Published in final edited form as:

Hum Pathol. 2018 January ; 71: 55–59. doi:10.1016/j.humpath.2017.07.013.

Arthroconidia in Lung Tissue: An Unusual Histopathological Finding in Pulmonary Coccidioidomycosis

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Abstract

Coccidioides immitis/posadasii presents in mycelial form with branching hyphae and arthroconidia when cultured in the laboratory. On histopathology, the presence of endospore-containing spherules is considered diagnostic of coccidioidomycosis. Here we report an unusual case of coccidioidomycosis with hyphae and arthroconidia in pulmonary tissue sections. A 49-year-old male patient with intermittently treated pulmonary coccidioidomycosis sought treatment for residual pulmonary complaints. A cavity in the left upper lobe was seen on CT scan. Due to minimal improvement of symptoms despite treatment with fluconazole, a left upper lobectomy was ultimately performed. *Coccidioides immitis/posadasii* was identified by culture and DNA-probe from the lobectomy specimen. The histopathology showed a fibro-cavitary lesion, with arthroconidia and hyphal structures, but no typical endospore-forming spherules. While an uncommon, *C. immitis/posadasii* may present with hyphae and arthroconidia on histopathology. Pathologists should be aware of this unusual presentations; culture remains the most reliable method for definitive diagnosis.

Keywords

Coccidioides immitis; Coccidioidomycosis; arthroconidia; histopathology of endemic fungi

1. Introduction

Coccidioides immitis and *C. posadasii* are thermally dimorphic saprophytic fungi that are known to cause a diverse spectrum of disease in humans [1]. These two *Coccidioides* species are endemic in the arid regions in the southwestern United States as well as parts of Mexico and Central and South America [2]. While *C. immitis* primarily occurs in central and southern California, with extension into Baja California, it is proposed that *C. posadasii* is commonly present throughout the entire southern and western United States, including

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Arizona and Texas, with extension to Central and South America. Both organisms are dimorphic fungi that grow in the environment (at “room temperature”) as molds with branching hyphae [1,2]. Alternating segments of the hyphae differentiate into spores, called arthroconidia, while the other, alternating segments ultimately undergo autolysis. By this process, the organism produces very stable, easily dispersible, barrel-shaped arthroconidia that are approximately 3 to 5 μm in size and may remain viable for many years [2]. In the southwestern U.S., the incidence of coccidioidomycosis varies greatly by geographic region and season. Smaller epidemics in these endemic regions have been described following events that facilitate the dispersion of arthroconidia, such as dust storms, earthquakes, and earth excavation activities [2].

When airborne arthroconidia are inhaled by humans, the organism initiates isotropic growth from the arthroconidium and transforms itself by remodeling its cell wall into a specialized structure called a spherule [1,2]. Over the following few days, the spherule expands in size and undergoes numerous nuclear divisions, producing large (approximately 100 μm in diameter) structures filled with up to 300 endospores. Upon maturation, the larger spherule ruptures, and the smaller endospores are released, subsequently developing into large spherules themselves. Small endospores can also be disseminated locally throughout the lungs (primary site of infection) or spread hematogenously to other organs.

Mold colonies of *Coccidioides* initially appear gray and glabrous but generally develop a white, floccose morphology as they age; some colonies may take on a red color [3]. Alternating arthroconidia are readily appreciated by microscopy of Lactophenol Cotton Blue stains prepared from the mold growing on routine laboratory fungal media. On tissue sections stained by Hematoxylin and Eosin [H&E] stain, or by special stains for fungi (e.g. Gomori Methenamine Silver [GMS] stain), the endospore-forming large spherules are readily appreciated [1–3].

Here we report an unusual case of *Coccidioides* presenting with an unusual histologic appearance (mycelial structures) in tissue sections from a pulmonary cavitory lesion.

2. Materials and Methods

Routine bacterial and fungal cultures from the initial BAL and TBB specimens, as well as from the subsequent partial lobectomy specimen, were performed in the BIDMC clinical microbiology laboratory, following BIDMC laboratory procedures as well as standard microbiological procedures [4]. Specimens were inoculated onto trypticase soy agar supplemented with sheep blood, chocolate agar, Sabouraud dextrose agar, and inhibitory mold agar. The routine agar plates for bacterial growth were incubated at 35°C with 5% CO_2 ; the agar plates for fungal cultures were incubated at 30°C in ambient air. Once mold growth was seen on the routine bacterial and fungal agar plates, a cellophane tape mount of the organism was prepared and stained with Lactophenol Cotton Blue. In addition, histopathologic examinations of the specimens were performed; tissue sections were stained with Hematoxylin and Eosin (H&E); Gomori Methenamine Silver (GMS); and Ziehl-Neelsen for Acid Fast Bacilli (AFB).

3. Case Report

Here we describe a 49-year-old male patient with a history of medically-treated pulmonary coccidioidomycosis. The patient was seen at the Infectious Diseases clinic at Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, for evaluation of a chronic productive cough. He had initially presented to care at a different hospital approximately five years earlier with similar symptoms, at which time computed tomography (CT) of the chest had revealed a cavitary left upper lobe lung lesion. *Coccidioides* was grown in sputum culture, and a serological test for *Coccidioides* was positive. Surgical resection was recommended, but the patient declined the procedure at that time and was treated with itraconazole, followed by fluconazole. However, the patient did not adhere consistently to the treatment protocol over the next several years. Upon presentation to the Infectious Diseases clinic at our hospital, the patient's physical examination and vital signs were unremarkable, with no abnormalities noted on auscultation of the lungs. Otherwise, his medical history was only notable for type II diabetes mellitus. He was originally from Massachusetts, where he was currently residing; however, upon further questioning, the patient noted that he had lived in Arizona for several years, while he had worked in construction. A chest CT at the time of current evaluation showed a $6.5 \times 3.5 \times 4.5$ cm irregularly-shaped, thick-walled cavitary lesion in the left pulmonary apex associated with fibrosis, bronchiectasis and pleural thickening.

The patient then underwent a bronchoalveolar lavage (BAL) and left upper lobe transbronchial biopsy (TBB). The primary aerobic bacterial culture from the biopsy specimen grew mixed bacterial flora; mycobacterial cultures were negative. The histopathologic examination, including H&E, GMS and AFB stains of the BAL and TBB specimens, were negative for bacterial, fungal, and/or mycobacterial organisms. For the corresponding bacterial and fungal culture from the initial BAL and TBB specimens, and following a three-day incubation period of agar plates, growth of a mold was observed on both standard bacterial agar plates, as well as on Sabouraud dextrose agar and inhibitory mold agar. Macroscopically, the colonies had a cottony, grayish to white aerial mycelium, which developed a slightly pinkish color after a few days. Microscopic examination of the Lactophenol cotton blue staining of the aerial mycelium revealed alternating arthroconidia (Figure 1), indicative of *Coccidioides*. The isolate was subsequently identified at the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio by DNA probe as *Coccidioides immitis/posadasii*. Susceptibility testing was performed at the Fungus Testing Laboratory using a microbroth dilution method following CLSI standard procedures. Minimum inhibitory concentrations (MICs) for the following antifungal agents were determined: fluconazole, 16 µg/mL; itraconazole, 1 µg/mL; amphotericin B, 2 µg/mL.

At this point, the patient was restarted on antifungal therapy for presumed coccidioidomycosis. A regimen of oral fluconazole at 200 mg twice daily was prescribed; however, despite compliance with this treatment, the patient continued to experience a residual cough. Approximately one month later, he elected to undergo left upper lobectomy. The left upper lobectomy specimen demonstrated a 7×4 cm cavitary lesion. Histopathology of the lesion demonstrated multifocal necrotizing granulomatous inflammation with abscess formation. Hyphal forms with alternating arthroconidia were identified on tissue sections of

the dominant lesion that were stained with both GMS stain and standard H&E stain (Figures 2 to 4). In addition, budding-yeast-like structures were also seen; however, no large, endospore-containing spherules were present.

The patient's cough significantly and rapidly improved following surgical intervention and continued antifungal therapy.

4. Discussion

Coccidioides immitis/posadasii is endemic in the western United States (Arizona, Texas, New Mexico, Nevada, California), as well as in Central and parts of South America. The incidence of coccidioidomycosis in endemic regions in the U.S. varies greatly. Infection with *Coccidioides* has been reported to be asymptomatic in approximately 60% of all cases [1]. Symptoms and clinical presentation in patients with coccidioidomycosis range from viral-like illness to acute pneumonia. While the infection primarily manifests as a respiratory illness, rapid dissemination or the development of chronic manifestations frequently occur, specifically in patients with certain co-morbidities. Patients infected with human immunodeficiency virus (HIV) and recipients of immune-modulating or immunosuppressive drugs, including high-dose corticosteroids, are at high risk to develop chronic or disseminated disease. In addition, chronic structural lung diseases, cardiopulmonary diseases, and diabetes mellitus are also significant risk factors for development of more severe and/or chronic infection.

However, an estimated 95% of primary pulmonary coccidioidomycosis cases may resolve spontaneously, with or without antifungal treatment [1,2]. Approximately 5% of patients who have resolution of the initial pulmonary symptoms can develop a more chronic illness, including pulmonary nodules or cavitary lesions [1]. Nodules due to *Coccidioides* are difficult to differentiate from pulmonary malignancies, specifically when coccidioidomycosis has not been considered in the differential diagnoses previously. In many of these cases, the initial exposure to the organism is so remote that patients often fail to recall travel or residence in endemic areas, as was the case with the patient presented in this case report. Cavitary lesions of coccidioidomycosis can present in one of three forms: asymptomatic, symptomatic, and/or ruptures [1,5]. The last presentation represents a more serious complication, as rupture into the pleural space can cause hydropneumothorax. Finally, a small percentage of patients, as in the case described here, may develop chronic fibrocavitary disease. These patients typically present with a several month history of cough, fever, and weight loss [2,5].

The laboratory diagnosis of coccidioidomycosis is usually achieved by culture of the organism in the microbiology laboratory, or by histopathologic examination of tissue sections from suspicious pulmonary lesions. *Coccidioides* species can be grown on most selective and nonselective laboratory media (e.g. Sabouraud dextrose agar, potato dextrose agar, or inhibitory mold agar) [3,6]. In addition, the organism grows on routine media used for bacterial culture, such as blood agar and chocolate agar. The time to growth on agar plates varies with an average of 4–5 days. The colonies initially have a moist, gray, and membranous appearance, but quickly develop a white, cottony mycelium, which becomes gray

or tan/brown with age. Microscopically, *Coccidioides* exhibits coarse, septate, and branched hyphae; with age, thick-walled, barrel-shaped arthroconidia can be appreciated, allowing a presumptive diagnosis of *Coccidioides* species. Definitive identification can be achieved by immunodiffusion of exoantigens, or conversion from mycelial to spherule phase [3,6]. The latter test requires special laboratory media or animal inoculation studies. More recently, a molecular-based, genus specific probe test (ACCUPROBE®, GenProbe, San Diego, CA) has become available. None of the above tests differentiate between the species *C. immitis* and *C. posadasii* [3]. However, differentiation is usually not relevant for clinical purposes as both prognosis and antifungal susceptibility are similar [7,8]. Based on epidemiological studies, our patient most likely was infected with *C. posadasii* [8].

However, in many cases of coccidioidomycosis, the diagnosis is made as an incidental histopathologic finding in pulmonary biopsies of lesions suspicious for malignancy. In such cases, the presence of endospore-containing spherules is diagnostic of coccidioidomycosis. The spherules can be seen on H&E stains, but are usually better visualized by histochemical stains such as GMS or periodic acid Schiff (PAS) [3]. GMS is considered the most sensitive stain for the detection of fungi, including *Coccidioides*, in histopathologic preparations [3]. Hyphal forms of *Coccidioides* are usually not seen in histopathologic sections; the detection of hyphal forms and arthroconidia in tissue sections in our case were an unexpected finding and previously only described in the literature in a few case reports [7–13].

Many of the early histopathological descriptions of *Coccidioides* infection are found in case reports of surgical resections from military personnel stationed in endemic regions. H.R. Barnes published the first report of hyphal forms of *Coccidioides* in pathologic specimens in 1946 [12]. Subsequently, additional case series were published, with the largest study by Puckett in 1954, describing 34 cases with cavitory lesions and 30 cases with solid coccidioidomas in which hyphal structures were found on histopathologic examination in a significant number of cases [10]. It is furthermore noteworthy that in several of the cases describing hyphae and arthroconidia in tissue sections, the classic spherule forms of *Coccidioides* were mostly absent. Similarly, in our current case, the typical spherules filled with endospores were not identified in the tissue sections. In addition to the presence of hyphae with arthroconidia (Figures 2 and 3), many of the fungal elements seen on both H&E and GMS stained sections in our case had the appearance of broad-based budding yeast forms and septate hyphae and only rarely alternating arthroconidia (Figures 3 and 4).

Notably, atypical forms of *Coccidioides* have been reported in several other studies found in the literature [14–16]. Kaufman et.al. described in their case report and review that immature spherules of *Coccidioides* are frequently indistinguishable from budding yeast as seen in *B. dermatitidis* or *H. capsulatum* [17]. In their specific case, serologic tests were negative, and fungal cultures were not pursued. Thus they relied only on the histopathologic findings for the diagnosis of coccidioidomycosis. Ultimately, the investigators applied a *C. immitis*-specific fluorescent-antibody staining method for the diagnostic confirmation. In their conclusions, the authors furthermore emphasized the potential need for use of multiple methods for the diagnosis of coccidioidomycosis [17]. There is no definitive explanation to date for the ability of *Coccidioides* to form hyphal structures and/or arthroconidia in tissue. Most proposed explanations theorize that increased oxygen levels in cavitory lesions,

especially in those connected to the bronchial tree, and perhaps higher levels of humidity, play a role in allowing hyphal growth and suppression of spherule formation [9].

Therapeutic options for the management of coccidioidomycosis depend on the site and severity of infection, and range from observation in the case of mild or asymptomatic disease to systemic antifungal therapy and surgical resection in the case of cavitary disease [1, 5]. Antifungal agents recommended for the treatment of *Coccidioides* species include intravenous amphotericin and oral fluconazole or itraconazole [5]. While there are no established interpretive breakpoints for *Coccidioides* species, *in vitro* studies have shown MIC ranges and MIC₉₀ values, respectively, of 2–64 µg/mL and 64 µg/mL for fluconazole, 0.125–2 µg/mL and 1 µg/mL for itraconazole, and 0.125–2 µg/mL and 0.5–1 µg/mL for amphotericin B [17–19]. The patient presented in our case report had a history of intermittent therapy with azoles; this patient's clinical course, coupled with elevated MICs (fluconazole, 16 µg/mL; itraconazole, 1 µg/mL; amphotericin B, 2 µg/mL), suggests that a more resistant sub-population of *Coccidioides immitis/posadasii* may have evolved during the prolonged period of discontinuous antifungal therapy.

5. Conclusion

Here we presented an unusual case of coccidioidomycosis in a patient with remote history of travel and residence in a region endemic for *C. immitis/posadasii*. This case emphasizes the fact that *Coccidioides immitis/posadasii* can present with diverse and unusual histopathologic manifestations, which could lead to misdiagnosis. While the more common histopathologic form is that of large spherules filled with endospores, in some cases, specifically those with fibrocavitary pulmonary lesions, hyphal forms, budding-yeast-like forms, and arthroconidia may be observed. It is equally important for laboratorians and pathologists to remember that serologic tests are frequently negative in patients with chronic coccidioidal cavitary lesions, and that fungal culture remains the most reliable means to establish a definitive diagnosis.

Acknowledgments

Thea Brennan-Krohn was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health pediatric infectious diseases research training grant, T32HD055148.

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Highlights

- Hyphae and arthroconidia are an uncommon finding on histopathology in coccidioidomycosis.
- On histopathology endospore-containing spherules are usually seen in coccidioidomycosis.
- Classic spherule forms are usually absent, when hyphae and arthroconidia are seen.

Figure 1. Cellophane tape mount of the mold from a Saboureaud Dextrose agar plate; stained with Lactophenol Cotton Blue (100X magnification), demonstrating alternating, barrel-shaped arthroconidia of *Coccidioides* species.

Figure 2. Representative H&E section (100× magnification) taken from the 7cm dominant lesion within the lobectomy specimen. The background is largely comprised of an amorphous, necrotic background with surrounding neutrophilic and lymphocytic inflammatory cells. Rare, but clearly visible alternating arthroconidia of *Coccidioides* are seen within the debris. Faintly-outlined spherical and ovoid endospores are also seen within the debris.

Figure 3. Higher-power representative H&E section (200× magnification) highlights the alternating pattern of the arthroconidia structures in greater detail.

Figure 4. Representative GMS section (100× magnification) highlights both arthroconidia and endospore structures of *Coccidioides* within the necrotic and inflammatory debris. Compared to the H&E sections, the details of the arthroconidia are somewhat obscured by the heavy silver-staining.