP of 95.1 mg/L, and a PCT of 6.46 ng/mL. Thus, compared with his previous test results, NEUs, CRP, and PCT increased, while PLT decreased. Sputum culture showed the growth of ESBL-producing *Klebsiella pneumoniae* subsp., as fungal (1-3)-β-D-glucopyranose was 290.71 pg/mL (the normal reference interval is 0–70 pg/mL); B-ultrasonography revealed a small amount of fluid in the intestinal space. Chest X-ray examination displayed progressive aggravation of pneumonia, and the abdominal portion was slightly thickened and stiff on *X*-ray. According to the examination results, we recognized that the child possessed a serious infection, and meropenem and fluconazole were administered as anti-infectives.

The boy's condition further deteriorated from the 19th to 22nd days after birth. During this period, fever occurred for the first time; and even under continuous mechanical ventilation, his complexion remained cyanotic, and his $TcSO_2$

Abbreviations: CRP, C-reactive protein; HGB, hemoglobin; IVF, *in vitro* fertilization; IVF-ET, *in vitro* fertilization and embryo transfer; *M. tuberculosis, Mycobacterium tuberculosis;* NEUs, neutrophils; PCT, procalcitonin; Plts, platelets; $TcSO_2$, transcutaneous oxygen saturation; WBCs, white blood cells.

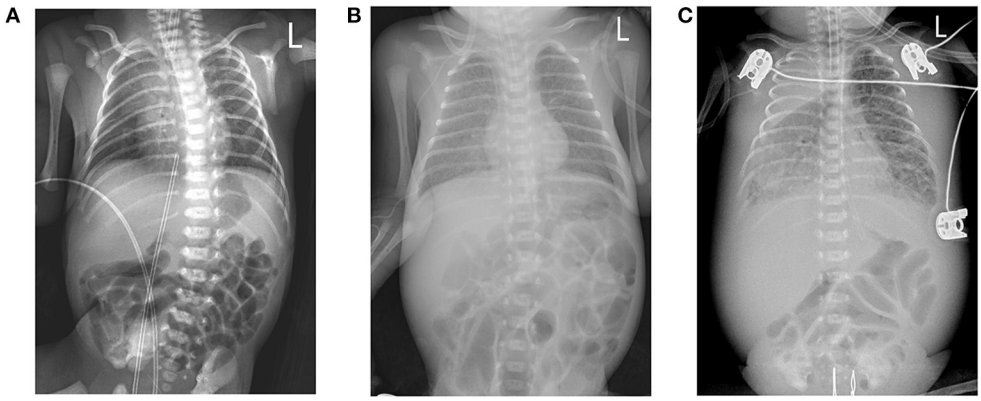

FIGURE 1
(A) Chest X-ray image shows a bilateral thickening of lung markings, with patchy and blurred shadows in the lower lung fields. **(B)** Chest X-ray image depicts thickening and blurring of bilateral lung markings, with small patches of fuzzy shadows in the lung fields. **(C)** Chest X-ray image shows that the texture of both lungs was thickened, increased, and blurred. Large dense shadows of uniform density were observed in the right upper lung field, and small patchy blurred shadows were found in the remaining lung fields. There was a narrow band of increased density in the field of the right lower lung that contained a clear boundary.

was difficult to maintain at 90%. The difference in percutaneous oxygen saturation between the right upper extremity ductus arteriosus and the right lower extremity ductus arteriosus is larger than 10%. The partial pressure of oxygen (PO_2) values from the blood gas analysis results on days 19–22 were 37.4 mmHg, 40.3 mmHg, 28.0 mmHg, and 34.6 mmHg, respectively, indicating that the child was hypoxemic. On day 22, blood test results showed that Plts decreased to 28×10^9/L and CRP increased to 134.8 mg/L. Chest X-ray examination on the same day showed large, dense opacities in the right upper lung field; small patchy opaque shadows in both lung fields; an air bronchogram sign; and a small amount of pleural effusion on the right side. We also noted bowel dilatation in the abdomen, and that the intestinal septum was slightly thickened, the left abdominal bowel was stiff, and the necrotizing enterocolitis (NEC) was aggravated (Figure 1C). The ventilator parameters were adjusted upward, nitric oxide and sildenafil were administered, and PS and other treatments were repeated. According to previously reported cases, the aforementioned treatment strategies should have achieved a better treatment effect, but the child's condition became progressively worse, and we investigated whether the condition was complicated by another uncommon pathogenic bacterial infection. We collected the child's serum for pathogenic microorganism metagene analysis, and our results indicated that the number of sequences detected by the growth of the *Mycobacterium tuberculosis* complex was 2,541 (100%) (Figure 2). As a result of the metagene results, the endotracheal aspirate of the child was collected for acid-fast bacilli examination, and the results showed that the endotracheal aspirate was positive for acid-fast bacilli (++) (Figure 3). Therefore, combined with the clinical manifestations and examination results, the child was diagnosed with congenital tuberculosis, and anti-tuberculotic treatment with isoniazid and rifampicin was applied.

The child was, however, found to be in critical condition on day 24 after birth, and due to multiple organ-system dysfunctions and the family's withdrawal of treatment, the child was ultimately declared clinically dead.

The neonate's parents

The neonate's father was 33 years old at the time of the boy's birth and in good health, and the boy's mother was 35 years old. There was a history of pregnancy, with the mother having undergone IVF-ET procedures and producing twins, and with premature rupture of membranes at 20 weeks of gestation. During the current pregnancy, the mother suffered from connective tissue disease and underwent premature rupture of membranes more than 6 days before delivery; and the child was born with clear amniotic fluid of approximately 30 ml. There had been numerous cases of *M. tuberculosis* infection in the families of both parents, the mother had been infertile for many years; both of her pregnancies were *via* IVF/ET, each resulting in preterm births. The mother complained that although other infectious diseases were investigated during her IVF treatment, tuberculosis was not evaluated; and there was a history of persistent cough 1 month before delivery. We therefore recommend that a mother suspected of having tuberculosis be sent to a higher specialized hospital as soon as possible to complete the relevant examinations. The mother was ultimately diagnosed with pelvic tuberculosis at the Guangzhou Chest Hospital (a superior hospital) and required conventional treatment.

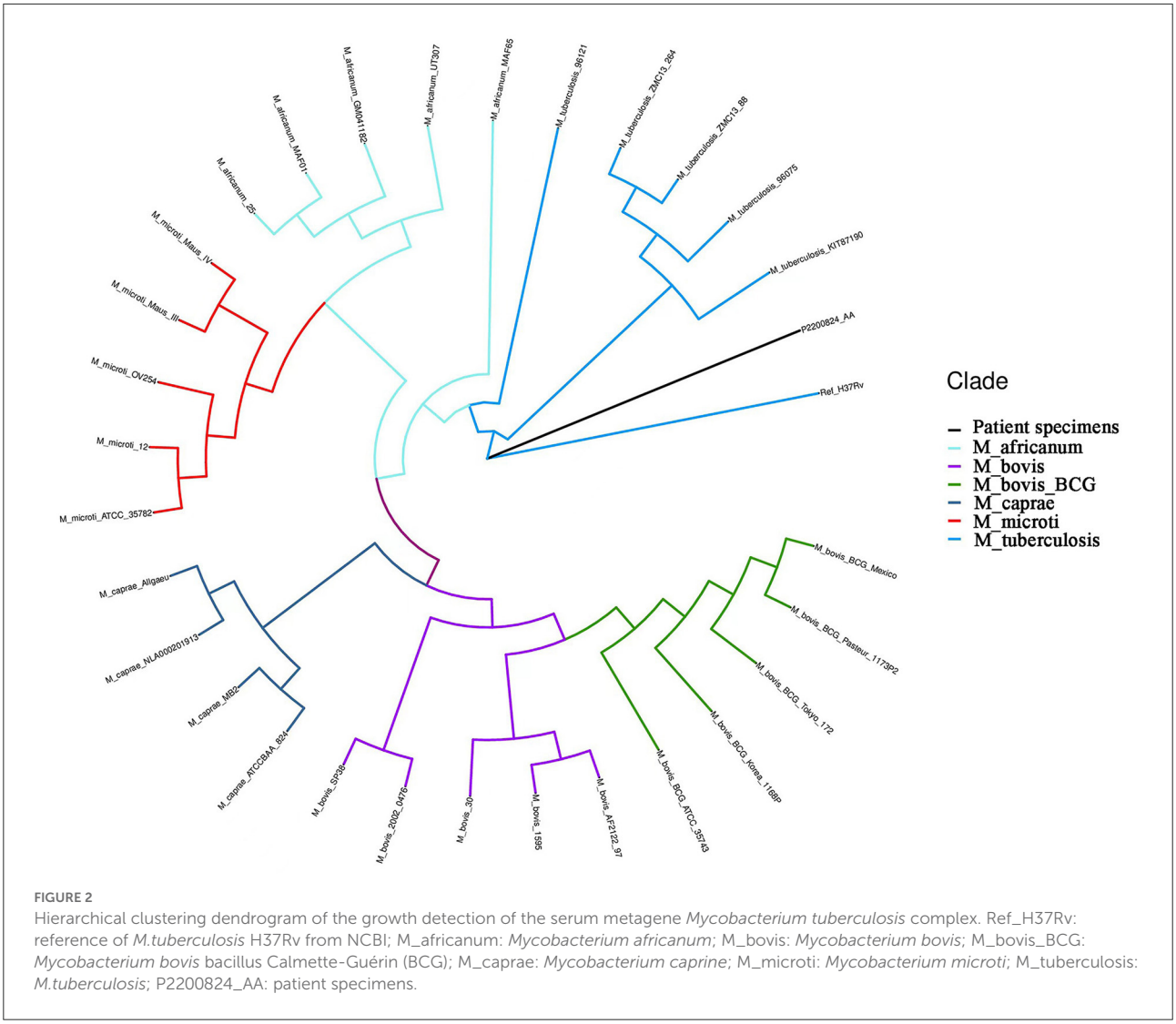

FIGURE 2
Hierarchical clustering dendrogram of the growth detection of the serum metagene *Mycobacterium tuberculosis* complex. Ref_H37Rv: reference of *M.tuberculosis* H37Rv from NCBI; M_africanum: *Mycobacterium africanum*; M_bovis: *Mycobacterium bovis*; M_bovis_BCG: *Mycobacterium bovis* bacillus Calmette-Guérin (BCG); M_caprae: *Mycobacterium caprine*; M_microti: *Mycobacterium microti*; M_tuberculosis: *M.tuberculosis*; P2200824_AA: patient specimens.

Discussion

Our current patient was an IVF-ET neonate with congenital tuberculosis, a rare clinical manifestation. The neonate manifested hypopnea at his initial clinical appointment, was not febrile for 14 days after birth, and we noted no abnormality in the inspection results with regard to infection indicators; only the chest X-ray showed lung changes and neonatal pneumonia in this premature infant. Due to the unsatisfactory response to treatment and the worsening severity of his condition, serum metagenomic testing was considered in combination with evaluation for other uncommon pathogenic bacterial infections, and our results revealed *M. tuberculosis* complex. Upon receiving the metagenomic results, sputum was collected to detect acid-fast bacilli to confirm tuberculosis infection, and the mother was ultimately diagnosed with pelvic tuberculosis from the diagnosis of neonatal congenital tuberculosis.

Congenital tuberculosis is relatively rare in clinical practice, particularly in neonates born from IVF-ET. Furthermore, due to different clinical manifestations, misdiagnosis is more likely to occur and result in fatal consequences. While respiratory distress and fever are the principal symptoms of congenital tuberculosis (5, 6), shallow breathing was in our case the initial clinical manifestation, and the condition was relatively stable over the next 14 days. By not noting any fever and by observing normal breastfeeding and significant weight gain of the child, we postulate that clinicians may thereby relax their vigilance regarding the diagnosis and severity of the neonate's disease.

Congenital tuberculosis is thought to be transmitted through the placenta or *via* inhalation of infected amniotic fluid at birth and the ingestion of infected substances (7). Because congenital tuberculosis often requires surgery or an autopsy to determine its mode of transmission, we often do not know the specific transmission route. In the present case, as no autopsy was

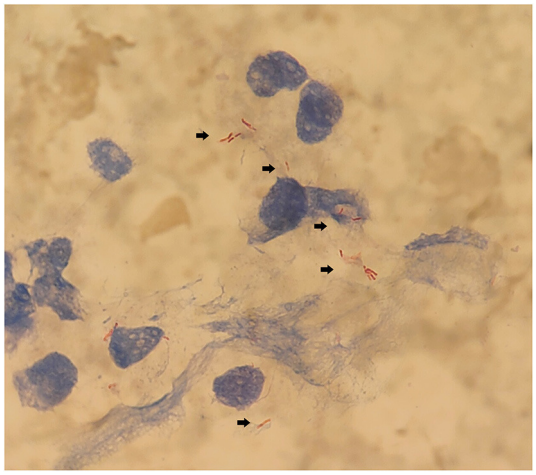

FIGURE 3
Acid-fast bacilli are shown in the neonatal sputum.

performed on the child, it remained unclear as to how the neonate was infected with congenital tuberculosis.

When our neonatal patient was diagnosed with congenital tuberculosis, his chest radiograph showed a large dense opacity in the right upper lung field, small patchy fuzzy opacities in both lung fields, and a small amount of pleural effusion on the right side. Serum metagene analysis subsequently revealed the *M. tuberculosis* complex. The sputum of the child was collected as a result of the metagenomic analysis, and the results showed the presence of acid-fast bacilli. Beizke (8) established criteria in 1935 to distinguish between congenital tuberculosis and postnatally acquired tuberculosis, and our case met Beizke's criteria for the diagnosis of congenital tuberculosis; i.e., the isolation of *M. tuberculosis* from the boy.

Previous studies have shown that a majority of mothers with congenital tuberculosis do not possess a previous history of tuberculosis infection (3, 9) and that most mothers are diagnosed with tuberculosis postpartum or when their children are diagnosed with congenital tuberculosis (10). These previous findings are consistent with the final diagnosis of pelvic tuberculosis in the mother in our case after her child was diagnosed with congenital tuberculosis. In the present case, the mother exhibited no history of tuberculosis infection, and only continued to cough for ∼1 month prior to delivery; this did not attract the attention of the obstetrician and resulted in the onset of congenital tuberculosis. We therefore suggest that pregnant women who manifest no obvious symptoms of infection during pregnancy, but continue to cough, should remain vigilant; and, if necessary, pregnant women should be evaluated for *M. tuberculosis*.

Infertility over 12 months has been reported to occur earlier in developing countries and ranges from 6.9 to 9.3% (11). In a prospective study in India, the investigators found that the incidence of infertility generated by genital tuberculosis was 3% (12). While IVF has become a relatively common treatment method used for infertile women in recent years, many women are not tested for *M. tuberculosis* before undergoing IVF protocols (10, 13). The application of IVF may thereby emerge as a potential risk factor for congenital tuberculosis (14). Samedi et al. (15) reported a case of congenital tuberculosis following IVF in which the mother exhibited uncontrollable epileptic seizures during preterm birth; these authors isolated *M. tuberculosis* in the maternal placenta, urine, gastric aspirates, and sputum. However, in the present case, the mother displayed a persistent cough only 1 month before delivery; and after the baby was diagnosed with congenital tuberculosis, a detailed examination of the mother revealed her pelvic tuberculosis.

The use of glucocorticoids during maternal preparations for IVF can sensitize the ovaries to gonadotropin stimulation (16, 17), and due to the increased level of estradiol after ovulation induction, both hormones can suppress the immune system and reduce maternal immunoresistance. In addition, changes to maternal hormone concentrations during pregnancy (especially the increase in estrogen) are capable of inhibiting the immune function of maternal lymphocytes (18), thus reducing maternal resistance and thereby precipitating tuberculosis or its recurrence in pregnant women. The appearance of this condition may have been due to the recurrence of pelvic tuberculosis caused by a diminution in maternal resistance during IVF treatment and pregnancy, which ultimately provoked the onset of congenital tuberculosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author/s.

Ethics statement

This study was approved by the Ethics Committee of the Maternal and Child Health Hospital of Huadu District (no. 2022-035). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

GZ and WL conceived the study. WL drafted the manuscript. LQ revised the manuscript. LY collected patient medical records. HZ supported laboratory data collection. All

authors participated in the interpretation of the results and approved the version of the final manuscript.

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Conflict 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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Supplementary material

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EDITED BY
Hans Van Rostenberghe,
Universiti Sains Malaysia (USM),
Malaysia

REVIEWED BY
Frieder Schaumburg,
University of Münster, Germany
Arturo Solis-Moya,
Dr. Carlos Sáenz Herrera National
Children's Hospital, Costa Rica

*CORRESPONDENCE
Wei Guo
guowei79656@126.com
Yongsheng Xu
xxyyss@126.com
Chunquan Cai
15122656313@126.com

†These authors have contributed equally to this work and share first authorship

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Successful treatment of pleural empyema and necrotizing pneumonia caused by methicillin-resistant *Staphylococcus aureus* infection following influenza A virus infection: A case report and literature review

Chunjiao Han[1,2†], Tongqiang Zhang[2†], Yidi Zhao[1], Lili Dong[2], Xiaole Li[2], Jiafeng Zheng[2], Wei Guo[2*], Yongsheng Xu[2*] and Chunquan Cai[3*]

[1]Clinical School of Pediatrics, Tianjin Medical University, Tianjin, China, [2]Department of Pulmonology, Tianjin Children's Hospital, Tianjin University Children's Hospital, Tianjin, China, [3]Institute of Pediatrics, Tianjin Children's Hospital, Tianjin University Children's Hospital, Tianjin, China

With the rapid increase in the number of infections, children with *Staphylococcus aureus* (*S. aureus*) infection secondary to Influenza A virus (IAV), appear to have a great possibility of causing severe complications and illness. Despite some cases and research findings regarding the death of children with IAV and *S. aureus,* coinfection included, there were few details about successful treatment of pleural empyema and necrotizing pneumonia caused by methicillin-resistant *Staphylococcus aureus* (MRSA) infection following IAV. In this case report, we describe the clinical symptoms and treatment of a teenager with pleural empyema and necrotizing pneumonia related to *S. aureus* secondary infection who was initially infected by IAV. This case highlights the importance of early recognition and application of thoracoscopy for this potentially fatal pleural empyema caused by MRSA and IAV coinfection. We conclude that this is a significant case that contributes to raising awareness regarding rarely occurring severe respiratory infections by MRSA in a child with normal immune function after IAV. In addition, further studies are needed to explore risk factors for IAV coinfection with *S. aureus*.

KEYWORDS

***Staphylococcus aureus*, influenza A virus, pleural empyema, necrotizing pneumonia, bronchoscopy**

Introduction

Retrospective studies of samples from the four pandemic influenza outbreaks of the last century have identified secondary bacterial infections as the fatal cause of co-morbidity and co-mortality, which reportedly manifested especially in the following week after viral infection symptoms are manifested, in 40–95% of influenza A -associated cases (1). IAV infections are associated with increased susceptibility to secondary bacterial infections, such as *S. aureus* and *Streptococcus* infections, wherein morbidity and mortality increase significantly (2). Children with IAV and *S. aureus* coinfection appear to have a great possibility of causing severe complications, and the mortality rate is very high as well. However, the details about the successful treatment of complications caused by coinfection of IAV and *S. aureus* have been rarely reported. In the present study, we report a unique case of pleural empyema and necrotizing pneumonia related to MRSA in a 13-year-old boy who was initially infected by IAV. Meanwhile, based on previous literature reports, we summarized the clinical characteristics and treatment of IAV and *S. aureus* coinfection.

Case description

A previously healthy 13-year-old boy was hospitalized with a three-day history of cough and fever. He was diagnosed with IAV infection in the local hospital. On admission, he was in respiratory distress and complained of left-sided chest pain. Laboratory indicators were shown in **Table 1**. His oxygen saturation level was 89–94% with an oxygen supply and he could not even lie down. The computed tomography (CT) scan of his chest showed atelectasis and a small amount of pleural effusion (**Figure 1A**). He was provided with oxygen support, anti-infection therapy and nutritional support. A diagrammatic representation of the treatment and outcome was presented in **Figure 2**.

The following day, the patient was not doing well and developed a stridor; hence, the decision was made to perform a flexible bronchoscopy with bronchoalveolar lavage (BAL) under ECG monitoring and respiratory oxygen support after employing local anesthesia (**Figure 1B**). Because the patient's severe pneumonia progressed rapidly and even endangered his life, an early and accurate etiological diagnosis was very important for the implementation of pathogen-specific treatment. Therefore, the next-generation sequencing analysis from BAL fluid was performed, and it indicated *S. aureus* as the infectious pathogen residing in the patient's lungs. Blood culture for microbial infection was also found positive for *S. aureus*. On the fourth day of hospitalization, the chest X-ray showed no improvement, so the bronchoscope was continued to be used for BAL. Tracheal aspirate showed heavy growth of MRSA sensitive

TABLE 1 Changes of various laboratory values.

Date of examination	WBC ($\times 10^9$/L; 4.0–10.0)	Neutrophils (%; 45–77)	CRP (mg/L; 0–8)	Hb (g/L; 110–160)	PLT ($\times$109/L; 100–300)	PCT (ng/ml)	IL-6 (pg/ml)	D-dimer (mg/L)	CK (U/L; 50–310)	CKMB (U/L; 0–24)	LDH (U/L; 120–300)	AST (U/L; 15–40)
1st	6.9	8.14	12.33	135	188	NA	NA	NA	NA	NA	NA	NA
4th	5.23	88	237.1	126	117	NA	NA	NA	508	20	355	33
5th	5.78	91.2	202.8	151	115	49.12	424.6	NA	Normal	NA	NA	NA
6th	6.65	82	64.4	124	163	8.66	NA	0.2	NA	NA	458	NA
8th	11.77	72	14.7	136	248	NA	NA	NA	NA	NA	NA	NA
11th	19.27	89	17.9	150	426	0.5	31.59	0.4	NA	NA	355	NA
13th	18.86	82	45	135	299	NA	NA	NA	NA	NA	NA	NA
16th	11.26	77	66.2	129	283	NA	NA	NA	NA	NA	NA	NA
18th	7.96	74	22.2	111	298	NA	NA	NA	NA	NA	NA	NA
19th	7.05	66	10.4	116	318	0.09	13.44	0.9	NA	NA	NA	NA
22th	4.69	60	<2.5	114	299	NA	NA	NA	NA	NA	NA	NA

WBC, white blood cell; PCT, procalcitonin; CRP, c-reactive protein; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; CK, creatine Kinase; CKMB, creatine kinase Mb isoenzyme.

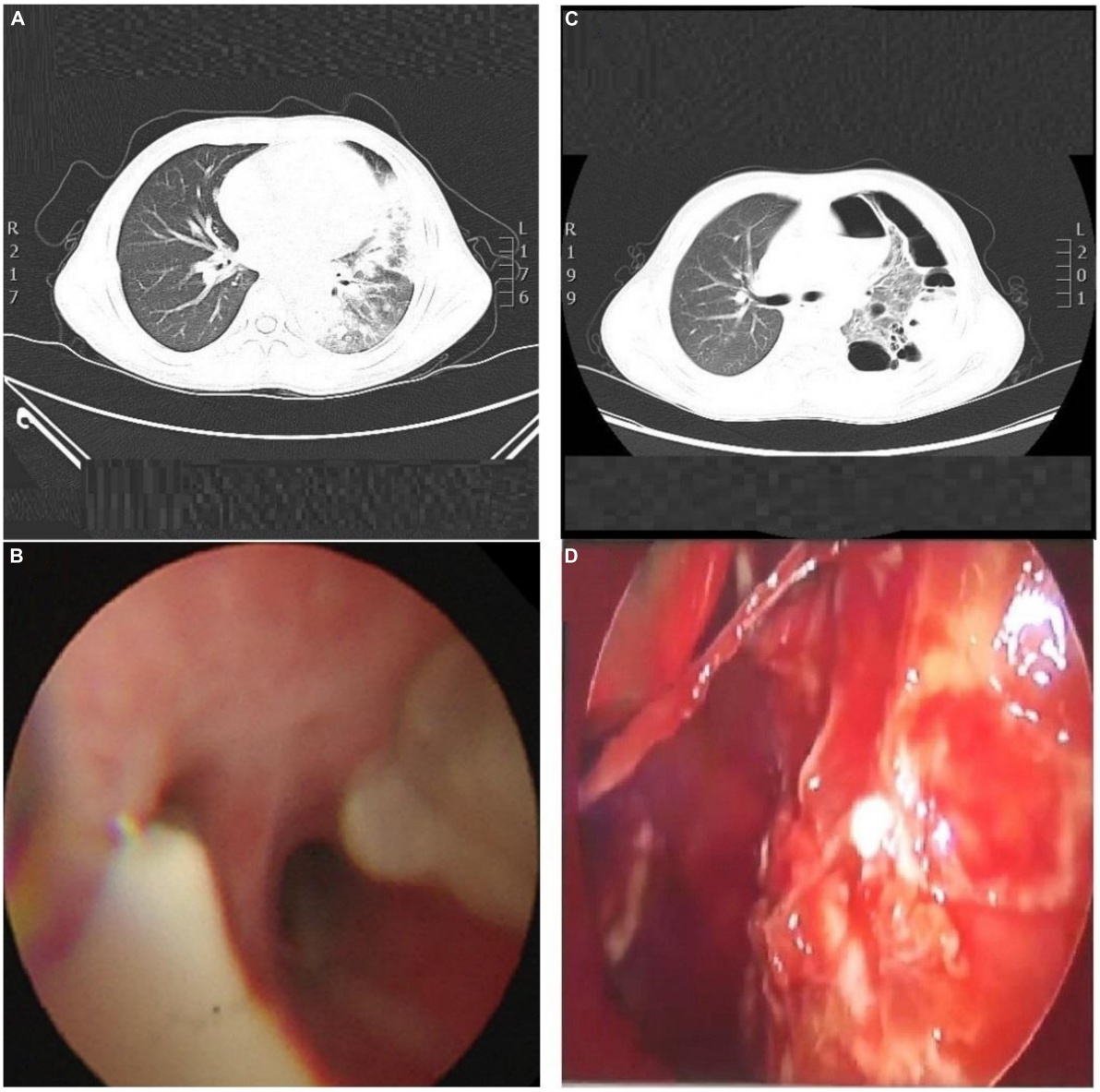

FIGURE 1
(A) CT scan of his chest, show the left lung and the right lower lobe consolidation, with atelectasis in the lower lobe of the left lung. The left pleural cavity showed a small amount of pleural effusion, and the lumen of the bronchial branch of the left lower lobe was not unobstructed. **(B)** Bronchoscopy, show the basal segment of left lower lobe, the bronchial mucosa was rough, a large number of yellow and white mucus plugs were found in the opening, and the ventilation was not smooth. **(C)** Chest CT scan. Indicate consolidation of left and right upper and lower lobes with signs of atelectasis and multiple cavities in the left lung, partially wrapped left pneumothorax. **(D)** Thoracoscopy, show the lung surface covered with a yellow purulent moss-like layer.

to linezolid, vancomycin, and rifampicin. Meanwhile, it was resistant to tetracyclines and quinolones.

Unfortunately, the patient was very ill, remained pyrexial (38.8°C) and developed a left-sided pyopneumothorax on day 8 of hospitalization with recurring fever, and the body temperature increased to 38.8°C. The patient complained of chest pain related to breathing abnormality, accompanied by apparent breath-holding. B-scan ultrasound and CT imaging (Figure 1C) of thorax revealed massive bilateral pleural effusions and multiple cavities in the left lung. Based on the laboratory and imaging diagnostic investigations, thoracentesis was performed on the patient's left chest, and the chest tube was placed on the same side.

Even after 12 days of hospitalization and treatment, the patient's condition did not improve due to poor drainage of empyema as a result of cellulose and thick pus accumulation.

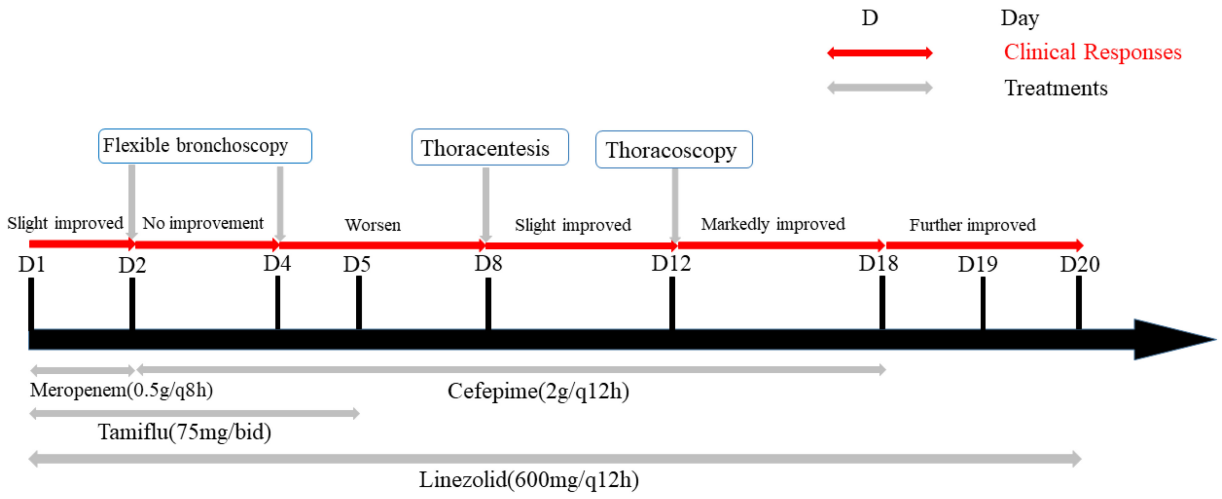

FIGURE 2
Diagrammatic representation of the treatment and outcome.

The patient underwent thoracoscopy to diagnose and treat pleural effusion (**Figure 1D**). After thoracoscopic investigations of the pleural cavity, the lung surface was cleared of the thick yellow moss-like layer of pus, and the left chest cavity was repeatedly flushed until the outflow was clear. At the end of the surgical procedure, the light bloody liquid could be seen in the chest bottle, and the chest tube was connected to the closed thoracic drainage system to prevent further pleural effusions.

On the 19th day of hospitalization, the chest drainage tube was removed. His cough condition was improved, and his breathing rate was stable without the occurrence of shortness of breath. The patient was discharged without any complaints of breathing discomfort. The patient was followed up for a chest X-ray examination after ten months of hospital discharge. The prognosis was good and the patient was in good physical condition.

Discussion

Influenza is considered as a known potential risk factor for Staphylococcal diseases (3). There are strong and consistent pieces of evidence of epidemiologically and clinically important interactions between the influenza virus and secondary bacterial respiratory pathogens (4). A study in the United States (5) showed that *S. aureus* was the most common bacterial pathogen (44%) among the 36 children who died of bacterial co-infection reported during the 2004–2007 influenza season, while MRSA accounted for 60%. The complication of co-infection progresses rapidly, and children cannot get accurate and timely treatment, which is one of the reasons for its high mortality rate.

There are several hypothetical mechanisms of bacterial co-infection secondary to influenza virus infection. It is reported that the influenza virus can increase the adhesion of bacteria by destroying the epithelial layer of the tracheobronchial tree and neuraminidase activity (6, 7). At the same time, Panton-Valentine leukocidin (PVL) is a pore-forming cytotoxin produced by *S. aureus* genes, acting synergistically to induce a strong lytic effect on host defense cells, notably with poly-morphonuclear leucocytes but especially on neutrophils (8). Previously infected influenza virus can enhance the proinflammatory and cytotoxic effects of PVL on neutrophils. Disintegration of the epithelial airway results in hemorrhage and tissue damage, which leads to the development of necrotizing pneumonia (7). In addition, nasal carriage of *S. aureus* is a significant risk factor for secondary staphylococcal pneumonia in IAV (9). Therefore, children with a history of *S. aureus* infection should be more alert to the risk of IAV and *S. aureus* co-infection. Finelli et al. found that compared with children without *S. aureus* infection, children with *S. aureus* infection were more prone to pneumonia and acute respiratory distress syndrome during the influenza season (5).

We reviewed 16 clinical reports of IAV and *S. aureus* coinfection; however, five of them lack detailed clinical information (**Table 2**). Reviewing the literature of 16 children with IAV and *S. aureus* co-infection, we can find that *S. aureus* is almost always characterized by MRSA. Most of these children are older than 10 years old, which is consistent with the result of Finelli et al. (5). Complications in these children mainly include sepsis, empyema, DIC, pneumothorax, lung abscess, emphysema, necrotizing pneumonia, and so on. However, most detection of virulence factors of *S. aureus* were missing.

TABLE 2 The clinical characteristics and results of the literature review of the IAV and *S. aureus* coinfection.

References	Cases in article	Age at the diagnosis	Sex	Duration of hospitalization (days)	Complication		Drug	Outcome	*S. aureus* drug resistance	Virulence factor
Boettger et al.(11)	1	21-month-old	NA	3	Sepsis, atelectasis, and pleural effusion	Orotracheal tube, central venous catheter insertion, and ventilatory support	Oseltamivir, Ceftriaxone, and clarithromycin	Death	MRSA	icaA, icaB, icaD, SeiO, hla and hlb(+); PVL and TSST-1(−)
Sharp et al.(12)	1	9-year-old	Male	12	Sepsis and circulatory collapse	ECMO, bronchoscopy	Vancomycin, meropenem, and enteral oseltamivir	Death	MRSA	NA
Pugh(13)	1	5-year-old	Male	30 more	Empyema	Chest tube insertion	Vancomycin, clindamycin, and cefepime	Recovery	MRSA	NA
Thomas et al.(14)	1	13-year-old	Female	8	Acute necrotizing tracheobronchitis	None	Amoxicillin and codeine	Death	Penicillin susceptible	NA
Obando et al.(15)	1	12-year-old	Male	28	Empyema and pneumothorax	Thorascopic surgery and chest tube insertion	Ceftriaxone, vancomycin, clarithromycin, linezolid, and oseltamivir	Recovery	MRSA	PVL(+), ACME(+)
Barrett et al.(16)	1	17-year-old	Male	17	Sepsis	None	Clarithromycin and co-amoxiclav	Recovery	MSSA	PVL(+)
Thomas et al.(17)	1	8-month-old	Male	25	Pleural effusion	Chest tube insertion	Ampicillin sodium, gentamicin sulfate, and nafcillin sodium	Recovery	NA	NA
Tanaka et al.(18)	1	11-month-old	Female	37	Empyema, DIC	Thoracentesis and thoracoscopic decortication	Oseltamivir phosphate	Recovery	MSSA	NA

(Continued)

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TABLE 2 (Continued)

References	Cases in article	Age at the diagnosis	Sex	Duration of hospitalization (days)	Complication		Drug	Outcome	*S. aureus* drug resistance	Virulence factor
CDC (19)	3	10-year-old	Male	3	Hypotension and hypoxia	Endotracheal intubation	Ceftriaxone and vancomycin	Death	MRSA	NA
		14-year-old	Male	2	Sepsis, DIC, and necrotizing pneumonia	Endotracheal intubation	Clarithromycin, penicillin, oseltamivir, ceftriaxone, and vancomycin	Death	MRSA	NA
		8-year-old	Female	20	Renal and hepatic failure, a subpulmonic abscess, respiratory distress, and sepsis	En dotracheal intubation	Azithromycin, dexamethasone, and ceftriaxone	Death	MRSA	NA
CDC(20)	5	14-year-old	Female	19	NA	NA	Oseltamivir	Death	MRSA	NA
		13-year-old	Male	5	NA	NA	Oseltamivir	Death	MRSA	NA
		15-year-old	Male	2	NA	NA	None	Death	NA	NA
		9-year-old	Female	15	NA	NA	Oseltamivir	Death	MRSA	NA
		15-year-old	Male	7	NA	NA	Oseltamivir	Death	MRSA	NA

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Two cases presented PVL (+) and one presented PVL (−). A study suggested that an early confirmed presence of the PVL toxin is particularly important in choosing antibiotics and administrating immunoglobulin that inhibits PVL toxin release in early stage (10). Notably, the application of bronchoscopy was important for early detection of the patient's respiratory conditions. However, bronchoscopic results have rarely been reported and utilized in treating children with *S. aureus* co-infection that is secondary to IAV. In this case, we removed the obstruction of the trachea and lung with the assistance of bronchoscopy findings. Moreover, we could wash the diseased area of alveoli through alveolar lavage and subsequently analyzed the BAL fluid for bacterial infection. Bronchoscopy has also been reported in the case of Sharp et al. (12). Copious cloudy secretions, fibrinous debris, and patchy plaques were found in the main bronchi and distal trachea. We should pay more attention to the application opportunity of bronchoscope. Thoracoscopy was also the key for this potentially fatal pleural empyema caused by MRSA and IAV coinfection when patient developed pleural adhesion and multiple cavities. In addition, the application of drugs was also critical. In our case, we covered the positive cocci in the initial treatment. We timely adjusted antibiotics by detecting the drug resistance of *S. aureus*, which effectively controlled the *S. aureus* infection. Due to the application of flexible bronchoscopy, thoracoscopy and adequate anti-infective treatment, we could successfully relieve the patient's uncomfortable respiratory conditions, and let the patient be discharged within 3-weeks of hospitalization.

In conclusion, due to the synergistic pathogenic effects between the influenza virus and coinfecting respiratory bacteria, we should raise awareness regarding the rarely occurring severe respiratory infections by *S. aureus,* following influenza for early diagnosis and rapid recovery from respiratory complications in children. In addition, this study suggests a need for further research about risk factors of secondary *S. aureus* infection of IAV.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consents were obtained from the parents for publication of this report.

Author contributions

TZ and XL cared for patients. YZ and LD collected the data. CH and JZ drafted the article. WG and YX revised it for intellectual content. CH and CC approved the final completed article. All authors read and approved the final manuscript.

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Conflict 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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EDITED BY
Hans Van Rostenberghe,
Universiti Sains Malaysia (USM), Malaysia

REVIEWED BY
Mohammad Bagher Rahmati,
Hormozgan University of Medical Sciences, Iran
Hua Gao,
Peking University People's Hospital, China

*CORRESPONDENCE
Zhimin Chen
zmchen@zju.edu.cn

†These authors have contributed equally to this work

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An isolated pulmonary nodule secondary to *Streptococcus intermedius* infection in an otherwise healthy 10-year-old boy: A case report and literature review

Meixia Huang†, Shuxian Li†, Xiling Wu, Dan Xu, Lanfang Tang and Zhimin Chen*

Department of Pulmonology, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, China

Streptococcus intermedius, as a Gram-positive commensal bacterium, tends to cause various infections, such as brain and liver abscesses, endocarditis, and empyema, especially in immunocompromised patients. However, an isolated pulmonary nodule caused by *S. intermedius* in previously healthy individuals without traditional risk factors is rarely reported. Herein, we present a case of a 10-year-old immunocompetent boy referred to our department with a 5-day history of intermittent, left-sided chest pain. Chest X-ray and computed tomography revealed a left lung nodule. Although his blood, sputum, and bronchoalveolar lavage fluid cultures were negative, metagenomic next-generation sequencing (mNGS) showed only the presence of *S. intermedius* in ultrasonography-guided lung biopsy tissue and pleural fluid (416 and 110 reads, respectively). He was then successfully treated with appropriate intravenous antibiotics and avoided surgical intervention. To the best of our knowledge, this is the first report of *S. intermedius*-related pulmonary nodule confirmed by mNGS analysis in healthy children. For achieving proper diagnosis and treatment, infection with *S. intermedius* should be included in the differential diagnosis when coming across such a similar pulmonary nodule. mNGS, as a valuable supplement to conventional culture methods, is an essential diagnostic tool for identifying pathogens without typical characteristics.

KEYWORDS

***Streptococcus intermedius*, infection, pulmonary nodule, metagenomics next-generation sequencing, ultrasonography-guided lung biopsy, case report**

Introduction

Solitary pulmonary nodules are rare in children. Identifying the etiology of pulmonary nodules can present a challenge to clinicians owing to the diversity of pathologies, such as hamartochondroma, *Cryptococcus neoformans*, abscesses, vasculitides, malignancy, and granulomatous diseases (1, 2). Consequently, a conscientious and fastidious diagnosis is essential to avoid inappropriate treatment.

Streptococcus intermedius (*S. intermedius*), as a member of the *Streptococcus anginosus* group (SAG), is a β-hemolytic Gram-positive microaerophilic coccus (3). Despite being a normal flora of the oral cavity, gastrointestinal, respiratory, and female urogenital tracts, *S. intermedius* is notorious for abscess formation, particularly in the brain (3). Underlying risk factors for *S. intermedius* infection include sinusitis, congenital heart disease, dental illness, oral manipulation, undergoing surgery, and chronic obstructive pulmonary disease (3). It is noteworthy that *S. intermedius* infection was more frequently identified in older patients with predisposing conditions (3). Still, healthy individuals typically do not develop an invasive infection with *S. intermedius*. Additionally, *S. intermedius* is most frequently associated with head and neck infections in children (4). Until now, there have been very few reported cases of *S. intermedius* causing isolated pulmonary nodules in the literature review (5), especially in children.

Herein, we describe a 10-year-old immunocompetent boy with a pulmonary nodule, which was ultimately confirmed by analysis of lung biopsy tissue with metagenomic next-generation sequencing (mNGS). Our case is extremely rare, as our patient is a young child without possible risk factors and he initially did not report fever, cough, or any other symptoms that may indicate lung infection and represented a diagnostic dilemma.

Case presentation

A previously healthy 10-year-old boy presented to our department with a 5-day history of intermittent, left-sided chest pain exacerbated by deep breathing. He denied any current fever, cough, hemoptysis, nausea, vomiting, diarrhea, palpitations, night sweats, or weight loss. Additionally, he also denied previous surgery, trauma, sick contacts, dental work, aspiration episodes, and exposure to hazardous and/or infectious materials. He had no remarkable medical history and had received all scheduled vaccines, including BCG, without any serious adverse events. There was no family history of asthma, diabetes, immunodeficiency, malignancy, and tuberculosis. On day 3 of chest pain, he sought treatment at a local hospital. Chest radiography demonstrated left lobe pneumonia (Figure 1A), while blood examination revealed a white blood cell count (WBC) of 10.4×10^9/L with 58.5% neutrophils and C-reactive protein levels $<$ 0.50 mg/L. Thus, he was initially treated for community-acquired pneumonia with ceftriaxone and azithromycin for 2 days without relief. A subsequent plane computed tomography (CT) scan of the chest disclosed a round soft tissue mass and a small amount of pleural effusion in the left lung (Figures 1B,C). Then, the patient was transferred to our hospital for further evaluation and treatment at the request of his parents.

His vital signs upon arriving to us were as follows: body temperature, 37.1°C; pulse rate, 92/min; respiratory rate, 28/min; and blood pressure, 113/67 mmHg. Physical examination showed no abnormalities. Blood routine and biochemical tests were all within normal ranges. Empiric antibiotics with azithromycin and amoxicillin/clavulanate were given. The HIV test, viral hepatitis serologies, antinuclear antibody, serum parasite IgG, serum galactomannan, serum 1, 3-β-D-glucan, serum Cryptococcus antigen test, T-spot test, oncological biomarkers, Legionella antigen, *Mycoplasma pneumoniae* antigen, and respiratory virus antigen were all negative. Due to concern of endocarditis and other occult sources of infection, echocardiogram and abdominal ultrasonography (US) were also ordered and were unremarkable. The patient underwent conventional flexible bronchoscopy and bronchoalveolar lavage (BAL). The utility of bronchoscopy revealed no unsuspected endobronchial lesions. BAL fluid (BALF) yielded only benign bronchial epithelial cells and macrophages, with no bacterial, viral, or fungal inclusions. The initial blood, sputum, and BALF specimens were all negative on both Gram stain and culture. These results yield no clear etiology for the formation of the nodule. However, his symptoms persisted. On hospital day 5, a chest X-ray revealed a left lung nodule measuring 2 cm in diameter (Figure 1D), which was similar to the previous. The thoracic US showed a hypoechoic lesion, 2.2 cm × 1.9 cm × 1.6 cm. This was confirmed by a contrast-enhanced CT scan revealing a rim-enhancing lesion in the left lower lobe lung (Figures 1E,F). The pulmonary abscess was highly suspected based on these findings. He was then sent for a US-guided lung biopsy on hospital day 7. Metagenomic next-generation sequencing (mNGS) showed 416 sequence reads of *S. intermedius* in lung biopsy tissue. Consistently, 5 days later, the culture of the specimen yielded pure growth of *S. intermedius*, with the pathological evaluation of the mass demonstrating fibroblast proliferation, no evidence of malignancy, lymphoma, or soft tissue tumor. The microorganism isolated from the lesion was susceptible to penicillin G, ceftriaxone, vancomycin, linezolid, levofloxacin, and chloramphenicol. Antibiotics were then narrowed to amoxicillin/clavulanate. However, following the biopsy, he had an intermittent slight fever since hospital day 7. A repeated chest X-ray showed partial resolution of the mass (Figure 2A) on hospital day 8, but an ultrasound revealed pleural effusion on hospital day 11. Considering the unsatisfactory effectiveness of amoxicillin/clavulanate, his antibiotic regimen was switched to linezolid on day 11. A thoracentesis was

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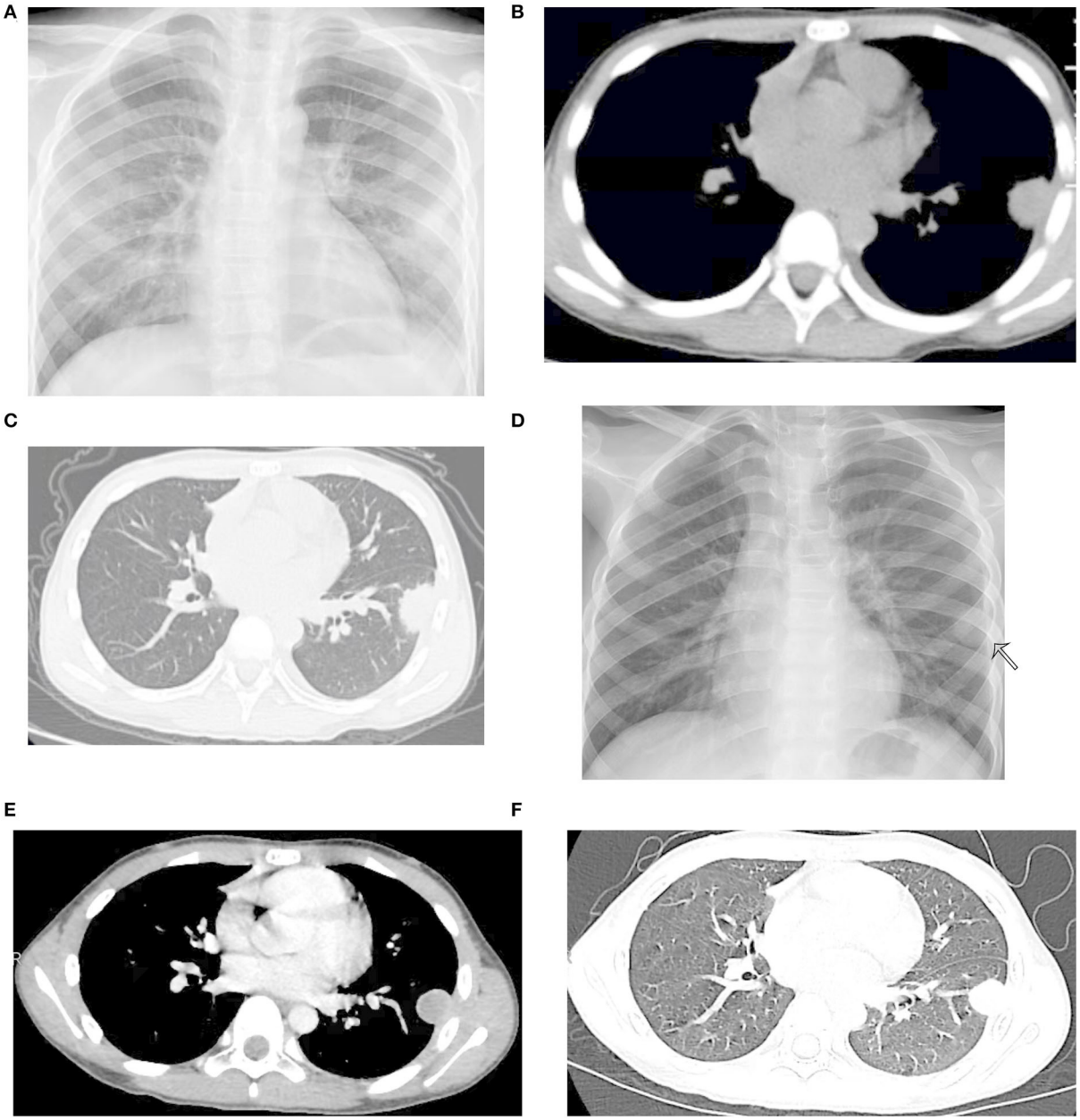

FIGURE 1
Radiological images before ultransonography (US)-guided lung biopsy. **(A)** Signs of infiltration present in the left lower lung field. **(B,C)** Plane computed tomography (CT) at the previous hospital revealing a round soft tissue mass and a small amount of pleural effusion in the left lung. **(D)** Chest X-ray revealed a left lung nodule measuring 2 cm in diameter. **(E,F)** Contrast-enhanced CT of the chest showing a rim-enhancing lesion in the left lower lobe lung.

performed evacuating 190 ml of reddish-brown purulent pleural fluid (Figure 2B). Pleural fluid glucose was 6.07 mmol/L, protein 54.4 g/L, lactic dehydrogenase (LDH) 240 U/L, triglyceride 0.42 mmol/L, adenosine deaminase (ADA) 16.2 U/L, and white blood cell count 6,836.0 $\times 10^6$/L (64.0% polymorphonuclear cells and 36.0% mononuclear cells). Accordingly, pleural fluid analysis with mNGS reported 110 sequence reads of *S. intermedius*. The pleural fluid culture was negative. Clinically, his fever subsided on hospital day 13, and the patient was also much improved. A repeat chest scan confirmed the obvious resolution of the lung nodule and disappearance of the pleural effusion on hospital day 20 (Figures 2C,D). The patient was then discharged home with the plan to finish a 4-week course of linezolid and continued to be asymptomatic. Follow-up CT

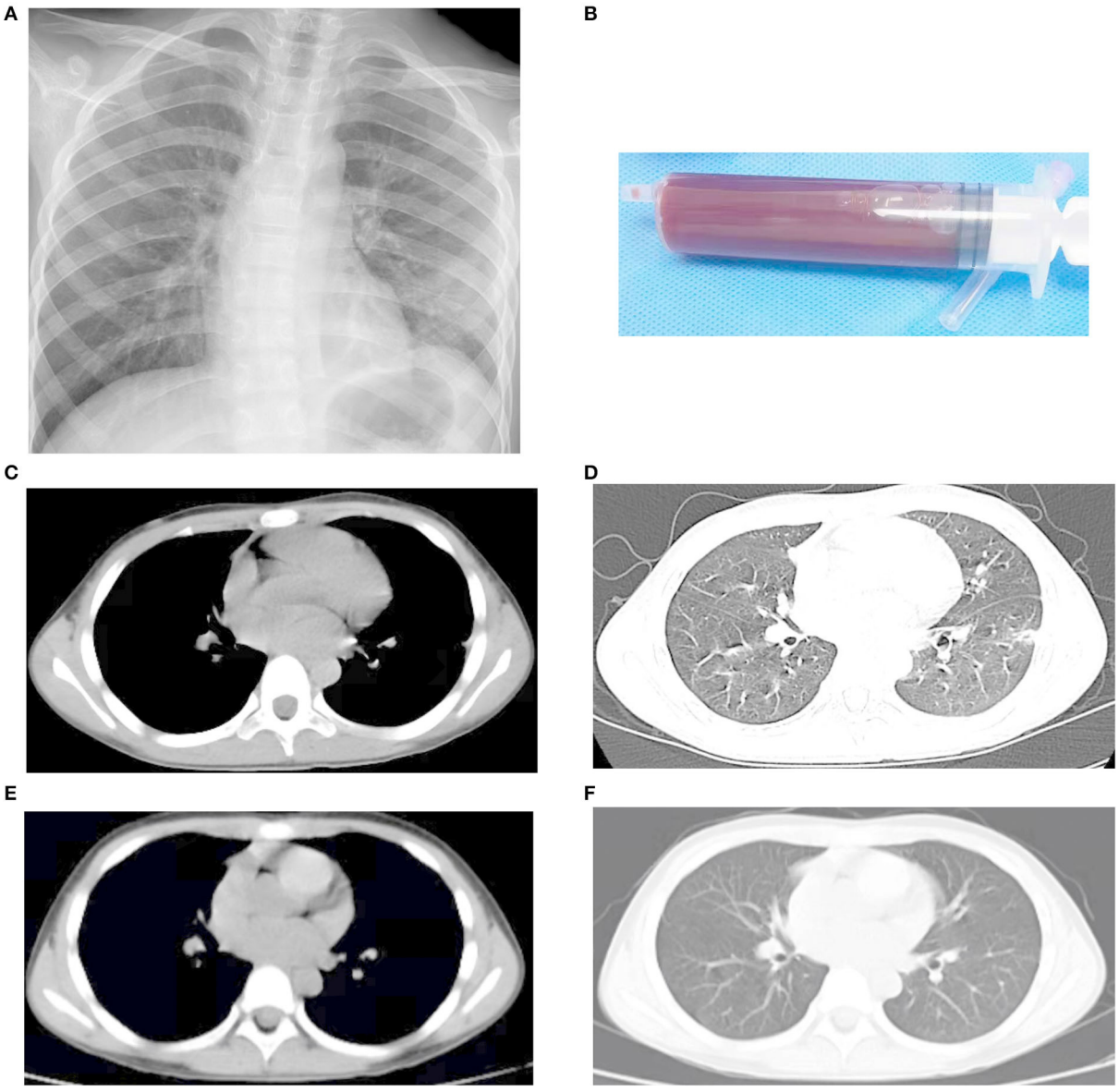

FIGURE 2
Radiological images after US-guided lung biopsy and appropriate treatment. **(A)** A repeated chest X-ray demonstrating partial resolution of the mass on hospital day 8. **(B)** Reddish-brown purulent pleural fluid. **(C,D)** A repeat chest scan confirming obvious resolution of the lung nodule on hospital day 20. **(E,F)** Follow-up CT after 3 months showing nodule resolution without any residual lesion.

after 3 months showed nodule resolution without any residual lesion (Figures 2E,F).

Discussion

Although *S. intermedius* has been recognized as an opportunistic pathogen, it has been implicated in a variety of purulent infections with an apparent tropism for the brain and liver (3). Until now, only a few cases of pneumonia caused by *S. intermedius* have been published in the literature (6–11). The radiological findings shown in the patients with *S. intermedius* pneumonia varied broadly, including consolidation, isolated pleural effusion, lung abscess, and empyema (6–11). Notably, only one case of *S. intermedius* pulmonary nodules in a 29-year-old male was documented (5). Moreover, most of the reported cases were in the adult population (5–11). Therefore, to the best of our knowledge, our case, as a rare phenomenon, is the first ever reported study of isolated pulmonary nodules secondary to *S. intermedius* infection in

children. Collectively, these cases highlighted the characteristic property of *S. intermedius* of causing local as well as systemic abscesses (5, 11).

Multiple predisposing conditions were noted related to infection by *S. intermedius*, e.g., poor dental hygiene, chronic sinusitis, immunodeficiency, malignancy, diabetes, previous surgery, and trauma (3). Our case presented a dilemma because it lacked underlying medical problems. Likewise, Esposito et al. reported a 3-year-old girl, without any history of severe congenital or acquired diseases or known risk factors, who developed a 6 × 5.6 cm brain abscess with surgical drainage cultures positive for *S. intermedius* (12). Denby et al. illustrated an unusual case of life-threatening purulent pericarditis secondary to *S. intermedius* in an otherwise healthy male adolescent without typical risk factors (13). Despite the multiple known causes, these data highlight that the source of *S. intermedius* infection can remain unidentified, and *S. intermedius* could infect human subjects regardless of underlying diseases. Although our patient had neither predisposing factors nor a clear source of infection, the possible mechanism for pulmonary nodule formation is aspiration or seeding with transient bacteremia from an oral or gastrointestinal source. Although cell-associated virulence factor (e.g., antigen I/II), extracellular virulence factors (e.g., hyaluronidase, deoxyribonuclease, and chondroitin sulfatase), and intermedilysin contribute to the pathogenic potential of *S. intermedius* (14), the wide range of presentations and radiological findings described in literatures (5–11) raised the question of whether emergent *S. intermedius* species have acquired novel molecular mechanisms of pathogenesis suitable for scientific exploration. Therefore, further identification of disease-specific virulence factors of *S. intermedius* will provide new insight into *S. intermedius* pathogenesis.

Since clinical features of *S. intermedius* infection are often non-specific, it can cause a delay in diagnosis (3, 14, 15). Our patient showed neither fever on admission nor elevated WBC or CRP. Similarly, fever is not present even in severe respiratory disease (e.g., empyema and necrotizing pneumonia) caused by *S. intermedius*, and both WBC and CRP were in the normal range as demonstrated in published cases (9, 11). Therefore, the absence of fever should not dissuade the physician from the seriousness of the infection.

Both X-ray imaging and CT scan showed a nodule of the left lung in our case; however, those imaging modalities were unable to determine the characteristics of the nodule and diagnose definitively. The radiological finding in our case was difficult to distinguish from malignancy, *Cryptococcus neoformans*, and tuberculous nodule, driving us to further perform a US-guided lung biopsy to explore the pathophysiology of the disease. Therefore, a physician should be in high suspicion of *S. intermedius* infection whenever coming across such a similar radiological manifestation. Using US-guided tissue biopsy, we were able to determine the pathology and etiology of the nodule, indicating that the US is a useful technique with low radiation risk for clinical diagnosis and improves the accuracy of the diagnosis. Identification of the causative pathogen is important for appropriate antimicrobial treatment, and susceptibility tests are important. Culture yield and pathological findings with tissue biopsy led us to a final diagnosis, indicating that a tissue biopsy remains to be the best way to definitively diagnose and should be performed if the diagnosis is uncertain. Thus, the approach to determining the etiology of pulmonary nodules should be an overall consideration of clinical presentation, patient history, radiological imaging, laboratory test results, and multidisciplinary collaboration (e.g., US-assisted tissue biopsy) if necessary.

For diagnostic purposes, the diagnosis of *S. intermedius* infection requires isolation of the organism from clinical specimens. Although blood, sputum, and BALF specimen cultures were negative in our case, still, those findings should not exclude the possibility of *S. intermedius* infection. To some extent, the pathogenic role of *S. intermedius* may be underestimated because *S. intermedius* is not commonly discounted as a pathogen but a contaminant using sputum cultivation (16). Additionally, *S. intermedius* is more frequently reported in studies using specimens obtained from transthoracic needle aspiration, intracerebral aspiration, thoracentesis, or percutaneous lung needle aspiration (9, 16–18). Our study and these studies indicate that further lung biopsy and pleural fluid analysis should be performed if *S. intermedius* infection is highly suspected. Physicians should be alert to *S. intermedius* isolated from usual sterile sites or from abscesses.

Pathogen identification in bacterial infections is of great importance for establishing the correct diagnosis and appropriate selection of more targeted treatment. Reported laboratory testing for *S. intermedius* includes culture and 16S rRNA gene sequencing (10, 17). However, culture is time-consuming, and we frequently experience negative culture results, especially for patients who have received antibacterial therapy at the time of specimen sampling. Although 16S rRNA gene sequencing can serve as a useful method for pathogen discovery and identification since the 1990s, it is not used as a routine laboratory test (19). Currently, mNGS, as a revolutionary development for microbiological diagnosis, has been increasingly applied in medical microbiology detection of various diseases, including pneumonia, meningitis, and sepsis (20). It has been proven to be unbiased, culture-independent, high-throughput, and less affected by antibiotic exposure and fast methodology for detecting pathogens, especially for identifying rare, novel, and difficult-to-detect pathogens (21). We revealed the isolated pulmonary nodule secondary to *S. intermedius* infection diagnosed by mNGS. In this way, we showed that mNGS is a valuable supplement to conventional methods for identifying the pathogen responsible for infections with atypical clinical symptoms.

In conclusion, an isolated pulmonary nodule caused by *S. intermedius* infection in an immunocompetent child is an uncommon case with few reports published in the literature. Our case reminds us to be aware of *S. intermedius* infection as a differential diagnosis when physicians encounter a similar pulmonary nodule. In addition, our case report highlights the usefulness of mNGS to identify the primary causative organism of such pulmonary nodules, especially for patients who do not display typical clinical features. Further studies are required to more precisely determine the prevalence and range of clinical features caused by *S. intermedius* infection.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

MH and SL collected the data, drafted, edited the manuscript, and contributed equally to this study. XW, DX, and LT revised the manuscript. ZC supervised this study. All authors critically reviewed, revised, approved the final manuscript, and agreed to be responsible for all aspects of this study.

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Conflict 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.

Publisher's note

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EDITED BY
Kazumichi Fujioka,
Kobe University, Japan

REVIEWED BY
Fernando Almeida-Silva,
Oswaldo Cruz Foundation (Fiocruz), Brazil
Retno Wahyuningsih,
Universitas Kristen Indonesia, Indonesia
Gianpaolo Tessari,
Integrated University Hospital Verona, Italy

*CORRESPONDENCE
Brian Chang
brianchang@mednet.ucla.edu
Karin Nielsen-Saines
knielsen@mednet.ucla.edu

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Case report: Disseminated histoplasmosis in a renal transplant recipient from a non-endemic region

Brian Chang[1]*, Tawny Saleh[2], Cameron Wales[3], Lawrence Kuklinski[3], Prerana Malla[1], Shangxin Yang[3], David Fuller[4] and Karin Nielsen-Saines[2]*

[1]Department of Pediatrics, UCLA Mattel Children's Hospital, Los Angeles, CA, United States, [2]Department of Pediatrics, Pediatric Infectious Disease Division, UCLA Mattel Children's Hospital, Los Angeles, CA, United States, [3]UCLA Pathology and Laboratory Medicine, Ronald Reagan UCLA Medical Center, Los Angeles, CA, United States, [4]Department of Medicine, Infectious Diseases Division, Ronald Reagan UCLA Medical Center, Los Angeles, CA, United States

Histoplasmosis is the most common endemic fungal infection in the USA. The majority of cases are asymptomatic and have clear exposure to endemic regions. In contrast, we present an adolescent immunocompromised patient with systemic and relatively non-specific symptoms including abdominal pain, weight loss, lower extremity edema, and scabbing skin lesions, without known exposure to endemic areas for histoplasmosis. Histologic analysis of gastrointestinal and skin biopsies eventually revealed a diagnosis of disseminated histoplasmosis; the patient was successfully treated with amphotericin B followed by itraconazole maintenance therapy. Ultimately, a high bar of suspicion for fungal disease must be maintained in immunosuppressed individuals even without apparent exposure history to endemic areas. This case report serves as a valuable reference for practitioners evaluating differential diagnosis of infections in immunocompromised patients.

KEYWORDS
disseminated histoplasmosis, pediatric, renal transplant, immunosuppression, pathology

Introduction

Histoplasmosis is a relatively widespread fungal disease and is often asymptomatic in immunocompetent individuals. However, it can present systemically in the immunocompromised, with a single center retrospective study estimating that of pediatric patients with histoplasmosis in an endemic area, roughly half of immunocompromised patients had disseminated disease affecting the pulmonary, gastrointestinal, integumentary, and nervous systems (1). Organ transplants in particular are a risk for histoplasmosis wherein the first year following transplant is the period of highest risk for histoplasmosis, and given the relative prevalence of kidney transplants, there are multiple case reports of histoplasmosis in renal transplant patients (2, 3).

The differential for histoplasmosis is broad given the large variety of relatively non-specific symptomatology and encompasses a range of infectious, autoimmune, and

hematologic/oncologic etiologies as well as system-specific diagnoses (4). As such, a comprehensive evaluation is necessary involving laboratory testing, advanced imaging, and biopsies of affected areas (5). While case reports exist of disseminated histoplasmosis in immunosuppressed patients, to our knowledge none exist in the absence of high-risk exposures (6). We thus present an exceptional case of disseminated histoplasmosis in a renal transplant patient without known exposure to endemic areas highlighting the utility of molecular diagnostics in unclear clinical scenarios.

Patient information

A 16-year-old male with a history of Bardet-Biedl syndrome and live donor kidney transplant in 2013 for end-stage renal disease due to dysplastic kidneys presented with abdominal pain and diarrhea. For the last one and a half months, he endorsed intermittent generalized abdominal pain often associated with bowel movements, which alternated between constipation and diarrhea. In the days leading to hospitalization, stools became consistently loose and watery. The patient also noted a painless, erythematous papule on his upper abdomen for the past 4 months which had become ulcerated without discharge. His mother reported recent fatigue, anorexia, and approximately ten pounds of weight loss over the past few weeks. He denied fevers, chills, nausea, vomiting, or hematochezia.

Historically, the patient was born in Egypt, lived primarily in Qatar until 2017, then emigrated to California. His renal transplant was performed in Egypt. Travel history included brief travel of up to 2 weeks to New York City, the United Kingdom, and Germany in 2015. Of note, the patient had been receiving treatment for chronic antibody-mediated rejection of his transplant with monthly tocilizumab and IVIG for the previous 6 months. In the week before admission, he had a renal biopsy consistent with worsening rejection. His immunosuppressive regimen was further expanded to include tacrolimus, mycophenolate, and prednisone.

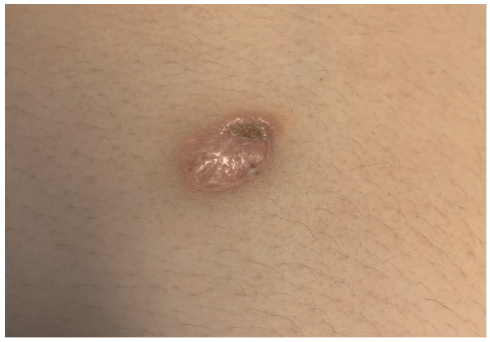
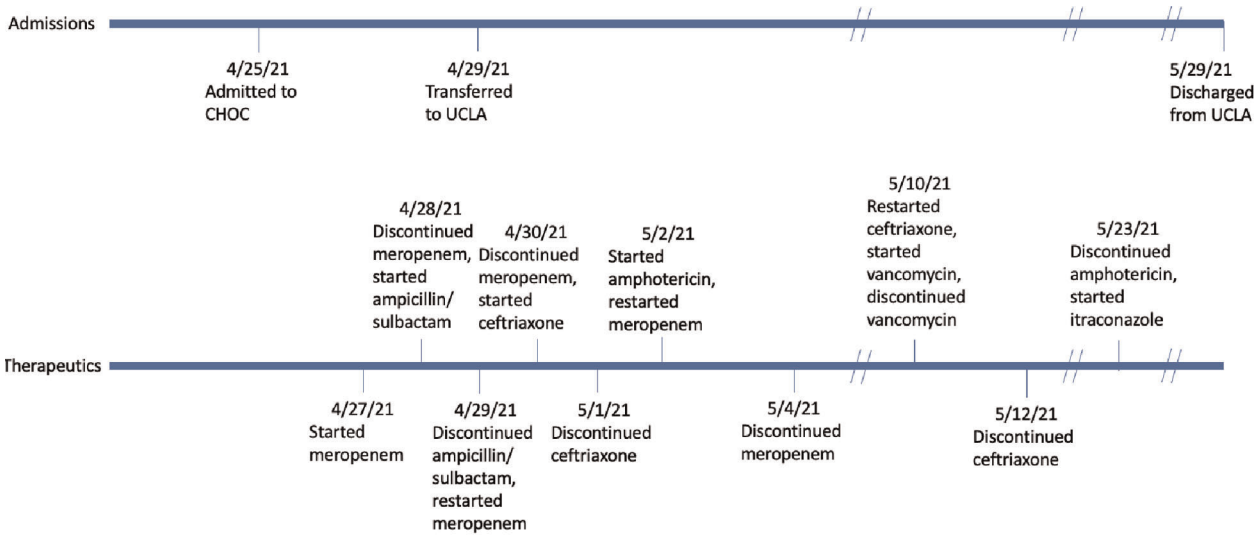


FIGURE 1
Skin lesion on upper abdomen at the time of presentation.

Clinical findings

On presentation, the patient was afebrile with normal vital signs. Physical exam was significant for abdominal distension, and edema noted throughout the right upper extremity and lower extremities bilaterally. Skin lesion was an approximately two- by three-centimeter ovoid plaque with accentuated border and erosion/crusting at the superior aspect (**Figure 1A**).

Timeline



Diagnostic assessment

Labs were significant for WBC 3.26×10^3/μl with 70% neutrophils, 14.4% lymphocytes, and 0.9% eosinophils, platelet count 107×10^3/μl, creatinine 1.31 mg/dl (eGFR 47.7 ml/min/1.73 m^2), CRP 2.1 mg/dl, CMV DNA PCR was positive but below the 137 copies/ml cutoff, and urinalysis demonstrated 14 WBC/HPF but negative nitrites and leukocyte esterase. Comprehensive stool studies to evaluate for infectious etiologies of diarrhea were unrevealing. Chest x-ray showed patchy retrocardiac opacities, central opacities, opacity along the left lateral thorax, appearance of a widened mediastinum/cardiac silhouette, and soft tissue edema along the right inferior abdominal wall. An echocardiogram demonstrated small posterior and inferior pericardial effusions but no significant regurgitation. An esophagogastroduodenoscopy and colonoscopy demonstrated an erythematous duodenal bulb with villous blunting, erythematous terminal ileum, small ulcerations throughout the colon, and a rectal mass. Biopsies were collected throughout the gastrointestinal (GI) tract and sent for pathology (**Figure 2**). After the procedure, the patient became febrile to 38.3 °C and developed increased work of breathing concerning for an aspiration pneumonia.

The patient subsequently developed abdominal wall swelling affecting his right lower quadrant, and a CT abdomen and pelvis imaging study with contrast revealed an edematous right colon, bibasilar pulmonary infiltrates, mediastinal and mesenteric lymphadenopathy, moderate hydronephrosis of transplanted kidney with perinephric urinoma, hepatosplenomegaly, and extensive edema affecting the adjacent retroperitoneum, mesentery, and abdominal wall. Preliminary GI pathology results were concerning for visceral leishmaniasis but Karius (Karius Inc, Redwood City, CA) molecular testing on blood detected *Histoplasma*. Serum and urine *Histoplasma* antigen testing (MiraVista Diagnostics, Indianapolis, IN) returned positive at 2.34 ng/ml and 5.50 ng/ml, respectively. While fungal tissue culture from his biopsies ultimately returned negative, which could be considered a potential limitation, GI pathology was subsequently reviewed by the Parasitology Branch of the Centers for Disease Control and Prevention who determined the histological appearance was consistent with *Histoplasma* rather than *Leishmania*. Additionally, biopsy of the abdominal skin lesion demonstrated yeast consistent with Histoplasma (**Figure 3**). Histology revealed epidermal ulceration with underlying granulomatous inflammation with associated necrosis, as well as multiple intra- and extracellular yeast, measuring approximately 2–4 microns with narrow based budding. These findings were confirmed on GMS and PAS special stains. Cerebrospinal fluid (CSF) antigen testing was conducted given headaches and was initially weakly positive. However, repeat testing of CSF IgG and IgM were undetectable twice and CNS histoplasmosis was thought to be unlikely.

Ultimately a diagnosis of disseminated histoplasmosis with gastrointestinal, cutaneous, and probable pulmonary involvement was made. Diagnostic work-up for additional pathogens as part of the differential included tuberculosis, coccidioidomycosis, and *Leishmania* and were all negative. Challenges to the diagnosis included a lack of historical exposure to areas endemic for histoplasmosis and extended duration between symptom onset following transplantation. Additionally, diagnosis was confounded by the need for multiple sampling of tissue specimens to evaluate for pathogenic organisms and disseminated organ loci. Finally, diagnosis was delayed as cultures and specialized antigen/antibody testing required extended turnaround time and advanced processing facilities.

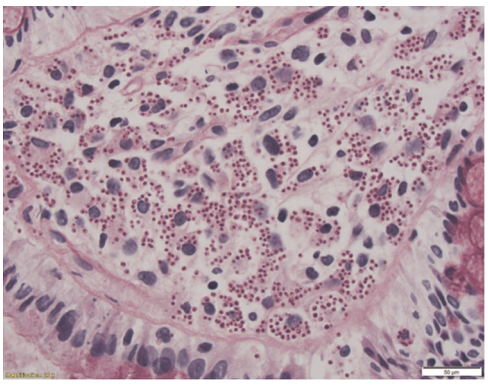


FIGURE 2
PAS-stained rectal biopsy showing abundant histiocytes within the lamina propria laden with PAS positive organisms.

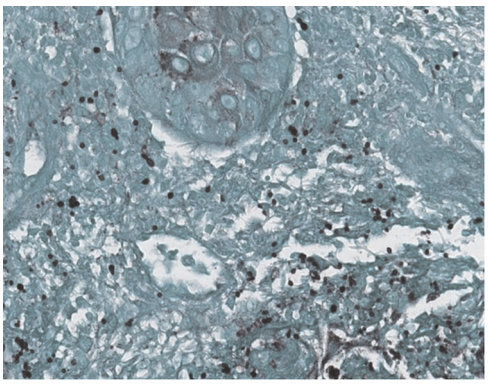


FIGURE 3
GMS-stained skin biopsy demonstrating numerous budding yeast within the dermis.

Therapeutic intervention

Given fever after GI biopsies, the patient was started on meropenem (unknown dosage) empirically on 4/27, which was eventually transitioned to ampicillin/sulbactam (unknown dosage) on 4/28 after serum bacterial cultures did not demonstrate any growth. On transfer to UCLA on 4/29, ampicillin/sulbactam was discontinued and meropenem 1 g every eight hours was initiated and administered from 4/29 to 4/30 and 5/2-5/4 given continued clinical instability. During the course of empiric treatment and prior to diagnosis of histoplasmosis, he also received intermittent ceftriaxone 1 g daily (4/30-5/1, 5/10-5/12) and one dose of vancomycin 750 mg on 5/10. The patient was eventually started on liposomal amphotericin B 4 mg/kg daily, given findings consistent with disseminated histoplasmosis. Twenty-two days of IV liposomal amphotericin B were completed prior to transitioning to oral itraconazole therapy 200 mg twice daily. Itraconazole was increased to 300 mg PO twice daily two months later due to low trough levels; the patient was maintained therapeutic itraconazole levels on this dose thereafter.

Follow-up and outcomes

Per IDSA guidelines for histoplasmosis treatment monitoring, changes in blood and/or urine antigen levels can be used as indicators for response to therapy. Antigen testing has a sensitivity of 100% in urine and 92.3% in serum with a specificity of 99%. Antigen levels should be measured before treatment is initiated, at two weeks, at one month, every three months during therapy, and for at least six months after treatment is stopped. These markers should also be measured subsequently if treatment failure or relapse is suspected.

Outpatient monitoring by pediatric infectious disease and nephrology teams revealed undetectable serum antigen after four weeks of treatment and detectable urine antigen at lower levels compared to hospitalization. Concurrently, serum itraconazole trough levels and hepatotoxicity were evaluated at regular intervals and adjusted as needed. The regimen was well tolerated with good patient adherence and no adverse effects. He will maintain lifelong treatment with an antifungal agent given his immunosuppressed state and will be periodically monitored for *Histoplasma* disease relapse.

Discussion

Human histoplasmosis is caused by two varieties of dimorphic fungi: *H capsulatum* var. *capsulatum* and *H capsulatum* var. *duboisii*. In the US, being in an urban metropolitan area like New York City as was the case in our patient is not commonly associated with increased risk of histoplasmosis, which is generally found in the Ohio and Mississippi River Valleys. However, with the use of new immunosuppressive medications and other immunosuppressive conditions, increased spread of histoplasmosis has been reported in states including Texas, New York, Colorado, and California (7). Indeed, serum testing suggests that histoplasmosis is more prevalent worldwide than we would suspect, though its clinical significance is unclear (8). Egypt, where our patient once lived, is not generally recognized as an endemic area for histoplasmosis, with further research needed to address this question. Due to this uncertainty, a potential source of exposure in Egypt cannot be completely ruled out.

Most patients afflicted with histoplasmosis remain asymptomatic throughout the disease course, especially if immunocompetent and otherwise healthy. For immunocompromised patients and pediatric patients, histoplasmosis may present with more acute and severe disease: a study by Garcia-Boyano et al. found that children with HIV diagnosed with disseminated histoplasmosis required prolonged hospitalization (9). If symptomatic, histoplasmosis in pediatric populations predominantly manifests as acute pulmonary histoplasmosis. However, hematogenous spread can result in disseminated disease, and other isolated single-organ infections have been rarely reported including meningeal involvement (4). Signs and symptoms of pulmonary involvement include fever, chest pain, and cough and may include anorexia, weight loss, lymphadenopathy, hepatosplenomegaly, and skin findings. Given these relatively non-specific findings, the differential for histoplasmosis is broad and includes other infectious causes (leishmaniasis, blastomycosis, atypical pneumonias, and tuberculosis), inflammatory (sarcoidosis), and oncologic causes. Indeed, these conditions—especially tuberculosis—can be concurrent in certain endemic areas (10).

Depending on the initial presentation, preliminary laboratory testing may include CBC, CMP, ESR, CRP, procalcitonin, LDH, ferritin, galactomannan, (1-3)-beta-D-glucan, tuberculosis testing, and urine, serum, and CSF testing. Careful radiologic examination should be conducted as appropriate to identify systemic involvement. Of note, common radiologic findings in CNS histoplasmosis include focal mass lesions, diffuse white matter changes, and areas of restricted diffusion (11). Definitive diagnosis includes antigen testing and histologic analysis of tissue cultures, especially blood, liver, skin lesions, CSF, urine, or any other potential sites of involvement (5). In terms of histopathology in histoplasmosis, numerous plasma cells, histiocytes and lymphocytes may be seen with budding yeast forms on H&E staining. Giemsa stain may reveal phagocytic cells containing oval organisms approximately 3–4 microns in diameter with a cap surrounded by a small light halo (12). Periodic Acid Schiff stain demonstrates a similar halo pattern with red-violet

coloration of the yeast. In our patient's specific case, leishmaniasis was suspected because of our patient's early travel to Qatar and the similarity in histopathologic features to histoplasmosis, including its small size and intracellular location. However, *Leishmania* can be differentiated from *Histoplasma* by the presence of a dense collection of DNA in the mitochondria known as kinetoplasts (13). More generally, the morphologic and serologic findings seen in this case can potentially be seen in infections by other types of dimorphic fungi, and so definitive diagnosis should be made in conjunction with culture and morphologic, biochemical, mass spectrometry, or nucleic acid studies.

For disseminated histoplasmosis in an immunocompetent host, treatment should include amphotericin B for 2–4 weeks followed by itraconazole for a total of 3 months. In immunosuppressed hosts, long term suppressive therapy may be merited if immunosuppression cannot be discontinued. For CNS involvement, the Infectious Disease Society of America recommends liposomal amphotericin B for 4–6 weeks followed by itraconazole for at least one year or until resolution of CNS symptoms (14). In patients who survive the first month after diagnosis, treatment with an amphotericin formulation followed by an azole for 12 months is usually successful, with only a rare relapse.

We thus present a case of disseminated histoplasmosis in a kidney transplant patient. While there have been previous reports of this in the literature, our case is unique in our patient's underlying genetic condition, travel history, and diagnosis with no known clear exposure to *Histoplasma* endemic areas (2, 15). With the use of novel diagnostic techniques including Karius, serum metagenomics, and next-generation sequencing, we were eventually able to identify histoplasmosis, a feat that would have been difficult with cultures alone (16). A high index of suspicion for fungal infection should thus be maintained in cases of undifferentiated symptoms in immunosuppressed patients, and workup should be comprehensive including laboratory microbiology studies, radiology, and pathology.

Patient perspective

Patient perspective was unable to be elicited as patient has an intellectual delay.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

BC, TS, and DF performed the background research and wrote the paper. PM contributed data and edited the paper. CW, KK, and SY contributed to histologic analysis of samples. DF and KNS conceived the paper. All authors contributed to the article and approved the submitted version.

Conflict 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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EDITED BY
Kazumichi Fujioka,
Kobe University, Japan

REVIEWED BY
Gabriela Maron,
St. Jude Children's Research Hospital, United States
Miriam Fulová,
Comenius University, Slovakia

*CORRESPONDENCE
Katherine Rodriguez
K6rodriguez@health.ucsd.edu

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Plasma cell free next-generation sequencing detects an unusual pneumonia pathogen in an immunocompetent adolescent with acute respiratory distress syndrome

Katherine M. Rodriguez[1,2]*, Nanda Ramchandar[3,4] and Nicole G. Coufal[1,2]

[1]Division of Pediatric Critical Care, Department of Pediatrics, University of California, San Diego, CA, United States, [2]Division of Pediatric Critical Care, Department of Pediatrics, Rady Children's Hospital San Diego, San Diego, CA, United States, [3]Division of Infectious Disease, Department of Pediatrics, Navy Medical Center, San Diego, CA, United States, [4]Division of Infectious Disease, Department of Pediatrics San Diego, Rady Children's Hospital San Diego, San Diego, CA, United States

This case details a rapid diagnosis of legionella pneumonia causing severe acute respiratory distress syndrome (ARDS) in an otherwise healthy adolescent through plasma microbial cell-free DNA next generation sequencing (mcfDNA-NGS). Diagnosis by mcfDNA-NGS of this unexpected pathogen led to narrowing of antimicrobials and the addition of glucocorticoids as adjunctive therapy for ARDS.

KEYWORDS

plasma microbial cell-free DNA next generation sequencing, pneumonia, acute respiratory distress syndrome, pediatric critical care, legionella

Introduction

Community acquired pneumonia (CAP) is a leading cause of hospitalization in children. Pediatric pneumonia is predominantly attributable to viral causes such as influenza and respiratory syncytial virus. Bacterial causes of pneumonia are primarily due to *Streptococcus pneumonia*, group A Streptococcus, or *Staphylococcus aureus*. The most common atypical cause of pneumonia is *Mycoplasma pneumonia* (1). Isolating bacterial pathogens in cases of pneumonia is historically challenging with only 17%–21% of blood cultures identifying a bacterial pathogen (2, 3). Sensitivity of blood culture is greatly affected by antibiotic pretreatment. Cultures from pleural fluid tend to have a higher diagnostic yield but invasive procedures, such as thoracentesis and bronchoalveolar lavage, are less commonly performed in pediatrics. This case details the detection of an unusual pathogen *via* plasma microbial cell-free DNA next generation sequencing (mcfDNA-NGS) in an immunocompetent patient with severe acute respiratory distress syndrome (ARDS).

Abbreviation
mcfDNA, NGS detects unusual pathogen in adolescent with ARDS.

Patient presentation

A previously healthy 14-year-old female presented to the Emergency Department with cough, shortness of breath, and fever to 38.8°C. The patient reported 12 days of cough and rhinorrhea with symptomatic worsening five days prior to presentation, including: fatigue, nausea, vomiting, diarrhea, abdominal pain, and headache. The patient was fully vaccinated (including against SARS-CoV-2), did not have known exposure to sick contacts, and denied any recent travel from her home in Southern California. She had no underlying immunodeficiency nor was she receiving immunosuppressive therapy. She denied exposure to unpasteurized dairy, water parks, freshwater swimming, or ocean swimming. She endorsed occasional remote hot tub use, with no use in the 2 weeks prior to presentation. The patient was admitted and initiated on supplemental high flow oxygen due to hypoxia and increased work of breathing. Initial laboratory studies were significant for a C-reactive protein of 31.4 mg/dl (ref 0–0.99 mg/dl), a procalcitonin of 29.57 ng/ml (ref <0.5 ng/ml), leukocytosis with neutrophilic predominance (WBC 17.3, 90% neutrophils, 7% bands), and a respiratory multiplex PCR panel (ePLEX, Genmark Diagnostics, Carlsbad, CA) from the nasopharynx positive for human rhinovirus/enterovirus. A chest radiograph (CXR) on admission was notable for bilateral opacities consistent with multifocal pneumonia (**Figure 1**). She was initiated on ceftriaxone for empiric coverage of CAP. On hospital day (HD) 2, due to persistent high fever (40°C) and worsening respiratory status, clindamycin was added for coverage of methicillin resistant staph aureus (MRSA) and anaerobic pathogens. Local MRSA isolates demonstrate 84% sensitivity to clindamycin.

On HD 3, the patient exhibited worsening respiratory failure necessitating transfer to the Pediatric Intensive Care Unit and bilevel positive airway pressure (BIPAP). She had worsening pleural effusions on CXR and clinical signs of fluid overload thus what started on intermittent intravenous furosemide. Ultimately, her hypoxia and work of breathing necessitated intubation on HD4. At that time, she met clinical criteria for severe ARDS with an initial oxygenation index (OI) of 30 and a maximum OI of 39, a PaO2/FiO2 ratio of 90, with a maximum positive end-expiratory pressure (PEEP) of 24. Adjuncts such as inhaled nitric oxide and prone positioning were required for refractory hypoxemia. Bronchoalveolar lavage was not performed for diagnostic purposes due hypoxia and the severity of her ARDS. She was hemodynamically stable during this period with intermittent use of low dose epinephrine infusion to support blood pressure and diuresis. On HD 3 levofloxacin was added to the antimicrobial regimen of ceftriaxone and clindamycin to cover for atypical organisms.

On HD 3 plasma mcfDNA-NGS (Karius Inc., Redwood City, CA) was sent, this is common practice at our institution for critically ill patients where infectious cause is suspected but the pathogen has not been identified by standard testing. Plasma mcfDNA-NGS resulted positive after 46 h on HD 5 for *Legionella pneumophilia*. The organism met the sequencing coverage threshold for reporting, but not for

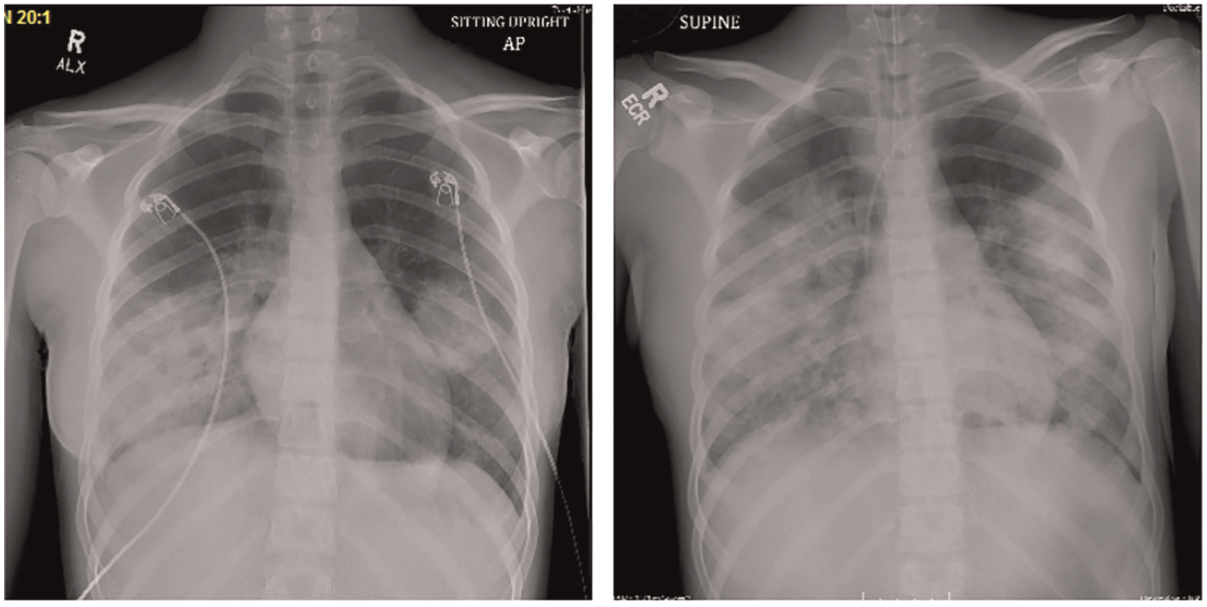

FIGURE 1
Chest radiograph on HD 1 (left). Chest radiograph on HD 4, post-intubation (right).

quantification and thus molecules per microliter was not given. Once the pathogen was identified, antimicrobials were narrowed to levofloxacin alone, the treatment of choice for severe legionella infection (4). The patient was also initiated on high dose glucocorticoids as there is some evidence of benefit in severe ARDS secondary to legionella (4). Pathogen identification was later confirmed *via* an atypical pneumonia PCR panel (Quest Diagnostics, San Juan Capistrano, CA) sent from an endotracheal tube aspirate after intubation and a urine legionella antigen test (Quest Diagnostics, San Juan Capistrano, CA). These tests were sent on HD 4 and resulted after 66 and 73 h respectively on HD7 after mcfDNA-NGS results were known. Legionella was not detected *via* standard blood culture or bacterial respiratory smear/culture.

Given the unusual Legionella diagnosis, an immunology consultation was obtained, identifying a slightly low immunoglobulin G of 647 mg/dl (ref 749–1,640 mg/dl) and lymphopenia with absolute lymphocyte count of 957 cells/ul (ref 1200–5200 cells/ul), both determined to be most likely attributable to the acute infection. An HIV antigen/antibody 4th generation test was non-reactive, and immunoglobulin A and immunoglobulin M antibody levels were normal. After five days of mechanical ventilation the patient was successfully extubated. She was treated for a total 14-day course of levofloxacin and was weaned off all respiratory support prior to discharge. An investigation by local public health authorities failed to identify a source for her legionella infection.

Discussion

Pneumonia is common in the pediatric population, though rarely caused by Legionella. In the pediatric population legionella pneumonia can occur in infants or patients with immunosuppression and is often nosocomially acquired (5). The patient described had a severe and unusual course for a healthy child without any specific exposures, although hot tub usage is a known risk in individuals over age 45 or heavy smokers (6). Additionally, this case was reported to the county health department and an associated cluster of legionella infection was not identified. Empiric parenteral therapy commonly prescribed for CAP such as ampicillin or ceftriaxone are inactive against this intracellular pathogen. Macrolides, such as azithromycin, can be effective in the treatment of mild to moderate disease. Fluoroquinolones such as levofloxacin provide the best intrinsic activity against Legionella and are recommended in severe illness (4). The low sensitivity of blood cultures and standard respiratory cultures for detecting Legionella may result in missed diagnoses, and in serious cases, may result in increased morbidity due to inadequate empiric therapy or overly broad empiric therapy.

Traditional methods to diagnose Legionella include lower respiratory tract culture, urinary antigen testing, and quantitative PCR testing of serum, urine, or respiratory samples. Lower respiratory tract culture is considered the gold standard but has a limited and variable sensitivity of 10%–80% (4, 7) and can take up to 7 days to result. Urinary antigen detection has a sensitivity of 56%–99% (7) but is most useful in detecting the Lp1 subtype and has decreased sensitivity with other subtypes. PCR sensitivity ranges from 33%–70% depending on sample source and is highest in lower respiratory tract samples. All methods have high specificity of 98%–100% (4). There are 3 cases of legionella diagnosed by metagenomic NGS (mNGS) described in the literature including one immunosuppressed child (8) and two adults (9, 10). In the case described by Wang et al. (8) evidence of legionella infection was also found in the bronchial alveolar lavage (BAL) fluid which was positive by gram stain, mNGS, and culture. Legionella was confirmed by Yi et al. (9) with positive mNGS and PCR testing from sputum and pleural fluid. Yue et al. (10) also detected legionella by mNGS of BAL fluid and later detected anti-legionella IgM antibodies. Blood cultures were negative in all three cases.

In case series where plasma mcfDNA-NGS has been applied in complicated CAP, the addition of mcfDNA-NGS to standard of care diagnostics improved bacterial diagnosis from 27% by conventional culture methods to 86% by mcfDNA-NGS (11). Identifying a causative organism allows antibiotic selection to be narrowed limiting the patient exposure to unnecessarily broad antibiotics. Importantly, the rapid pathogen identification allowed the clinical team to make decisions about initiation of adjunctive treatment with steroids, which are currently not routinely recommended in the pediatric ARDS treatment guidelines (12). The turn-around time for the mcfDNA-NGS test was less than 48-h and allowed the clinical team to rapidly optimize therapy.

Legionella infection, while not uncommon in adults, is quite rare in children. While currently not the standard of care, the application of plasma mcfDNA-NGS in critically ill children with pneumonia can aid in pathogen identification, especially where disease severity or clinical complications precludes other well established but invasive diagnostic modalities. It was through the quick and effective application of plasma mcfDNA-NGS that this pathogen was detected. Early detection allowed for narrowing of antibiotics and facilitated management of severe ARDS leading to a favorable outcome.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Author contributions

The draft manuscript was prepared by KR The manuscript was reviewed and edited by NR and NGC. All authors contributed to the article and approved the submitted version.

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EDITED BY
Kazumichi Fujioka,
Kobe University, Japan

REVIEWED BY
Oliver Schildgen,
Kliniken der Stadt Köln, Germany
Maria Söderlund-Venermo,
University of Helsinki, Finland

*CORRESPONDENCE
Na Zang
zangna1214@126.com

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Respiratory tract infection of fatal severe human bocavirus 1 in a 13-month-old child: A case report and literature review

Jing Liao, Zhongying Yang, Yu He, Jianhua Wei, Luo Ren, Enmei Liu and Na Zang*

Department of Respiratory Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China

Human bocavirus 1 (HBoV1) belongs to the family Parvoviridae and it is acknowledged that HBoV1 is a respiratory pathogen. We report the case of a 13-month-old boy who presented with a cough, shortness of breath, and wheezing, and who eventually died of severe pneumonia and acute respiratory distress syndrome (ARDS). Metagenomics next-generation sequencing (mNGS) showed that HBoV1 was the only detected pathogen. The nasopharyngeal aspirate viral load was 2.08×10^{10} copies/ml and the serum viral load was 2.37×10^{5} copies/ml. The child was still oxygen deficient under mechanical ventilation. Chest imaging suggested diffuse lesions in both lungs, an injury caused by ARDS. In this case, the clinical symptoms and signs of the child, the high viral load, viremia, and the detection of mNGS in the tracheal aspirate all supported that HBoV1 could cause severe acute respiratory tract infection in children without other pathogen infections.

KEYWORDS

human bocavirus 1, severe infection, children, respiratory, *STAT1* variant

Introduction

Human bocavirus 1 (HBoV1) was first discovered in respiratory secretions by Allander et al. in 2005 (1); later, three other bocaviruses (HBoV2, 3, and 4) were successively found in fecal samples (2). HBoV1, which belongs to the family of Parvoviridae and the genus of *Bocaparvovirus*, comprises a non-enveloped capsid with a single-stranded linear DNA and is the second known human parvovirus that can replicate autonomously after parvovirus B19 (3, 4). The clinical manifestations of HBoV1 infection are often atypical and similar to other respiratory virus infections, such as rhinitis, acute otitis media, pneumonia, bronchiolitis, and asthma exacerbation (5). The symptoms of HBoV1 infection are mild and self-limited and are easy to be ignored by clinicians. HBoV1 is often combined with other respiratory viruses, and the co-infection rate is as high as 75% (6, 7). The pathogenicity of HBoV1 was earlier considered controversial because HBoV1 DNA can persist for months in airway

Abbreviations
HBoV1: human bocavirus 1; ARDS, acute respiratory distress syndrome; NPA, nasopharyngeal aspirate; mNGS, metagenomics next-generation sequencing; CT, computed tomography; PCR, polymerase chain reaction.

secretions following primary infection and can also be shed in the nasopharyngeal aspirates (NPAs) of asymptomatic healthy children. However, increasing evidence supports that HBoV1 is associated with respiratory symptoms without other pathogens detected and can even cause severe lower respiratory tract infections (8). A case of severe pneumonia, acute respiratory distress syndrome (ARDS), and respiratory failure brought on by HBoV1 infection was reported in our study, along with a review of the literature.

Clinical data

A 13-month-old boy, the second child of consanguineous parents, was admitted to the intensive care unit (ICU) of the Children's Hospital of Chongqing Medical University in October 2018. He presented with a paroxysmal cough for 1 month, aggravated with shortness of breath and wheezing for 1 week. Regarding the patient's family history, there was nothing unusual in the immune system. The child was delivered naturally at full term and has received his BCG vaccination. There was no history of feeding difficulties or repeated respiratory tract infections. He had not been exposed to toxic substances, gases, smog, or allergens.

A chest CT scan at a local hospital revealed a bilateral lung infection. The treatment of cefminox and interferon-α1b was not effective. The cyanosis was difficult to relieve. The patient was then admitted to our hospital with endotracheal intubation for further treatment. At admission, the boy had malnutrition with a normal body temperature and oxygen saturation of 94% under assisted ventilation (other vital signs were as follows: heart rate of 147 beats/min, the respiration rate of 30 beats/min, bodyweight of 5.5 kg, blood pressure of 77/50 mmHg). Nasal flaring, cyanosis around the lips, and an inspiratory triple concave sign were observed. Chest auscultation revealed bilateral coarse crackles.

Chest radiographs showed decreased translucency in both lungs and an extensively increased density shadow in the alveoli, showing ground-glass changes. Some showed a mesh change, a slightly dilated bronchus, and localized hyperinflation of the upper lobe of the left lung. Lung injury caused by a diffuse interstitial lesion of both lungs and ARDS were considered (**Figure 1**). The blood routine on admission showed that the white blood cell counts were elevated (18.91×10^9/L) (reference value: $4.3–11.3 \times 10^9$/L), neutrophils mainly, and a thrombocyte count of 490×10^9/L (reference value: $100–453 \times 10^9$/L), a hemoglobin concentration of 121 g/L (reference value: $118–156 \times 10^9$ g/L), and a slightly elevated level of C-reactive protein (CRP) (9 mg/L) (reference value: <8 mg/L); other markers were within the reference ranges. After treatment with piperacillin-tazobactam for 4 days and meropenem for 6 days, the inflammatory indicators gradually decreased. However, on the 10th day of hospitalization, the

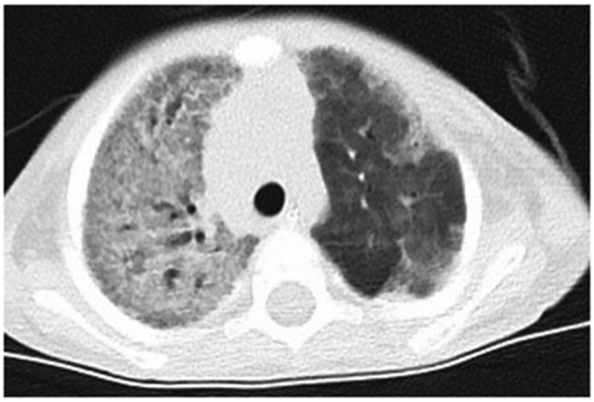

FIGURE 1
The chest-CT on the 14th day after hospitalization showed both lungs decreased translucency, extensive parenchymal opacities, and ground glass change. Some showed grid change and slightly dilated bronchus throughout the course.

white cell count and the neutrophil percentage increased again, and the level of CRP increased to 20 mg/L. The platelet count increased with the continuous decrease of red blood cells and hemoglobin (81–121 g/L), and the child had no bleeding tendencies. Amphotericin B, meropenem, and cefoperazone-sulbactam were administered as treatment, but the patient's condition did not improve. The level of CRP had no obvious change (18–20 mg/L), the white blood cell count continued to rise ($12.25–18.45 \times 10^9$/L), and the level of hemoglobin decreased to 77 g/L. Arterial blood gas analysis indicated type 2 respiratory failure ($PO_2$53 mmHg, $PCO_2$62 mmHg). After admission, the assisted ventilation was continued, and the patient still had persistent oxygenation disorders. The child had hypoproteinemia, increased lactate dehydrogenase, and a decrease in cholinesterase. Immunoglobulin levels (including IgM, IgG, and IgA) were in the normal range. The C3 complement was 0.43 g/L. Tumor markers were negative. Flow cytometry was used for the detection of T cell, B cell, NK cell and other lymphocyte subsets were determined, with no obvious abnormalities (CD4/CD8 2.37, CD3 + 1610.88/μl, CD3 + CD8 + 457.59/μl, CD3 + CD4 + 1084.62/μl, NK cell 53/μl). The TREC gene was detected by fluorescence probe PCR screening for severe combined immunodeficiency diseases. The result was >1,000 copies/μl (<10 copies/μl is considered to be severe combined immune deficiency, 10–1,000 copies/μl is considered to be a primary immune syndrome or normal, and >1,000 copies/μl is considered normal). The fungal D-glucan test, T-SPOT test, purified protein derivative test, *Mycoplasma pneumonia/Chlamydia* PCR, sputum bacterial and fungal culture, and double blood were negative. Pneumocystis was not detected in the next-generation sequencing (NGS) of bronchoalveolar lavage (BAL) fluid, and CT findings did not support pneumocystis Jiroveci pneumonia. All tests were

negative for influenza virus A and B, respiratory syncytial, adenovirus, parainfluenza 1, 2, and 3, coronaviruses (including HKU-1, OC43, 229E, NL63), rhino/enterovirus, parechovirus, Hanta pulmonary syndrome, and fungi. The results of metagenomics NGS (mNGS) in serum and respiratory secretions suggested that HBoV had a high detection sequence of 112,786 reads and 100% gene coverage. The coverage of *Acinetobacter baumannii*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* were 0.889%, 0.058%, and 0.005%, respectively, and the reads were 370, 14, and 2, which were considered colonized bacteria. The mNGS was carried out by a core facility (Kindstar Global, Wuhan, China). HBoV was the only viral pathogen detected. No other pathogens were detected. Quantitative PCR (qPCR) showed that the viral load of NPA was 2.08×10^{10} copies/ml and the viral load of serum was 2.37×10^{5} copies/ml. Viral DNA and RNA were extracted from 200-μl aliquots of the NPA samples by the QIAamp MinElute Virus Spin kit (Qiagen, Hilden, Germany). The RNA was applied as the template for complementary DNA (cDNA) synthesis with the SuperScript III First-Strand Synthesis System (Invitrogen, California, USA). DNA and RNA extractions and cDNA products were used for the subsequent testing of respiratory viruses (9). HBoV1-specific primers were forward primer amplification of 5′-CCTATATAAGCTGCTGCACTTCCTG-3′ and reverse primer 5′-AAGCCATAGTAGACTCACCACAAG-3′ (10, 11). The plasmid amplified target fragment was cloned into the pMD19-T vector (TaKaRa Biotechonology, Dalian, China). The PCR process was performed exactly as described in the report (10), except for the AmpErase-UNG at 50°C. Each run included plasmid and negative controls. Standard precautions were taken throughout the PCR process to avoid cross-contamination. Negative controls and serial dilutions of the positive controls were included in every PCR assay. Therefore, HBoV was considered to be the pathogen in this case. Finally, the boy was diagnosed with severe pneumonia, ARDS, and diffuse pulmonary interstitial disease.

Due to the high suspicion of the possibility of respiratory system-related genetic diseases, complete exon sequencing (completed by Beijing Mygeno) was completed during hospitalization. It indicated that the patient had a heterozygous variant in the *STAT1* gene: C. 1127 + 10G > A, causing amino acid change splicing. After 16 days of treatment in the ICU, the patient's pulmonary symptoms were not alleviated, and the chest CT scan showed that the diffuse lesions in both lungs were still severe. The patient's family was required to give up treatment, and the patient died.

Discussion

Since 2005, there has been increasing evidence supporting HBoV1 as an actual human pathogen that causes mild to severe respiratory infections. HBoV1 DNA is detectable in respiratory secretions in 2%–20% of children with acute respiratory tract infections (12). However, HBoV is not used to routinely detect respiratory viruses in clinical practice, which can easily lead to the neglect of clinicians and a missed diagnosis. However, increasing cases of severe and even fatal respiratory HBoV1 infections have been reported in recent years. In this study, the clinical characteristics, risk factors, methods of detection, and treatment of severe HBoV1 infection are discussed. Here, we present a case of severe HBoV1 infection and review the literature to provide a reference for its clinical diagnosis and treatment.

The major manifestations of severe HBoV1 infection were a respiratory failure or respiratory distress, fever, and wheezing. A total of 17 cases of HBoV1-related pediatric severe respiratory infection admitted to the ICU were collected from 10 pieces of literature (8, 13–21) (**Table 1**). Among these 17 children, fever and wheezing were the first symptoms in most cases. Specifically, the symptoms included respiratory failure or respiratory distress ($n = 12$), cough ($n = 2$), wheezing ($n = 5$), fever ($n = 4$), and diarrhea ($n = 1$). The diagnosis was respiratory failure ($n = 5$), ARDS ($n = 4$), bronchiolitis ($n = 3$), atelectasis ($n = 2$), and status asthma ($n = 4$), suggesting that severe HBoV infection often leads to respiratory failure and ARDS. Except for a 4-year-old child and two newborn patients, the children were aged between 6 months and 2 years. Chest imaging suggested interstitial infiltration and atelectasis. Specifically, it included atelectasis ($n = 4$), infiltration ($n = 7$), and hyperinflation ($n = 1$), which were consistent with our case. Our patient was 13 months old, and his first symptoms were a cough and fever, aggravated by shortness of breath, cyanosis, and hypoxemia. The chest CT scan suggested ground-glass changes, with diffuse lesions in both lungs. In the laboratory tests, with the increase in disease severity, the proportion of neutropenia and lymphocytopenia significantly increased. Procalcitonin and CRP obviously increased. In severe cases reported in the past, the levels of CRP and leukocytes were also slightly elevated. Among them, HBoV1 was the only pathogen detected. Jula et al. (12) reported that a tiny amount of HRV RNA was detected. However, the copy number of HBoV1 DNA was significant, so it was still highly correlated with HBoV1 infection.

The risk factors for severe HBoV1 infections include underlying chronic conditions, such as congenital heart disease, chronic lung disease, premature birth, cancer, and immune deficiency (5). Of the 17 patients, 8 were premature infants, 6 had underlying pulmonary disease, 1 had patent ductus arteriosus (PDA), 2 had a history of wheezing, and 1 was an immunodeficient child. In terms of treatment, all the children were given oxygen: 8 cases were given antibiotic therapy, corticosteroids were given in 7 cases, mechanical ventilation was given in 14 cases, and ECMO in 2 cases. Four children died, and the remaining 13 survived. In this case, we

TABLE 1 The reported severe respiratory tract infection cases admitted to ICU were caused by HBoV1.

Author	Country, year	Age	Male/female	Manifestation	Chest image	Sample	Method	Serology	Co-infection	Mechanical ventilation	Clinical diagnosis	Viremia	Viral loads in serum	Viral loads in NPA	Treatment	Outcome	Fundamental disease
Tabatabai et al. (11)	Germany, 2019	6 months	1/0	Respiratory failure	Bilateral opacities	Blood, tracheal secretions	PCR/serology	HBoV IgM/IgG	No	Yes	ARDS	Yes	2×10^3	3.1×10^9	Oxygen, antibiotics, NO	Death	Immunodeficient (NIN gene mutation, T-cell defect)
Moesker et al. (8)	The Netherlands, 2015	24 months	3/4	Respiratory failure, ECMO indication	–	NPA	PCR/NGS	–	No	5/7, 1/7ECMO	ARDS, LRTI, BHR/PSA, Severe atelectasis with ARTI	–	–	–	Oxygen, Supplemental	Survival	3Pulmonary disease, 1premature
Korner et al. (12)	Germany, 2011	8 months	0/1	Hypoxia, respiratory distress, wheezing, cough, and fever	Diffuse bilateral infiltrates and total atelectasis of the right upper lung lobe	Blood, NPA	PCR/serology	HBoV IgM/IgG	No	No	Severe obstructive bronchitis	Yes	5.8×10^3	–	Oxygen, antibiotics	Survival	None
Eskola et al. (13)	Finland, 2017	9 months	1/0	Fever, wheezing, dyspnea	Interstitial infiltrates atelectasis of the right upper and lower lobes	Blood, tracheal aspirate	PCR/serology	HBoV IgM/IgG	No	Yes	Respiratory failure	Yes	1.7×10^3	–	Oxygen, corticosteroids, antibiotics, NO	Survival	Bronchiolitis at 6 months
Ursic et al. (15)	Slovenia, 2011	20 months	0/1	Respiratory distress	Hyperinflation with an infiltrate in the left lower lung field	Blood, NPA, tracheal aspirate	PCR	–	No	Yes	Acute bronchiolitis, pneumomediastinum, interstitial emphysema, and acute respiratory failure	Yes	1.8×10^6	8.6×10^9	Oxygen, corticosteroids, antibiotics	Survival	Premature
Edner et al. (14)	Sweden, 2011	4 years	0/1	Fever, wheezing, dyspnea	Subcutaneous emphysema, pneumomediastinum, and left-sided pneumothorax	Blood, tracheal aspirate	PCR/serology	HBoV IgM	No	Yes and ECMO	ARDS	Yes	0.45×10^4	1×10^9	Oxygen, corticosteroids, antibiotics	Survival	Wheezing at 1 year premature
Ursic et al. (16)	Slovenia, 2015	18 months	N/S	Respiratory distress	Hyperinflation and infiltrates of the right perihilar area, pulmonary edema	Blood, NPA, tracheal aspirate	PCR	–	No	Yes	Acute bronchiolitis with hypoxemia, diffuse hyperinflation, bilateral infiltrates, and possibly pulmonary edema	Yes	7.42×10^6	8.27×10^6	Oxygen, corticosteroids, antibiotics, dopamine	Death	Chronic respiratory insufficiency, premature
Jula et al. (10)	Finland, 2013	16 months	1/0	Rhinorrhea, cough, respiratory distress, tachypnea	Bilateral infiltrations and atelectasis of the upper right lobe	Blood, NPA	PCR/serology	HBoV IgM/IgG	low load HRV higher load HBoV1 in NPA	Yes	ARDS	Yes	3×10^3	1.6×10^{10}	Oxygen, corticosteroids, antibiotics	Survival	Severe bronchopulmonary dysplasia, premature

(*continued*)

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TABLE 1 Continued

Author	Country, year	Age	Male/ female	Manifestation	Chest image	Sample	Method	Serology	Co-infection	Mechanical ventilation	Clinical diagnosis	Viremia	Viral loads in serum	Viral loads in NPA	Treatment	Outcome	Fundamental disease
Calvo et al. (17)	Spain, 2008	1 months	1/0	Respiratory distress	Atelectasis	NPA	PCR	–	No	Yes	Respiratory failure	–	–	–	Oxygen, corticosteroids	Survival	Premature
		1 months	0/1	Wheezing, respiratory distress	Infiltrations	NPA	PCR	–	No	Yes	Respiratory failure	–	–	–	Oxygen, corticosteroids, antibiotics	Death	PDA/ bronchopulmonary dysplasia, premature
Ziyade et al. (18)	Turkey, 2015	5 months	0/1	Fever, wheezing, diarrhea	–	NPA	PCR	–	No	Yes	Respiratory failure	–	–	–	Oxygen, Supplemental	Death	Premature

HBoV1, human bocavirus 1; ARDS, acute respiratory distress syndrome; NPA, nasopharyngeal aspirate; mNGS, metagenomics next-generation sequencing; CT, computed tomography; PCR, polymerase chain reaction; ECMO, extracorporeal membrane oxygenation; LRTI, lower respiratory tract infections; BHR/PSA, bronchial hyperresponsiveness/ paediatric status asthmaticus; NIN, ninein; HRV, human rhinovirus.

performed qPCR detection of HBoV1 in the serum and NPA. The NGS and other PCR results showed that HBoV1 was the only detected pathogen. The viral load in the NPA was high, accompanied by viremia, suggesting that HBoV1 was the most likely cause of severe respiratory tract infection in this case. It is reported that the single detection of HBoV1 was more prevalent among children with a high viral load than those with a low viral load in severe acute cases (11). Christensen et al. (6) showed that mono-detection, high viral load, and viremia are associated with respiratory tract infection. The samples include blood, NPA, or tracheal intubation aspirates of the seven cases reviewed here for qPCR detection, and viremia was indicated in all seven critically ill patients. Unlike other markers (14, 22), the detection of HBoV1 DNA in the blood is more closely associated with the symptoms of the present infection. Therefore, blood testing is one of the important diagnostic methods for the study of HBoV1 (3, 5). It includes both PCR and serodiagnoses.

The results of whole exon sequencing showed a heterozygous variant in the *STAT1* gene: c.1127 + 10G > A, causing amino acid change splicing. According to the public database ClinVar, the clinical significance of this variant was considered to be uncertain significance. The family history was negative. STAT proteins are key transcription factors that regulate cellular responses to interferon (IFNs), cytokines, growth factors, and hormones and are associated with diseases related to regulating immune responses. The protein plays a vital role in immune responses to viral, fungal, and mycobacterium pathogens. The invasion of macrophages is the most common mechanism for infectious agents and *STAT1* is probably fundamental for the activation of the corresponding intracellular killing programs (23). Genetic variants in *STAT1* can lead to four different phenotypes. There are three outcomes of reduced or absent *STAT1* function (loss of function [LOF]) and one outcome of gained *STAT1* function (GOF). *STAT1* plays a crucial role in the cellular response to IFNA/IFNB (type I interferon) and IFNG (type III interferon). Autosomal dominant (AD) LOF STAT1 selectively affects the IFNG pathway but does not affect the IFNA/IFNB pathway and mainly leads to susceptibility to mycobacterial infections and no susceptibility to viral infections. AD LOF STAT1 has low penetrance, a mild clinical phenotype, and a good prognosis (24). However, AD GOF STAT1 with susceptibility to candida has a highly variable prognosis (25). Two patients with a heterozygous variant of *STAT1* have been reported to have an increased susceptibility to adult-onset herpes simplex encephalitis (HSE) without a history of other significant infections (24, 26). Autosomal recessive (AR) complete LOF STAT1 affects the IFNA/IFNB and IFNG pathways, leading to susceptibility to mycobacteria, Salmonella, and viruses, often leading to a severe course of the disease and fatal results (24). Patients with AR partial LOF STAT1 present with clinical insufficiency, and the severity of

illness is variable (23). However, we have not collected samples from the parents of the child for genetic testing, so we cannot determine the source of the genetic variant. The clinical pathogenicity of this gene variant is uncertain.

In this case, we noted a continuous decline in hemoglobin and red blood cells, presenting as small cell hypochromic anemia. Still, the child had no signs of bleeding, considered to be iron-deficiency anemia or related to iron death. Jayaweera et al. (27) found a significant correlation between the risk of acute respiratory tract infections and iron deficiency anemia in children. Iron supplementation in blood played a critical protective role in recurrent acute respiratory tract infections and gastroenteritis in children. The child, in this case, had severe malnutrition, low oxygen-carrying capacity in pulmonary vessels and lung parenchyma, and low protective immunity against invading pathogens as well as a significant decrease in hemoglobin from day 10 after admission, suggesting a severe infection (28).

In this study, we used qPCR and mNGS for etiological detection, which further confirmed that HBoV1 was the only pathogen that could cause severe respiratory tract infection. Because the PCR method is often targeted at suspected pathogens, unknown or rare pathogenic microorganisms cannot be quickly identified, and certain limitations exist. The mNGS high-throughput sequencing of nucleic acids in clinical samples is then compared with the database analysis. The detection range is more extensive and suitable for diagnosing severe infections (29). The routine clinical use of mNGS is still under development. Assume that NGS is added to clinical and routine laboratory data. In that case, it may be combined with the PCR detection method for clinical diagnosis in the future, which has high clinical specificity and broad application prospects (30). Our study, using mNGS, provides further evidence that HBoV1 can cause severe acute respiratory tract infections in children without other viral and bacterial infections.

It is reported that at least two of the following five factors should be present for the diagnosis of an acute primary HBoV1 infection: high DNA load by qPCR ($>10^6$ HBoV1 DNA copies/ml of NPA); HBoV1 mRNA in NPA; positive IgM; low IgG avidity; or a fourfold increase or more of IgG titre in paired serum samples (5). One of the limitations of this study is that we did not include the serological analysis of HBoV1. Second, as this study was a retrospective study, blood samples of the patient's parents were not collected; therefore, gene sequencing and variant analysis could not be performed for verification.

Conclusion

In this case report, the clinical symptoms and signs of the child and the high viral load, viremia, and mNGS detection in the tracheal aspirate all supported that HBoV1 could cause a severe acute respiratory tract infection in children without other viral and bacterial infections. This case suggested that bocavirus can cause severe infection, especially in immunodeficiency conditions, and more vigilance is needed.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The study procedure was approved by the ethics committee of the Children's Hospital of Chongqing Medical University, Chongqing, China. Written informed consent was obtained from the parent or guardian of all participants.

Author contributions

JL analyzed and interpreted the data, and wrote the manuscript. ZY, YH, and W collected the clinical information and assisted in the analysis. NZ contributed to the design of the study and assisted in the analysis and interpretation of data, and revised the manuscript. LR assisted with the revision of the manuscript. EL contributed to the conception, collection of clinical information, and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict 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.

Publisher's note

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EDITED BY
Kazumichi Fujioka,
Kobe University, Japan

REVIEWED BY
Heidi Flori,
University of Michigan, United States
Wei Guo,
Tianjin University Children' Hospital, China

*CORRESPONDENCE
Yanping Yu
✉ yyp757605@163.com

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Severe pediatric Mycoplasma pneumonia as the cause of diffuse alveolar hemorrhage requiring veno-venous extracorporeal membrane oxygenation: A case report

Xinjuan Zhang and Yanping Yu*

Department of Pediatric Intensive Care Unit, Affiliated to Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, China

Background: Diffuse alveolar hemorrhage (DAH) is an acute life-threatening disease often associated with immunocompromised patients and systemic disorders, such as infections, vasculitis, and toxins. *Mycoplasma pneumoniae* is one of the most common causes of community-acquired pneumonia in children, which rarely causes respiratory failure and fulminant disease; However, a rapid progression may occur in some patients. Mycoplasma pneumonia-associated DAH is rare.
Case Presentation: We report a case of severe pediatric mycoplasma pneumonia in an immuno-competent child. This patient's condition progressed rapidly, with severe lung lesions associated with pleural effusion, coagulopathy, diffuse alveolar haemorrhage and severe respiratory distress requiring ventilator and intravenous extracorporeal membrane oxygenation (VV-ECMO) support. She was discharged upon successful treatment.
Conclusion: Diffuse alveolar hemorrhage associated with *Mycoplasma pneumoniae* in children is very rare, and clinicians should be aware of the potential rapid onset of the disease. Early detection and diagnosis are very important. The main treatment measures include anti-infection and supportive measures such as mechanical ventilation, but as in our case, success with both prone positioning for more than 10 h per day and VV-ECMO was life-saving.

KEYWORDS
mycoplasma pneumoniae, diffuse alveolar hemorrhage, pediatric, acute respiratory distress syndrome, veno-venous extracorporeal membrane oxygenation, mNGS

Introduction

Diffuse alveolar hemorrhage (DAH) is a life-threatening emergency, and hospital mortality rates are reported to be 20%–100% (1). Its etiologies include vasculitis, thrombocytopenia, autoimmune diseases, coagulopathy, drugs, and infections (2). *Mycoplasma pneumoniae* (MP) is one of the most common causative organisms of community-acquired pneumonia in children. It rarely causes life-threatening disease,

but some patients can develop a rapid progression of MP-associated disease. MP-associated DAH is very rare and has been reported to occur more frequently in immunocompromised patients (3). We report a previously healthy child who suffered from severe mycoplasma pneumonia, complicated with DAH and severe respiratory distress syndrome. The patient required ventilator and extracorporeal membrane oxygenation (ECMO) support and was treated successfully.

Case presentation

An 8-year-old girl presented to a hospital pediatric emergency department with a 1-week history of cough, fever for 4 days, and dyspnea for 5 h, without any response to oral cephalosporins. When she was admitted to the hospital, she had shortness of breath, 50 beats per minute, fever, 38.3 °C, tachycardia, 154 beats per minute, and hypoxemia, 72% in ambient air. Physical examination revealed moist rales in the left lung and diminished breath sounds over the right lung. Leukocyte count was 19×10^9/L, indicating leukocytosis of mainly neutrophils, and C-reactive protein level was 0.2 mg/dl. Blood biochemical analysis showed that the level of alanine aminotransferase was 516 U/L, lactate dehydrogenase was 2113 U/L, and sodium ions was 122 mmol/L. Chest x-ray photograph showed diffuse opacification on the left lung, Atelectasis was observed in the right lung with a large effusion in the right pleural cavity (**Figure 1**). She received a combination of antibiotics (azithromycin and ceftriaxone) to treat severe pneumonia and was transferred to the pediatric intensive care unit (PICU) for further treatment. There, she was given high-flow warm humidified oxygen through the nose. A computed tomography (CT) scan of the chest showed bilateral lung infection and right lung consolidation density; Multiplelymph nodes in the mediastinum show that the area is slightly larger; Bilateral pleural effusion (**Figure 2A**). Ultrasonic examination also showed a large amount of pleural effusion in the right lung. Lower chest puncture drainage was performed under ultrasound guidance, and antibiotic therapy was escalated to cefoperazone sodium, sulbactam sodium, and azithromycin. Analysis of pleural effusion showed that a protein level of 38.2 g/L, glucose of 6.57 mmol/L, lactate dehydrogenase of 3082 U/L, and mononuclear phagocyte predominance was observed. Negative bacterial culture results for blood, endotracheal aspirate and pleural effusion. Antigen testing for respiratory pathogens (including adenovirus, respiratory syncytial virus, influenza virus and EB virus) is suggestive of negative results. Polymerase chain reaction (PCR) showed that MP was positive in endotracheal aspirate and pleural effusion. MP-specific IgM and IgG antibodies from blood showed negative results. Acquired mutations on the ribosomal

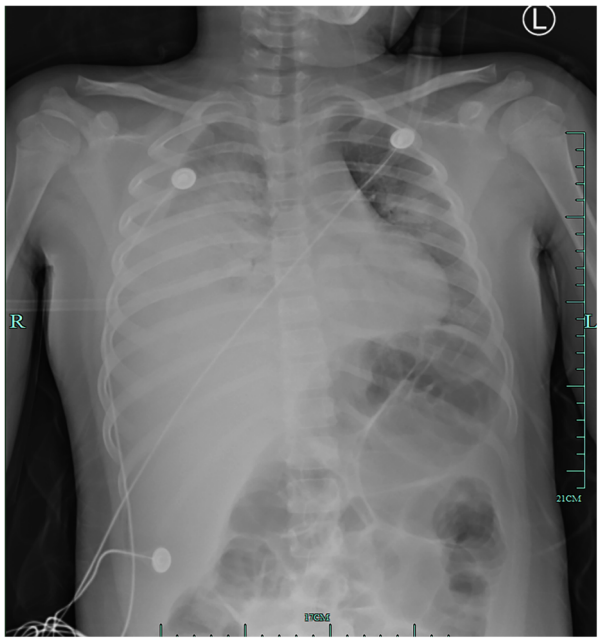

FIGURE 1
Initial chest radiograph on hospitalization day 1 showing diffuse opacification on the left lung, atelectasis in the right lung, and a large effusion in the right pleural cavity.

macrolide target were negative, suggesting that it was not a macrolide-resistant strain. Further medical history obtained from family members did not indicate any risk factors for tuberculosis; however, a gamma release assay was also sent for analysis. On the first day of admission, patient was found to have abnormal coagulation function, and bleeding spots appear on the skin the next day. In the coagulation profile, the international normalized ratio was 1.67, with a partial thromboplastin time of 31.5 s and a prothrombin time of 17.5 s. Thrombin time was 60.0 s. The fibrinogen function K value was 18.1 min, the fibrinogen and platelet function (angle) were 27.9, the platelet function was 21.2 mm, and the comprehensive index of coagulation function was −14.5. In order to exclude hematologic diseases, coagulation factors and plasma correction tests were added, and fresh frozen plasma and fibrinogen infusions were given, and the coagulation function was slightly improved. After 48 h, she remained febrile with worsening tachypnea and hypoxic respiratory failure, hemoptysis, and required intubation and ventilation. Blood was visible in the airway, and bronchoscopy and bronchoalveolar lavage revealed DAH. Therefore, a diagnosis of severe MP in conjunction with DAH, severe pediatric acute respiratory distress syndrome (ARDS), and acute hypoxic respiratory failure was established. Human blood immunoglobulin, epinephrine endotracheal instillation, and intravenous application of glucocorticoids were administered.

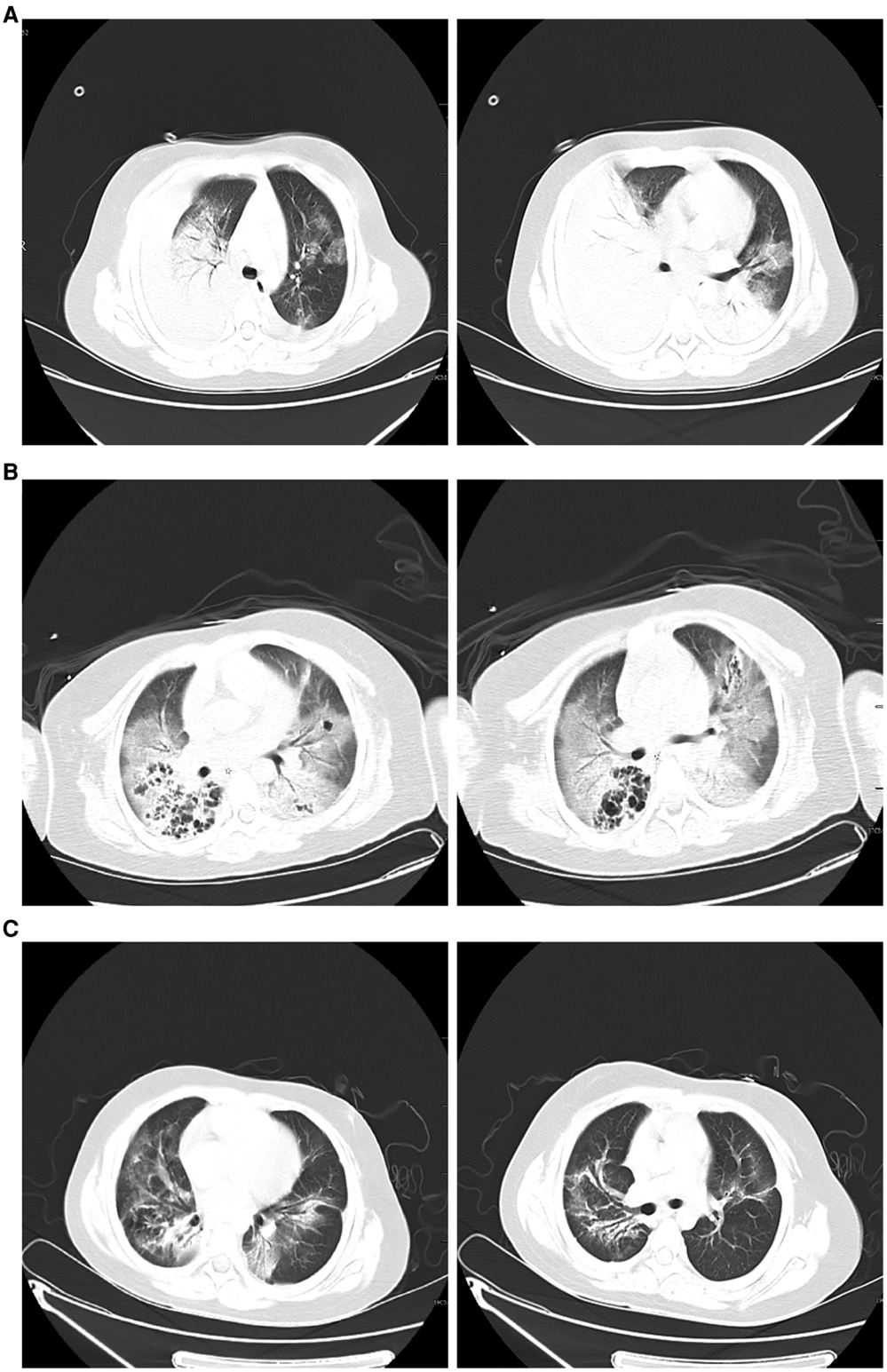

FIGURE 2
(A) Chest computed tomography scan on hospitalization day 1 showing double lung infection and right lung consolidation density; multiple lymph nodes in mediastinum the mediastinum show that the area is slightly larger; bilateral pleural effusion. (B) After 8 days of therapy, a computed tomography scan of the chest shows exudate changes in both lungs, partial consolidation in the right lung, similar lesions as initially observed, interstitial changes in the right lower lung with multiple bronchial cystic changes, and reactive lymphadenopathy. (C) Forty-five days after hospital discharge, the pulmonary lesions on computed tomography were significantly absorbed compared to earlier.

Because of ongoing hypoxia, she was cannulated onto veno-venous extracorporeal membrane oxygenation (VV-ECMO) on hospitalization day 3. The results of pleural fluid and blood tests for metagenomic next-generation sequencing (mNGS) suggested MP infection, with no other viral or bacterial infections. She was screened for rheumatological conditions, tuberculosis, autoimmune diseases, and tumour. Results did not suggest these situations.The patient stayed on ECMO for 5 d; Due to the significant prolongation of PT and APTT, anticoagulation was given without heparin or low-dose heparin regimen, and the anticoagulation target was gradually adjusted on the basis of fresh frozen plasma supplementation to prevent thrombosis in the extracorporeal membrane lung and pipeline. Laying in the prone position for for 10 h + per day during this period improved oxygenation. Ultrasound was used to assess lung exudation. The patient was ventilated for ten days. During this period, she received methylprednisolone 1–2 mg/kg/d and a 14-day tapering regimen for acute respiratory distress syndrome and azithromycin was given intravenously, for inflammation, three times a week. Simultaneously, further testing revealed that the patient's humoral and cellular immune function was disorder, the TBNK lymphocyte subsets were suggested that CD3 799×10^6/L(normal 960–3640), CD4 344×10^6/L(normal 550–2190), CD8 440×10^6/L(normal 260–1380), CD4/CD8 0.78(normal 0.72–2.88),natural killer cell 60×10^6/L(normal 80–680); Cytokine detection suggested that IL-6 1.33 pg/ml(normal 0–20), IL-10 27.56 pg/ml(normal 0–5.9); Immunoglobulins and complements were shown that IgA0.37 g/L(normal 0.52–2.16), IgM0.46 g/L(normal 6.09–12.85), C3 0.25 g/L (normal 0.79–1.52), C4 0.09 g/L(normal 0.12–0.36). Hemophagocytic lymphohistiocytosis was negative. Mycoplasma DNA continued to test positive on repeat workups.

On the 9th day, the patient had low-grade fever; sputum culture, blood mNGS testing, and G test suggested *Candida albicans* infection, and fluconazole antifungal therapy was administered for 10 d. Other infectious organism was not found in repeated respiratory tract, blood cultures andpleural cultures. After 8 d of therapy, CT of the chest revealed exudative changes in both lungs, partial consolidation in the right lung, similar to the range of lesions as before, interstitial changes in the right lower lung with multiple bronchial cystic changes, and reactive lymphadenopathy (**Figure 2B**). After 12 d of therapy, the patient clinically improved and became afebrile. Ventilator use was discontinued, and she was discharged from the hospital 15 d after extubation without supplemental oxygen. Pulmonary CT review 45 days after discharge indicated that the lesion was significantly absorbed compared to the lesion in the previous CT scan (**Figure 2C**).

Written informed consent to participate in this study was provided by the participant's legal guardian/next of kin. We obtained informed written consent from the patient's parent authorizing the publication of this clinical case and images.

Discussion

MP is one of the most common causes of community-acquired pneumonia in children. MP pneumonia is typically self-limiting and rarely requires mechanical ventilation or hospitalization. It rarely causes life-threatening diseases in children with normal immune ability but can be very severe (4–6). Severe mycoplasma infections are rare, and only 0.5%–2% of cases have a fulminant course (6). Key clinical and radiological findings of fulminant MP infection involve acute respiratory failure with diffuse consolidation or an abnormal interstitial pattern on chest radiograph. Izumikawa observed pleural effusion in 13.5% of cases with lymphocyte predominance (6). Herein, we describe a severe life-threatening case of infection with a macrolide-sensitive MP strain. This patient's condition progressed rapidly. We found this case remarkable because of (1) the approach to diagnosing MP, with pleural effusion samples and PCR/mNGS of the lower airways; (2) the presenting features of large pulmonary lesions and DAH, both rare in MP; and (3) the management strategies used, including methylprednisolone, prone position and VV-ECMO.

To our knowledge, DAH associated with MP is very rare; there are only five other published cases in adults, and there have been no reports in children (7–11). DAH is a life-threatening medical emergency with nonspecific symptoms and can result in respiratory failure and death. It is a pulmonary hemorrhagic syndrome caused by a disruption of the alveolar-capillary basement membrane due to injury to the microcirculation of the lungs, such as venules, alveolar capillaries, and arterioles (12). Hospital mortality rates are reported to be 20%–100% (1). Early diagnosis and treatment are critical to survival. The symptoms of DAH can present at any age, either with a previously diagnosed condition or as the first sign of a pre-existing condition. The most prevalent symptoms are hemoptysis, anemia, and new lung infiltrates. DAH is confirmed by hemorrhagic bronchoscopic bronchoalveolar lavage (BAL) on successive samples. The treatment is determined by the cause of the hemorrhage. The most prevalent cause of DAH is vasculitis, which is followed by thrombocytopenia, autoimmune illnesses, post-autologous stem cell transplantation, coagulation disorders, medications, and infections (2). The most common pathogens of infection are cytomegalovirus, Legionella, influenza A (H1N1), dengue, and staphylococcus. Reports of DHA due to Mycoplasma infection are rare and mostly affect individuals with weak immunity (3). We encountered an immunocompetent child with acute hypoxic respiratory failure due to MP-associated DAH requiring mechanical ventilation and VV-ECMO. Generally, pulmonary infections are infrequently related to DAH, but if left untreated, the condition has a high mortality rate and therefore should always be considered in the initial diagnosis (7).

Clinical characteristics and CT findings are not specific for detecting and diagnosing severe MP pneumonia initially. To minimize exacerbation of symptoms, early and exact laboratory diagnosis of MP infection is critical. Previous approaches, such as serological tests and mycoplasma culture, which might take a few weeks for the results to be available, are no longer feasible. The most sensitive and specific detection is real-time PCR of respiratory tract or nasopharynx samples, this is the preferred type of sample in most clinical settings (13–15). Early mNGS for MP utilizing pleural effusion fluid or serological fluid may be a viable option in individuals with dry cough without phlegm and when obtaining a lower respiratory tract sample is problematic. Studies have shown that PCR/mNGS of pleural effusion samples and lower respiratory tract can provide an early and rapid diagnosis of severe MP pneumonia with ARDS (16). In our case, in addition to collecting sputum, BAL fluid or endotracheal aspiration for PCR of MP, we also performed macro gene second-generation sequencing to detect *Mycoplasma* and other pathogens; the results are fast and sensitive. Related studies have found that mNGS technology can identify pathogens in patients with severe pneumonia at an early stage and guide the use of antimicrobials, thereby significantly reducing the 28- and 90-day case fatality rates of children with severe pneumonia (17). However, mNGS also has certain limitations as there is no international unified standard that can be used as a reference before the etiology is found.

DAH management entails an etiological diagnosis, vigorous supportive care, and treatment of any underlying systemic disease. In children with MP, macrolides are the treatment of choice; nonetheless, there are rising worries about the development of resistance (18). The only resistance mechanism reported is acquired mutations on the ribosomal macrolide target (19). Resistance may be present in up to a quarter of patients in Europe and the United States, whereas resistance may be present in more than 90% of patients in Japan and China (20). Our case, although fulminant, involved a non-resistant MP strain. Other underlying systemic diseases were excluded. Initially, the child received azithromycin combined with cephalosporin, low-dose glucocorticoid as an anti-inflammatory, and gamma globulin supportive therapy. A randomised controlled trial was conducted by Li et al. They randomly assigned children with refractory Mycoplasma pneumoniae pneumonia to group A [intravenous azithromycin (IA) + methylprednisolone (2 mg/kg/day for 3 days)], group B [IA + intravenous immunoglobulin (400 mg/kg/day for 3 days)] or group C (IA alone). After 7 days of treatment, the combined treatment groups A and B showed higher rates of infiltrative absorption, resolution of atelectasis and disappearance of pleural fluid compared to the control group C (21). Our report is similar to theirs, on the 5th day of admission, the child's body temperature returned to normal, and the pleural effusion was gradually absorbed. However, Zhang et al. reported that some children with severe or refractory MP required high-dose methylprednisolone (10–30 mg/kg/d) treatment with rapid recovery of pleural effusion and clinical symptoms (22).

Surveys have shown that some patients with MP develop severe hypoxic respiratory failure or severe ARDS and require ECMO rescue therapy (16). In the previous trial, the overall survival rate of mycoplasma pneumonia needing ECMO was 72.7%, suggesting that ECMO may be used to treat severe MP infection safely and successfully. In summary, the diagnosis of MP should be explored and studied in cases of DAH, severe ARDS, or other substantial cardiopulmonary illnesses, and unusual antimicrobial therapies should be undertaken. Because of the high percentages of survival shown in the literature and the Extracorporeal Life Support Organization database, ECMO should be considered in severe cases of MP (23). During ECMO, our patient was ventilated in the prone position (PP), and oxygenation improved significantly. Despite VV-ECMO support, patients with severe hypoxemia can consider PP. In patients with severe ARDS and COVID-19, PP under VV-ECMO improved the respiratory mechanics and oxygenation parameters, and the effects of PP on respiratory mechanics still existed after supine position reduction (24). There are more reports of ECMO combined with prone ventilation in adults, but there is a lack of data in children, we need a lot more clinical data on children to support it.

Studies have shown that lung lesions exceed two thirds of the lung volume, and the incidence of necrosis and embolism increases. Necrotizing pneumonia caused by MP is severe, although self-limiting and reversible. Good outcomes can be achieved with appropriate management (25). In our case, the child's CT suggested bronchial cystic changes without embolization. After treatment, the pulmonary lesions were obviously absorbed in the subsequent follow-up, and the child did not require oxygen support when discharged.

In conclusion, large lung lesions associated with MP and the presenting features of DAH are very rare and a high level of vigilance should be maintained for rapid outbreaks of this disease The rapid and early diagnosis of severe MP pneumonia can be realized by PCR/mNGS of samples from pleural effusions or lower respiratory tract. Diagnosis of DAH and other associated infections can be achieved by bronchoscopy and BAL. The main therapeutic measures include anti-infection and supportive measures such as mechanical ventilation, but as in our case, success in prone position and VV-ECMO was life-saving.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

YP designed the study and drafted the manuscript. XJ collected the literature and was a major contributor in writing the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict 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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EDITED BY
Hans Van Rostenberghe,
Universiti Sains Malaysia (USM), Malaysia

REVIEWED BY
Saba Battelino,
University Medical Centre Ljubljana, Slovenia
Raffaele Campisi,
Azienda Ospedaliera Universitaria Policlinico G. Rodolico-San Marco, Italy
Chenlong Yang,
Peking University Health Science Center, China

*CORRESPONDENCE
Oskar Feussner
✉ oskar.feussner@gmx.de

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Case report: Otitis media with subsequent mastoiditis and cerebral herniation in a patient with Arnold chiari malformation

Oskar Feussner*, Roland Haase and Jan Baier

Department for Operative and Nonoperative Pediatrics and Adolescent Medicine, Section for Neonatology and Pediatric Critical Care, University Hospital, Halle, Germany

We present the case of a 13-year-old boy who unexpectedly needed to be resuscitated at home after an assumed uncomplicated otitis media. Imaging at our clinic showed mastoiditis and a cystoid mass in the left cerebellopontine angle compressing the brainstem, as well as an Arnold-Chiari-Malformation. Both the laboratory examination of cerebrospinal fluid (CSF) and surgical biopsy with pathological evaluation of the mastoid supported the inflammatory etiology of the mass. Microbiologically, Streptococcus intermedius was detected in the blood culture and CSF. Due to brain death, which most likely already existed preclinically, the organs were released for donation during the course. Our case demonstrates a very rare lethal complication of acute otitis media on the basis of a cerebral malformation and emphasizes the need to stay alert when patients complain of symptoms after assumed resolution.

KEYWORDS

mastoiditis, otitis media, arnold-chiari-malformation, sudden death, brain absces

Introduction

Mastoiditis, despite being the most common severe complication of acute otitis media (AOM), is a nowadays rare condition (1, 2). However, since it is a potentially life-threatening disease, it is of great importance to recognize symptoms in time. Depending on age, these include predominantly a protruding ear, retroauricular erythema, swelling or pain, ear discharge, fever, and a deteriorated general condition (3). Particularly challenging are less symptomatic or even asymptomatic courses, which have been described especially in cases in which AOM was treated with an antibiotic (4). Diagnostics include a medical history, the physical examination and laboratory diagnostics of the blood. However, there is still no consensus on the need and timing of computer tomography (CT). For treatment, research demonstrated that in uncomplicated cases, conservative management (antibiotic treatment with or without myringotomy and ventilation tubes) is an efficient first-line treatment. However, mastoidectomy should be performed in case of failure of conservative therapy after 48–72 h or in the case of complications, e.g., epidural or subdural abscess, facial paralysis, sinus thrombosis or brain abscess. As a result, with appropriate therapy, the prognosis is generally favorable with few long-time complications (5).

Case description

We present the case of a 13-year-old boy with obesity and hypertension who unexpectedly needed to be resuscitated at home by his mother (for the timeline see **Figure 1**). When the emergency physician arrived half an hour later, pupils were already dilated. After more than

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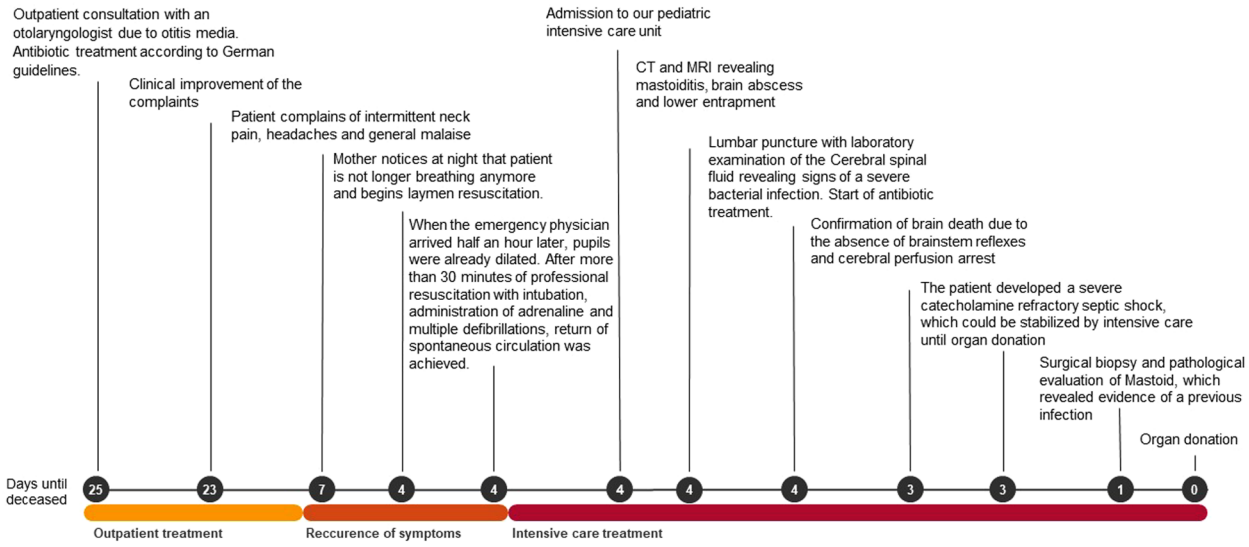

FIGURE 1
Timeline with chronological depiction of events from outpatient diagnosis of otitis media to organ donation.

30 minutes of professional resuscitation with intubation, administration of adrenaline and multiple defibrillations, spontaneous circulation was achieved. Intubated and ventilated, the patient was then admitted to our pediatric intensive care unit (PICU), where we performed a cranial computed tomography (CT) and, in the course, a cranial magnetic resonance imaging (MRI), in which the clinical suspicion of a lower entrapment was confirmed. The most likely cause appeared to be the caudal ectopia of the cerebellar tonsils and a cystoid mass in the left cerebellopontine angle compressing the brainstem, with prominent surrounding cerebellar edema and inflammatory changes in the area of the petrous bone and the left mastoid (see **Figure 2**). Laboratory results on admission are shown in **Table 1**.

A conversation with the mother revealed, that three weeks earlier, the patient was diagnosed with Acute Otitis media (AOM) and treated with oral antibiotics according to German guidelines. As a result, there had been a clinical improvement without symptom progression or fever. However, three days before admission to our PICU, the patient complained of intermittent neck pain and headache and was not feeling well. Because of this, he had slept in his mother's bed where he had been talking while sleeping, then suddenly paused and stopped breathing. Other symptoms in relation to possible neurological, oncological or infectious diseases were negated. The patient's medical history only revealed arterial hypertension treated with metoprolol, and a pronounced obesity of 110 kg body weight. In addition, the patient had repeatedly complained of headaches and decreased physical resilience for the last eight weeks. The physical examination on our PICU showed a mildly inflamed left tympanic membrane, consistent with an otitis media that had subsided a few weeks earlier. The patient was completely vaccinated according to the recommendations of the Robert Koch institute including two vaccinations with Comirnaty.

In the absence of brainstem reflexes and cerebral perfusion arrest, we confirmed brain death one day after admission according to the guidelines of the German medical association. We discussed the possibility of organ donation with the parents, who consented.

For differential diagnostic clarification of the etiology of the mass, we performed a lumbar puncture. Laboratory examination of the Cerebral spinal fluid (CSF) revealed signs of a severe bacterial infection, which supported the inflammatory etiology of the mass. Microbiologically, Streptococcus intermedius was detected both in the blood culture and in the CSF, which showed no resistances against standard antibiotics. Despite extensive diagnostics, there was no evidence of an acute viral infection. Myocarditis could be excluded by further investigations. Antibiotic treatment was started right after the diagnostic work-up.

In the meantime, the patient developed a severe catecholamine refractory septic shock, which could be stabilized by intensive care until organ donation. To rule out a malignant process of the left mastoid before organ transplantation, we performed surgical biopsy and pathological evaluation, which revealed evidence of a previous infection of the mastoid with fibrosed mucosa. A neoplastic process could be excluded. After clarification of the microbiological etiology of the cerebellar abscess, organ donation could proceed without complications.

Discussion

This case illustrates the rare but life-threatening presentation of mastoiditis with subsequent subdural empyema and cerebral herniation after AOM.

With a cumulative prevalence of more than 60% by the age of seven, AOM is not only a highly common disease (6) but it is also the most prevalent reason for antibiotic therapy in young children (7). Despite the high incidence, severe complications are rare (8). These include, with decreasing probability, mastoiditis,

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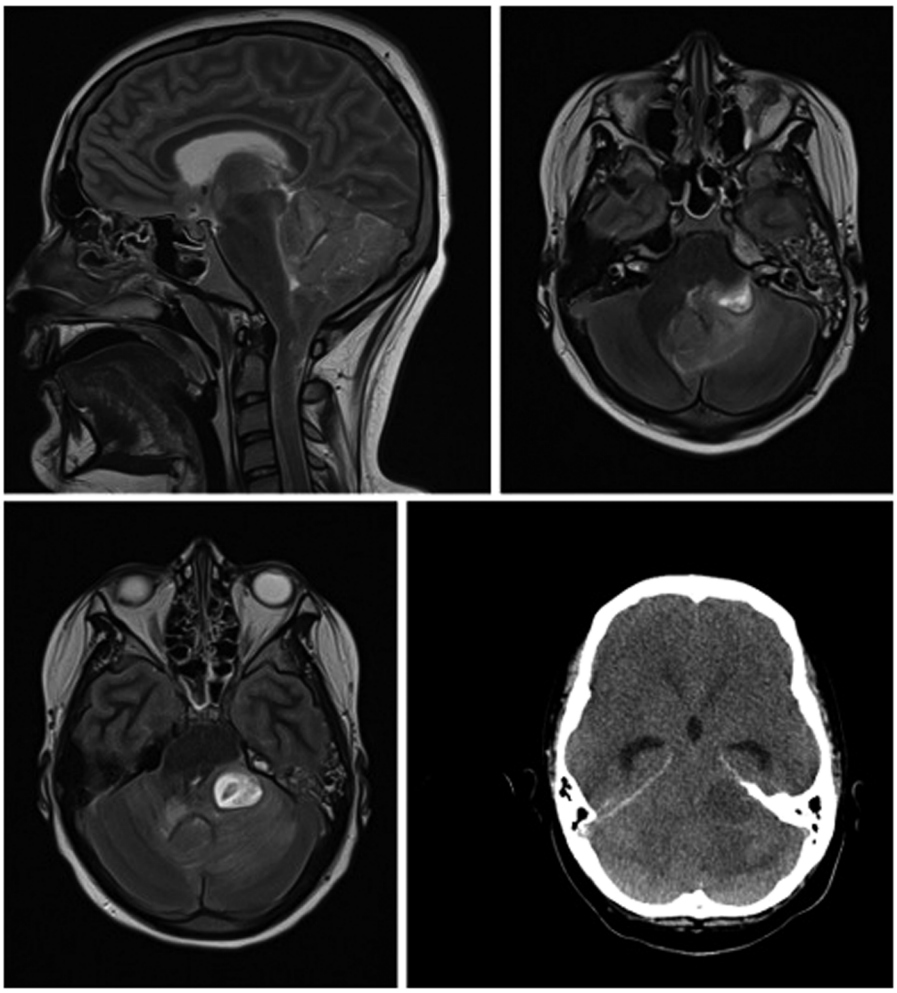

FIGURE 2
cMRI and cCT show lower entrapment most likely caused by caudal ectopia of the cerebellar tonsils and a cystoid mass in the left cerebellopontine angle compressing the brainstem, with prominent surrounding cerebellar edema and inflammatory changes in the area of the petrous bone and the left mastoid.

TABLE 1 Laboratory results on admission.

Blood sample	Liquor sample
Blood count: leucocytes 13.1 Gpt/L, haemoglobin 7.7 mmol/L, thrombocytes 402 Gpt/L	Cell count: leucocytes 5,396 Mpt/L, 99% Neutrophils, erythrocytes 1,196 Mpt/L
Coagulation tests: INR 1.26, PTT 27 s, fibrinogen 5.28 g/L, AT III 120%	Protein >6 g/L, Glucose <0,1 mmol/L, lactate 25 mmol/L
Further clinical chemical results: creatinine 74 µmol/L, Urea 5.5 mmol/L, ASAT 1.49 µcat/L, ALAT 2.30 µcat/L, GGT 1.98 µcat/L, CK 1.88 µcat/L, CK-MB 0.6 µcat/L, myoglobin 24 µg/L, troponin T 321 ng/L, NT-pro BNP 2300 ng/L, CRP 28,4 mg/L	

sub-periosteal abscess, facial nerve palsy, epidural abscess, sigmoid sinus and internal jugular vein thrombosis. Other complications such as elevated intracranial pressure, cerebral stroke and suppurative meningitis are rare and have been described only in single cases (1). Mastoiditis, as the most common complication, was observed in the United Kingdom in 1.8 per 10,000 AOM episodes in which antibiotics were administered and in 3.8 per 10,000 AOM episodes in which no antibiotic was administered (9), whereas a meta-analysis described 23.7 cases of mastoiditis per 10,000 episodes of AOM (10). Depending on the investigated country and time period, the incidence rate of Mastoiditis is reported to be 1.2–4.8/100,000 person-years (2, 11).

Regarding the dynamics of incidence in the last years and decades, there are conflicting findings. Several studies indicate an increase in incidence and an increase in surgical intervention and attribute this to the rising number of antibiotic resistances (12, 13). On the other hand, numerous studies report a constant or even decreasing incidence (2, 9, 11, 14). It should be noted, however, that an increase in the complication rate and the need for surgical intervention was observed here as well (14).

However, precise data on changes in incidence over the last two decades is important, as a possible rise in incidence has been associated with a more restrictive usage of antibiotics (2). This is supported by the fact, that Thompson and colleagues observed in their cohort study that antibiotic treatment for AOM reduced the risk of future mastoiditis by 50% (9). Arguing against the more liberal use of antibiotics in AOM, other studies have not only observed no effect of antibiotic administration on the risk of mastoiditis (15) but in some cases prior antibiotic administration was even associated with an increased risk for the need for surgical intervention (16). In addition, given the rarity of mastoiditis, the number needed to treat with an antibiotic to prevent one case of mastoiditis is estimated to be between 2,500 and 4,381 (9, 17). Here, however, the benefits bear no relation to the financial costs, the possible side effects and the development of bacterial resistance. Furthermore, our case supports the studies showing that despite antibiotic treatment, complete prevention is not provided and prior antibiotic administration may even mask mastoiditis (4, 18). In our case, however, it should be noted that the potential standard dosage of the antibiotic in an obese patient may have been a contributing factor to treatment failure.

The most common pathogens of mastoiditis include Streptococcus Pneumoniae, Pseudomonas aeruginosa, Streptococcus Pyogenes group A, Staphylococcus aureus, and Haemophilus influenzae (1, 11, 19). In addition, Fusobacterium Necrophorum has been detected more frequently in mastoiditis in recent years (14, 20), particularly in cases previously treated with antibiotics or where complications occurred and surgery was required (14, 19). Concerningly, a rising number of resistant infections have been found in mastoiditis in recent years as well (1, 13).

Streptococcus intermedius, a commensal microorganism of the oral flora and the bacterium we consider to be the most likely causative, does not appear to be a typical pathogen of mastoiditis. It does, however, appear to be a common pathogen associated with brain abscesses (21). There are case reports describing brain abscesses in children after mastoiditis caused by Streptococcus intermedius (22). Pathophysiologically, the brain abscess formation occurs after tissue damage, which is followed by bacterial colonization and subsequent tissue liquefaction and pus formation due to hyaluronidase activity of Streptococcus intermedius (21).

It is also worth mentioning that on MRI, in addition to the mass compressing the brainstem, there was a descent of the cerebellar tonsils, indicating a previously unknown Arnold-Chiari malformation Type 1. This as a rare craniovertebral junction malformation with caudal ectopia of the cerebellar tonsils through the Foramen Magnum that can lead to slowly progressive symptoms like headaches, neck pain, motor deficits, cranial nerve palsy, oropharyngeal dysfunctions and sleep disorders (23, 24). Epidemiologic data are scarce and with wide discrepancies. For instance, two retrospective analyses of MRI images reported incidences of 0.77 and 3.6%, respectively (25, 26). Matching our case, the disease was observed more frequently in children and young adults and the male sex (23). However, there is a minority of asymptomatic patients with an acute fatal onset (27, 28). Although, comparable to the case described by Stephany et al. the patient headaches, which he had described for several weeks can retrospectively be considered as a possible prodrome (29). Due to the ectopic position of the cerebellar tonsils through the foramen magnum into the spinal canal and the thus chronically constricted brainstem, patients with Arnold-Chiari malformation are particularly susceptible to cerebellar tonsillar impaction. Minor trauma, or as in our case, an intracerebral mass, can promptly decompensate the vulnerable system. Pathophysiologically, both direct mechanical entrapment of brain tissue and compression of cerebral vessels with subsequent ischemia can impair the function of the respiratory and circulator as well as the ascending reticular activating system with direct catastrophic consequences (23). In our case, this might have contributed to the rapid clinical deterioration and ultimately cardiovascular failure.

Although the coincidence of an Arnold-Chiari malformation and mastoiditis is rare, this case illustrates that even nowadays otitis media can have a severe and sometimes lethal outcome. Especially, recurring complaints such as headache, fever or neck pain should always raise the suspicion of unsuccessful treatment and require a conscientious investigation to initiate the appropriate diagnostics and subsequent therapy in time. To prevent a fatal course, as in our case, it is, therefore, crucial to educate the patient and parents about the need for immediate reappearance in the event of the above-mentioned complaints, some of which may occur after a latency of 8–12 weeks. Finally, this case also highlights that antibiotic therapy never reliably protects against serious complications and, in the worst case, can even lead to a delay in diagnosis because the treating physician is under a false sense of security.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

OF: wrote the manuscript in consultation with JB. RH: supervised the treatment of the patient and the elaboration of the manuscript. JB and RH: provided critical feedback. All authors contributed to the article and approved the submitted version.

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Conflict 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.

Publisher's note

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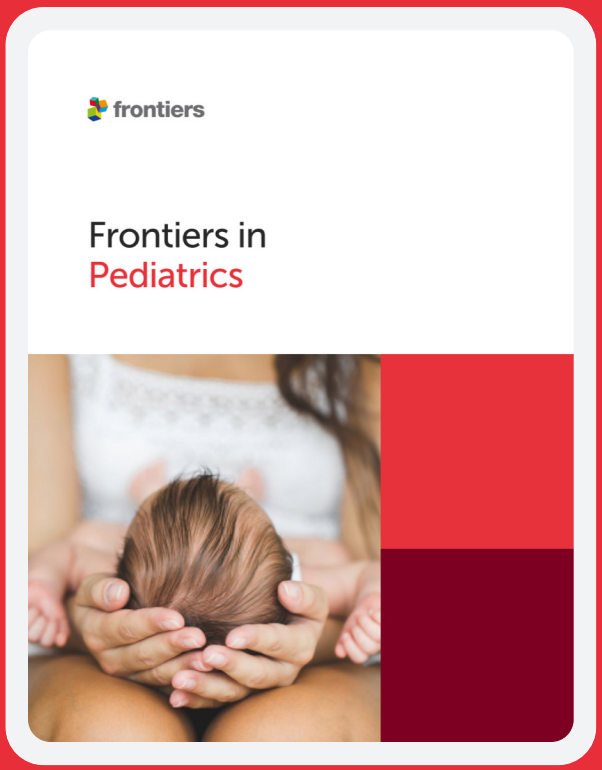

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