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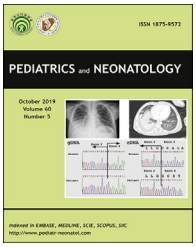

Short Communication

Pneumonia and empyema caused by *Methylobacterium* in a patient with X-linked chronic granulomatous disease

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1. Introduction

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease with an incidence of 1/250,000.[1] This disease is caused by genetic defects in the nicotinamide adenine dinucleotide phosphate oxidase complex, which includes gp91^{phox}, p22^{phox}, p40^{phox}, p47^{phox}, and p67^{phox}; mutations in these genes can lead to failure in the production of microbicidal superoxide anions and the associated phagocyte-derived reactive oxygen species.[2] These genetic defects can further result in the development of recurrent life-threatening fungal and/or bacterial infections, granuloma formation, and inflammatory processes. The most common pathogens associated with CGD are *Aspergillus* species, *Nocardia* species, *Burkholderia cepacia*, *Staphylococcus aureus*, *Serratia marcescens*, *Salmonella* species, and *Mycobacterium bovis* (bacillus Calmette-Guérin strain). Herein, we report a case of an unusual pathogen that resulted in pulmonary infection in a patient with CGD using 16S rRNA gene sequence analysis.

2. Case scenario

A 5-year-old boy presented with fever of unknown origin. Physical examination revealed no specific abnormalities. Results of laboratory tests are presented in Table S1. No active lung lesions were identified on serial chest radiographs, but there was a slight right-sided pleural thickening. The boy was discharged with oral amoxicillin/clavulanic acid after the fever had subsided; however, unfortunately, he developed chest pain 1 day after discharge. Chest radiograph (Fig. 1A) and computed tomography (Fig. 1B) revealed right lower lung consolidation and a right-sided pleural effusion. Lobar pneumonia with parapneumonic effusion was confirmed, and the boy was administered ceftriaxone. High fever persisted after the initial treatment, and a suspicion of atypical pathogens was considered. A review of his medical history revealed that he had recurrent infections, including perianal abscess, cellulitis, and lymphadenitis, since 1 year of age, which were frequently associated with

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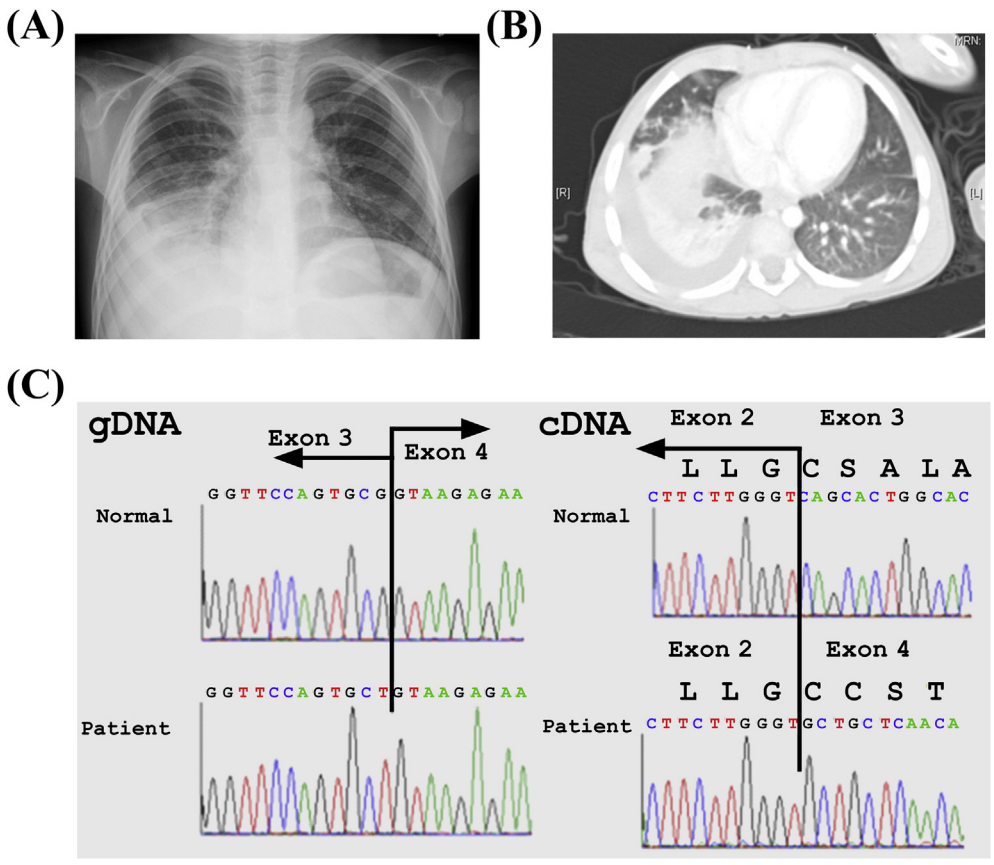

Figure 1 The images, past history, and genetic analysis of the patient. (A) Chest radiograph on the second admission revealed right lower lobe consolidation and pleural effusion. (B) Chest computed tomography revealed pneumonia in the right lower lobe with pleural effusion. (C) The genetic analysis of the patient.

uncommon pathogens such as *Bacteroides fragilis* and *B. cepacia* (Supplementary files). Based on the history of frequent infection, we considered immunodeficiency in this patient. Subsequently, serial testing was conducted to screen for primary immunodeficiency. Dihydrorhodamine (DHR) flow cytometry curve failed to shift after the stimulation of neutrophils of the patient, indicating the presence of a neutrophil function disorder. CGD was thus suspected. The antimicrobial therapy was switched to teicoplanin, meropenem, and voriconazole to cover the most common pathogens detected in patients with CGD. However, both blood and pleural effusion cultures showed negative results for bacteria and fungi. Therefore, the pleural fluid was subjected to DNA extraction. A pair of primers was used for amplifying the bacterial 16S rRNA gene. The sequence of the forward primer was 5′-AACGAGCGCAACCCTT-3′, and that of the reverse primer was 5′-TGACGGGCGGTGTGTACAAG-3′. The sequence was compared with published sequences in the NCBI GenBank database. *Methylobacterium* was then identified by sequencing the 16S rRNA amplicon. The patient was finally discharged after successful treatment with gentamicin, meropenem, and sulfamethoxazole—trimethoprim.

Whole exome sequencing revealed a c.252 G>T point mutation at the end nucleotide of exon 3 in the *CYBB* gene, which caused the splicing mutation leading to the deletion of exon 3. This c.252 G>T point mutation has been previously reported as the pathogenic mutation causing X-linked recessive CGD (XR-CGD).[1] XR-CGD was subsequently confirmed in our patient. The genomic DNA sequence and the complementary DNA sequence are shown in Fig. 1C. His family members also underwent genetic testing, and both his mother and elder sister were found as carriers of XR-CGD with the inherited c.252 G>T point mutation. The patient received an unrelated donor cord blood transplantation successfully with full donor-derived hematopoietic chimerism and is free of infection since then. The detailed hospital course of the patient is provided in Supplementary files.

3. Discussion

We have reported a case of pneumonia caused by *Methylobacterium* infection in a patient with XR-CGD. *Methylobacterium* was first reported by Patt et al., in 1976 and currently includes more than 50 different species.[3] *Methylobacterium* species are fastidious, pink-pigmented, slow-growing, Gram-negative bacilli that are positive to catalase, urease, and oxidase.[3] *Methylobacterium* species are common environmental organisms that are distributed in soil, tap water, sewage, and leaf surface. A case series

report from Taiwan had described these species in immunocompromized patients as opportunistic pathogens.[4] There is also a report of *Methylobacterium* that led to pneumonia in a 1-year-old patient with CGD who was successfully treated with a 7-month ceftriaxone regimen.[5]

Methylobacterium species have been defined as difficult-to-culture pathogens requiring atypical media and prolonged incubation.[5] Therefore, non-culture-based techniques are required for the identification of *Methylobacterium* species infection. DNA sequencing of the bacterial 16S rRNA gene sequence is a useful diagnostic tool that can be applied as a culture-independent technique for identifying difficult-to-culture pathogens. In our patient, universal primers were used for bacterial 16S rRNA gene amplification. *Methylobacterium* was confirmed after comparing the bacterial 16S rRNA gene sequence with published sequences in the NCBI GenBank database. The patient recovered after the complete treatment course with gentamicin, meropenem, and trimethoprim/sulfamethoxazole, which have been reported to be effective for treating *Methylobacterium* infection.[5]

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for CGD. Unrelated cord blood (UCB) could be a choice as the stem cell source in the absence of matched sibling donors. Successful UCB transplantation in patients with CGD has been reported in Taiwan and believed to be an ideal alternative donor source with the advantages of rapid availability and limited HLA matching requirements.[6,7] Nevertheless, HSCT must be performed before the development of severe infection in patients with CGD, and UCB provides an advantage for patients without a matched sibling donor.

In our case study, the patient suffered from progressive retinal degeneration since the age of 6 months before he had other typical CGD presentations. He developed esotropia, followed by retinal degeneration with macular pigmentation in the subsequent years, which resulted in visual impairment. CGD-related retinal degeneration and pigmentation were believed to be the cause, although no biopsy was obtained. The literature describes a case of retinal granuloma that was detected in a 2-year-old boy who was later diagnosed with CGD without other typical CGD presentations.[8] Therefore, physicians should be cautious about early-onset retinal lesions and consider them as CGD in the differential diagnoses.

In conclusion, *Methylobacterium* species could be an opportunistic pathogen causing pneumonia in patients with CGD. Diagnosis requires non-culture-based techniques. Sequencing of the bacterial 16S rRNA gene is a useful diagnostic tool that can be applied as a culture-independent technique for identifying difficult-to-culture pathogens.

Conflicts of interest

The authors have no conflicts of interest relevant to this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2019.08.002.