Case Report Pulmonary *Actinomyces graevenitzii* infection presenting as organizing pneumonia diagnosed by PCR analysis

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We report what is believed to be the first case of pulmonary *Actinomyces graevenitzii* infection presenting as organizing pneumonia. Fever and night sweats developed in a 69-year-old male. The only abnormal laboratory data were an elevated erythrocyte sedimentation rate and C-reactive protein level. On chest images, multiple consolidations with air bronchograms were seen in the bilateral lungs. Histological examination from lung biopsy revealed a pattern of organizing pneumonia with microabscesses, but definitive diagnosis was not obtained because culture from lung specimen was negative. *A. graevenitzii* was eventually identified in the lung biopsy specimen by detection of an *Actinomyces*-specific PCR product followed by 16S rRNA gene sequencing. The patient was treated with high-dose ampicillin intravenously for 1 month, followed by oral amoxicillin and clarithromycin for 6 months, and recovered. We suggest that actinomycosis can present as organizing pneumonia, and identification of infection by PCR analysis and rRNA gene sequencing is a useful strategy in cases that are difficult to diagnose.

**Introduction**

Pulmonary actinomycosis is a rare bacterial infection that is sometimes difficult to diagnose. Currently, PCR following 16S rRNA gene sequencing is the preferred method of detecting *Actinomyces* in clinical material (Hansen et al., 2009). This disease generally results in non-specific respiratory symptoms and various radiological findings. The typical histological appearance includes a variable number of abscesses composed of actinomycotic granules surrounded by fibrosing granulation tissue. In this article, we present a case of pulmonary *Actinomyces graevenitzii* infection presented as organizing pneumonia diagnosed by PCR and 16S rRNA gene sequencing.

**Case report**

A 69-year-old male presented with a 2-month history of low-grade fever and night sweats. He was a designer with a 60 pack-year smoking history. There was no pertinent past medical history or family history. Vital signs were as follows: temperature, 37.5 °C; respiratory rate, 10 breaths min⁻¹; systemic blood pressure, 120/60 mmHg. Admission laboratory results included an erythrocyte sedimentation rate of 130 mm h⁻¹ and C-reactive protein level of 10.68 mg dl⁻¹, suggesting inflammation. The chest X-ray revealed multiple areas of infiltration in the periphery of both lungs, and multiple consolidations with air bronchograms were seen in the lungs bilaterally on chest computed tomography (Fig. 1a). Initially, community-acquired pneumonia was suspected, and the patient was given 300 mg cefcapene pivoxil for a week and 500 mg levofloxacin for the following week. However, the infiltrations continued to increase in size. Because we could not rule out the possibility of malignancy, a video-assisted thoracoscopic lung biopsy was performed. Histological examination revealed a pattern of organizing pneumonia with micro-abscesses (Fig. 1b, c). We made a presumptive diagnosis of bacterial infection, but definitive diagnosis was not possible because biopsy tissue culture on Sabouraud’s dextrose agar medium at 25 °C or 35 °C for 3 weeks under aerobic conditions was negative. After the thoracoscopy, several antibiotics and steroid were administered. Firstly, oral doxycycline was administered for 1 month but lung infiltration did not diminish. Considering organizing pneumonia after bacterial pneumonia, 30 mg oral prednisolone was administered for 2 months. Steroid therapy slightly decreased lung infiltration; however, fever and increased lung infiltration were observed again. Additional aerobic culture from the lung biopsy specimen taken from the second fibro-optic bronchoscopy was negative. Administration of oral clarithromycin and intravenous meropenem for 1 month temporarily alleviated...
the patient’s fever and lung infiltration. One month later, after discontinuance of antibiotics, recurrent fever and recurrence of lung opacity were observed. We performed the third fiberoptic bronchoscopy examination and finally detected the microbe from a lung biopsy specimen by using a molecular genetic method. We performed molecular identification by PCR amplification and sequencing analysis of the 16S rRNA gene using DNA extracted from the lung biopsy specimen (Fig. 2). The universal primers for the Actinomycetales 16S rRNA gene (forward, 5′-GGKCTGCGGTGGGTACGGGC-3′; reverse, 5′-GGCTTTAAGGGATTCGCTCCRCCTCAC-3′) were used as described previously (Xia & Baumgartner, 2003). We performed sequencing analysis using a GenBank BLAST search and BiBi (http://pbil.univ-lyon1.fr/) phylogenetic tools. The sequence of the 16S rRNA gene (accession no. AB691580) was 99.7 % identical (581 bp over the entire 583 bp fragment) to that of the type strain of *A. graevenitzii* (CCUG 27294, accession no. AJ540309). No other bacteria or fungi were detected in this specimen by PCR (data not shown), indicating that contamination from the oral cavity was not a consideration. These results indicated that the organizing pneumonia in our patient was caused by *A. graevenitzii*.

The patient was treated with 8000 mg ampicillin intravenously for 1 month, followed by combined 2000 mg oral amoxicillin and 400 mg clarithromycin for 6 months. The clinical status gradually improved. All lung consolidations disappeared in a dramatic response to the antibiotics, and the patient was in good health during the 12-month follow-up period.

**Discussion**

Pulmonary actinomycosis is a chronic, suppurative, pulmonary or endobronchial infection caused by *Actinomyces* species. In general, age 20–60 years, male sex, diabetes and immunosuppression are the risk factors associated with *Actinomyces* infection. Men with poor dental hygiene appear...
to be at the greatest risk of developing pulmonary actinomycosis. Furthermore, a higher incidence has been reported in patients with underlying lung disorders, such as emphysema, chronic bronchitis and bronchiectasis (Wong et al., 2011). However, the patient in this report was immunocompetent and his oral hygiene was good. Since we could not determine the reason for his susceptibility to Actinomyces infection, we speculate that he may have an unknown immunological disorder.

A. graevenitzii was first isolated in 1997 from respiratory and bone specimens (Ramos et al., 1997). Cases of pulmonary infection and bacteremia caused by A. graevenitzii have been previously reported (Tietz et al., 2005; Hwang et al., 2011). Making the diagnosis of actinomycosis is usually difficult because of previous or current administration of antibiotics, indicating that many patients are misdiagnosed or experience delayed diagnosis. Definitive diagnosis is made by anaerobic culture of the bacteria, but often a presumptive diagnosis is made by histological observation of the characteristic sulfur granules. In this case, we did not detect any bacteria or fungi by conventional culture methods, and did not find typical features, such as sulfur granules, in the pathological specimen. In practice, PCR and 16S rRNA gene sequencing are required to detect rare or unusual bacteria; these are useful for definitive diagnosis of culture-negative specimens, as in this case (Hall, 2008). These new molecular genetic methods are available for more rapid and accurate identification of actinomycosis (Hansen et al., 2009; Wong et al., 2011). The main advantage of the PCR assay is that it detects small amounts of bacteria from specimens in cases where the conventional culture result is negative after several attempts of sampling even though infection is suspected from the clinical course.

We think that the possibility of contamination from the oral cavity would be zero in this case because of the following two reasons. Firstly, we used endobronchial ultrasonography (EBUS) with a guide sheath (GS) as a guide for transbronchial lung biopsy and bronchial washing. The probe was inserted into a GS, and the GS-covered probe was inserted through the working channel of the fibro-optic bronchoscope and advanced to the peripheral pulmonary lesion in order to obtain an EBUS image. The probe and GS were confirmed to have reached the lesion by X-ray fluoroscopy. After localizing the lesion using EBUS imaging, the GS remained in the peripheral lesion. Biopsy forceps were introduced via the GS to perform pathological and cytological examination (Kurimoto et al., 2002; Kikuchi et al., 2004). We used an antiseptic GS; therefore, it prevented the risk of track contamination with bacteria, especially oral bacteria. Secondly, we detected only one bacterial strain (A. graevenitzii) from the specimen by the PCR analysis, which could recognize all bacteria and fungi. If there was any contamination from the oral cavity, we would have detected several oral bacterial strains other than Actinomyces.

Organizing pneumonia is a pulmonary response pattern that can be either cryptogenic or associated with a variety of causes. To our knowledge, there is presently only one report in the literature of organizing pneumonia associated with Actinomyces infection (Alfaro et al., 2011). Our case is remarkable not only for the diagnosis by PCR analysis, but also for the associated histological finding. We suggest that actinomycosis should be included in the differential diagnosis for organizing pneumonia.

Conclusions

Actinomycosis can present as organizing pneumonia, and identification of infection by PCR analysis and rRNA gene sequencing is a useful strategy in cases that are difficult to diagnose.

References