Chronic progressive pulmonary paracoccidioidomycosis in a female immigrant from Venezuela

Ther Adv Respir Dis
2019, Vol. 13: 1–6
DOI: 10.1177/1753466619894913

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***Abstract*:** Paracoccidioidomycosis (PCM) is a fungal infection caused by *Paracoccidioides brasiliensis* and *P. lutzii*. It is endemic to South and Central America. While PCM frequently remains latent, the disease can reactivate years after the initial infection. As the disease is rare outside the endemic area, and symptoms can mimic other pulmonary diseases, correct diagnosis can be challenging for clinicians in developed countries. In this report, we present the case of a 57-year-old female Venezuelan immigrant with PCM. She was initially misdiagnosed with sarcoidosis and treated with corticosteroids, leading to an exacerbation of the infection requiring intensive care. Because cultivation of *Paracoccidioides* sp. is slow and unsensitive, we opted for microscopic observation of fungal elements and molecular testing on a tissue biopsy and *bronchoalveolar lavage* (BAL) together with antibody detection. This allowed the diagnosis of PCM, enabling specific management. PCM and other imported mycoses should be considered as a differential diagnosis in patients originating from South and Central America displaying symptoms suggestive of sarcoidosis.

The reviews of this paper are available via the supplemental material section.

Keywords: female, lung, paracoccidioidomycosis, sarcoidosis, Venezuela

Received: 2 July 2019; revised manuscript accepted: 15 November 2019.

Clinical observations

A 57-year-old woman was hospitalized due to dyspnoea, cough, and bilateral interstitial infiltrates on chest X-ray (Figure 1a). She moved from Venezuela to Germany a year prior to presentation. A chest computed tomography (CT) showed diffuse interstitial changes (Figure 1b). Flexible bronchoscopy revealed a black exophytic mass migrating through the left main bronchus wall. Subsequently, a peripheral lung biopsy was obtained using video-assisted thoracoscopy (VATS). Histological analysis of the peripheral lung biopsy by local pathology showed large interstitial granulomas, giant cells, and no necrosis or signs of vasculitis, compatible with sarcoidosis. In combination with elevated serum interleukin-2-receptor levels (1654 U/ml, normal 220–710 U/ml), pulmonary sarcoidosis was diagnosed and systemic corticosteroid therapy (1 mg per kg bodyweight) was initiated; 3 months later clinic and chest CT remained unchanged (Figure 1c), as did the lesion in the left main bronchus under bronchoscopy. A lesional biopsy revealed necrosis and inflammation. Viridans streptococci were isolated from the broncheoalveolar lavage (BAL), as well as low levels of unspecified yeast after 7 days of incubation. The latter was not found on microscopic analysis of the BAL or, at the time of testing, in the lesional biopsy. Meropenem was initiated but the patient developed progressive respiratory failure requiring noninvasive ventilation (NIV) and was transferred to our university clinic.

Subsequent re-examination of the initial (VATS) lung biopsy by our pathologists led to the detection of intracellular Grocott positive particles of 2–5 µm, accompanied by giant cell pneumonia, suggestive of a yeast infection. While initial stains showed small yeasts more in line with Histoplasmosis, more in depth examination of the

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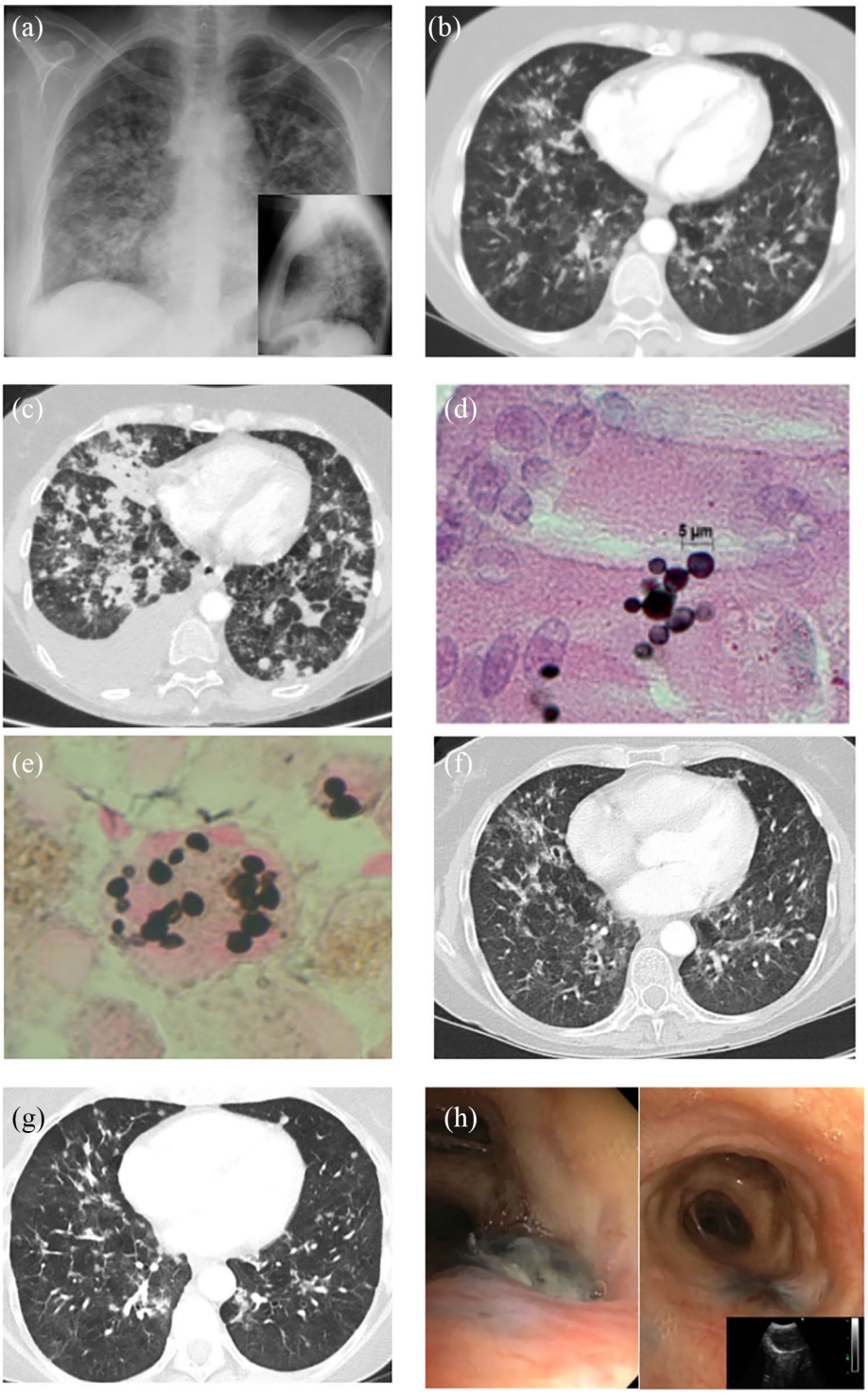

Figure 1. (a) Chest X-ray at initial presentation; (b) Chest-CT-Scan at initial presentation; (c) Chest-CT-Scan at readmission 3 months later, following 3 month treatment with prednisone (1 mg/kg bodyweight); (d) Histology of lung samples obtained during VATS at initial presentation, haematoxylin&eosin stain plus Grocott stain (dark nodules) demonstrate yeast cells with multipolar budding, bar indicates 5 µm; (e) Cytology of BAL sample obtained shortly after transfer to our clinic, Grocott stain (dark nodules); (f) Chest-CT-Scan 8 weeks after initiation of antifungal therapy; (g) Chest-CT-Scan 6 months after initiation of antifungal therapy; and (h) Endobronchial photography in the left main bronchus, showing an exophytic lesion before antifungal therapy (left panel) and on control bronchoscopy after 6 months of antifungal therapy (right panel).
BAL, broncheoalveolar lavage; CT, computed tomography; VATS, video-assisted thoracoscopy.

samples showed multi-budded yeasts (Figure 1d) more closely resembling Paracoccidioides, albeit not matching the textbook description of a multipolar 'pilot's wheel' configuration. Due to this ambiguity, we were unable to differentiate between these fungi by histology alone.

Due to the histologic finding, and since the patient had recently moved from a region endemic for dimorphic fungal pathogens, serologic testing was performed for histoplasmosis using immunodiffusion (ID) and complement fixing (CF) methods, as well as for paracoccidioidomycosis (ID) and coccidioidomycosis (ID, CF). Histoplasma and Paracoccidioides antibodies were detected by ID. However, a lack of antibodies against Histoplasma in the CF-test was interpreted as a likely cross-reaction in Histoplasma ID and evidence for paracoccidioidomycosis. CF testing for PCM was not available at our laboratory. In addition, a specific qPCR assay targeting the ITS-1 region of Histoplasma and broad range PCR assays targeting the 28S and ITS-2 region were performed from the formalin-fixed, paraffin-embedded (FFPE) lung biopsy as described previously.[1,2] While correct DNA extraction was shown by amplification of the human 18S rRNA gene, and an internal amplification control excluded PCR inhibition, both broadrange fungal PCRs amplified DNA. Negative Mastermix-, and extraction controls ruled out contamination with fungal DNA. Sequencing of both broad range PCR amplicons showed 99.7% pairwise identity with sequences of *Paracoccidioides brasiliensis* as the causative pathogen. The specific Histoplasma PCR did not amplify DNA.

In conclusion, the patient had paracoccidioidomycosis as suggested by the detection of antibodies, histology (Figure 1d,e) and PCR from FFPE tissue. Cerebral or abdominal involvement was ruled out by cMRI and abdominal ultrasound. Sarcoidosis was excluded after histopathological re-evaluation. We initiated a therapy using liposomal amphotericin B 150 mg once per day. Within 3 days, the respiratory situation improved and non-invasive ventilation was discontinued. After 3 weeks, therapy was switched to oral itraconazole 200 mg twice daily, and, after 8 weeks of antifungal treatment, oxygenation had returned to baseline and no additional oxygen supplementation was required. Chest CT showed decreasing pulmonary infiltrates (Figure 1f) which resolved completely after 6 months (Figure 1g). To monitor the lesion in the left main bronchus, another flexible bronchoscopy was performed after 6 months, showing a decrease in size of the fungal lesion (Figure 1h). However, in the BAL, yeasts were still detected by microscopy but fungal culture and PCR remained negative. Treatment with itraconazole 200 mg twice daily was continued.

Discussion

PCM is caused by *P. brasiliensis* and *P. lutzii*. Both PCM and histoplasmosis are endemic in South America, and are the most prevalent causes of systemic fungal infection in South America.[3] PCM is especially prevalent in rural areas of Venezuela, Colombia, and Brazil.[4] The infectious, conidia-forming forms of *Paracoccidioides* reside in the soil, and infection occurs upon inhalation along with dust, for example when working on or living close to a field.[4] A Brazilian study found that 93.7% of PCM patients had a history of prolonged living or working in rural areas.[5] By contrast, no evidence of host-to-host transmission has been found to date.[4] Annual incidence rates in endemic countries ranged from 1–4/100,000 inhabitants in Brazil, 0.8/100,000 inhabitants (Argentina), 0.81–3.08/100,000 (Colombia), and 0.52/100,000 (Paraguay).[6] In Brazil, the mortality of PCM has been estimated 1.45 per million inhabitants, with a lethality among PCM infected patients between 6.1 and 7.6% per year.[7,8] An Argentinian study from the 1980s found a post-treatment lethality of 2.2% in PCM patients treated with Ketoconazole[6,9]; however there are currently no more recent data available regarding PCM lethality and mortality outside Brazil.

In Europe, PCM was exclusively diagnosed in patients previously living in endemic areas, while travel-associated infections appear to be very rare.[10,11] Females are affected less often than males, most likely due to the presence of growth-inhibiting β-estradiol membrane receptors on the surface of *P. brasiliensis*.[12]

Besides histoplasmosis, sarcoidosis is one of the most important differential diagnoses, due to clinical, radiologic, and histologic similarities. In fact, the cutaneous multifocal infiltrative subtype of PCM has been explicitly described as sarcoid-like in the literature.[13,14] However, sarcoidosis has a lower prevalence in South America than in Europe or North America.[15,16] Therefore, a history of living in South America is more suggestive for PCM than for

sarcoidosis. While there are no previous case reports of concomitant PCM and sarcoidosis, sarcoidosis and PCM are unlikely to be mutually exclusive. While severe infections are fortunately relatively rare in sarcoidosis patients, fungi have been shown to be the second most common cause of infections following mycobacteria in sarcoidosis patients.[17] While studies so far have implicated globally more common fungal pathogens like *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Pneumocystis jiroveci*, and *Histoplasma capsulatum*,[17,18] simultaneous PCM and sarcoidosis is not inconceivable, thus complicating proper diagnosis, especially in patients from regions endemic for *Paracoccidioides*.

Another important feature impeding the diagnosis of PCM is its long latency period: While 1–2% of patients infected with PCM develop symptoms shortly after infection, in most cases, PCM infection remains inapparent.[8,19] Chronic PCM is usually asymptomatic and may present as pulmonary infiltrates and localized fibrosis on X-ray or CT-scans. However, a chronic infection can reactivate after months to years of latency. While unifocal PCM infections occur in up to a quarter of patients, with the lung being the most commonly affected organ, the majority of patients suffer from multifocal PCM infections. Aside from the lung, the upper airway mucosa, thoracic lymph nodes, brain, adrenal glands and skin are most often affected. PCM has been reported to disseminate both hematogenously and *via* the lymphatic system from its primary infection site.[20]

PCM can be diagnosed by direct microscopic detection of typical fungal elements, that is, yeasts of varying size with multipolar budding, referred to as a 'pilots wheel' in some yeasts but this form may not be present in samples containing a low amount of fungi. As an example, the fungal morphology observed in the histology of our patient displayed only one or two buds, resembling the description by Guarner and colleagues, instead of the classic multi-budded 'pilots' wheel' configuration.[21] That PCM may not show the growth pattern in histology formerly thought to be pathognomonic highlights the importance of a multipronged diagnostic approach of serology, PCR and histology for reciprocal confirmation.

Immunohistochemistry using antibodies specific for fungal antigens may help achieving the diagnosis in the absence of a characteristic tissue morphology.[21] Due to the slow growth rate of *Paracoccidioides* of up to 4 weeks *in vitro*, histopathology results may often be available earlier than culture results.[20,21] In addition, molecular methods such as PCR[22] can be used on FFPE tissue specimens to indicate the presence of suspected fungal pathogens, allowing for a diagnosis in the absence of a typical histomorphology of paracoccidioidomycosis.[21] In many cases, where it is not possible to isolate the fungus in culture or histopathological tests, or where additional diagnostic certainty is desired in the face of histopathological findings that are suggestive, but not definitive, for PCM, serological tests are great tools that aid in the diagnosis of PCM. Immunodiffusion and complement fixing tests are frequently used to diagnose PCM and differentiate this infection from Histoplasmosis. However, cross reactivity between both fungi, and the potential for false negative antibody tests (i.e. in PCM caused by *C. lutzii*, in localized infections or in immunocompromised patients), may impair their usefulness.[23]

All in all, PCM is a rarity in Europe, and, therefore, requires a high index of suspicion for timely diagnosis, and thus for effective therapeutic intervention. We used intravenous liposomal Amphotericin B for the acute therapy of PCM. Azol-derivates like Itraconazole and Voriconazole or sulfonamides have been shown to be effective treatment alternatives.[24–27] Maintenance therapy for 6–24 months is mandatory.[28] After transitioning to oral therapy, drug level tests should be performed.[29] Following acute infection, 60% of patients develop pulmonary fibrosis.[30]

In retrospect, it seems likely that our patient was infected years ago in Venezuela and remained initially asymptomatic. After initiation of systemic steroid therapy for suspected sarcoidosis, the infection was likely accelerated, leading to the need for readmission and ultimately noninvasive ventilation therapy 3 months later.

Conclusion

In this case report, we present the history of a Venezuelan patient with PCM who was initially misdiagnosed with sarcoidosis due to her combination of respiratory symptoms and fitting radiologic and histologic findings. After 3 months of corticoid therapy, her condition worsened progressively, ultimately requiring intensive care. Following a reassessment of her case and an extensive immunologic and histopathologic search the diagnosis of PCM, a mycotic infection endemic in

South and Central America, was made. Antifungal therapy was initiated, and the patient's symptoms improved. When infection with PCM becomes symptomatic, it frequently mimics sarcoidosis both clinically and in the histopathology. Therefore, imported mycotic infections including PCM should be considered as differential diagnoses in patients originating from South and Central America displaying symptoms of sarcoidosis.

Authors' contributions

Authors Moritz Kayser and Volker Rickerts contributed equally.

Funding

The author(s) received no financial support for the research, authorship, and publication of this article.

Conflict of interest statement

The author(s) declare that there is no conflict of interest.

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Supplemental material

The reviews of this paper are available via the supplemental material section.

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