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Cokeromyces recurvatus Identified in Lung Biopsy: Case Report of a Non-pathogenic Fungus, Highlighting Its Potential Histologic Mimics

Maksim Agaronov[1], Irene Ratkiewicz[1], Michael Lawlor[2], Richard W. Cartun[1], Jaber Aslanzadeh[1], and Mary Fiel-Gan[1]

[1]Department of Pathology and Laboratory Medicine and [2]Department of Infectious Disease, Hartford Hospital, Hartford, CT, USA

Abstract. We report a case of aspiration in a patient with gastric outlet obstruction due to pancreatic adenocarcinoma, in which three large yeasts were identified on tissue biopsy of the lung infiltrate. The histologic sections of the yeasts showed densely eosinophilic, round to oval, thick-walled structures with frayed borders and intra-cystic bluish inclusions. There was a background of mixed neutrophilic and eosinophilic infiltrate along with focal tissue necrosis. Our initial differential diagnoses included the usual large yeasts such as *Cryptococcus*, *Coccidioides*, and *Blastomyces*. Immunohistochemistry revealed reactivity to the Blastomyces antibody. Mycology studies eventually identified the organism as *Cokeromyces recurvatus*. Anti-fungal treatment was withheld with spontaneous resolution of the infiltrates. This case demonstrates the importance of using culture to speciate organisms identified on tissue, separating pathogens from non-pathogens and non-living artifacts in order for appropriate management.

Case Report

Our patient presented to the hospital with one-month history of heartburn, abdominal pain, nausea, and vomiting. The past medical history was significant for hypertension, hypothyroidism, and hypercholesterolemia. The patient is not known to have diabetes mellitus. The patient was a lifelong resident of Connecticut, U.S. with no recent history of travel within the country or internationally. After being admitted to the hospital, the patient underwent an upper GI endoscopy but during the procedure was found to have excessive gastric fluid and vomited repeatedly, resulting in termination of the procedure. Immediately subsequent to the endoscopy procedure, a chest X-ray to rule out an aspiration injury was performed but showed no acute findings. On his second day in the hospital, the patient had a CT scan of the chest, abdomen, and pelvis with contrast, which showed a 3.5 cm mass in the head of the pancreas.

Address correspondence to Jaber Aslanzadeh, PhD; Director Division of Microbiology, Department of Pathology and Laboratory Medicine, Hartford Hospital, 80 Seymour St., Hartford, CT 06102, USA; phone: 860 545 4128; e mail: Jaber.Aslanzadeh@clpct.com

A week after admission, a fine needle aspiration of the mass confirmed the diagnosis of pancreatic adenocarcinoma. On hospital day 8, a CT scan of the chest was performed as part of the staging work-up; it revealed numerous, variably-sized nodules scattered in both lungs, as well as patchy areas of ground-glass opacities predominantly involving the middle and upper lobes, suggestive of a metastatic, infectious, or an inflammatory process. On hospital day 9, further evaluation of the pulmonary nodules by CT-guided needle core biopsy was performed.

Microscopic Studies. Biopsy material from multiple cores of a single representative nodule was evaluated by tissue hematoxylin and eosin (H&E) stain to reveal focal areas of necrosis with an inflammatory process composed of a predominantly neutrophilic infiltrate with increased numbers of eosinophils and a scattering of lymphocytes **(Figure 1)**. The neutrophils and eosinophils were predominantly intra-alveolar with a minor component in the interstitium appearing to be a host response to the yeast. Associated with this inflammation were three non-budding yeasts, raising the concern for a

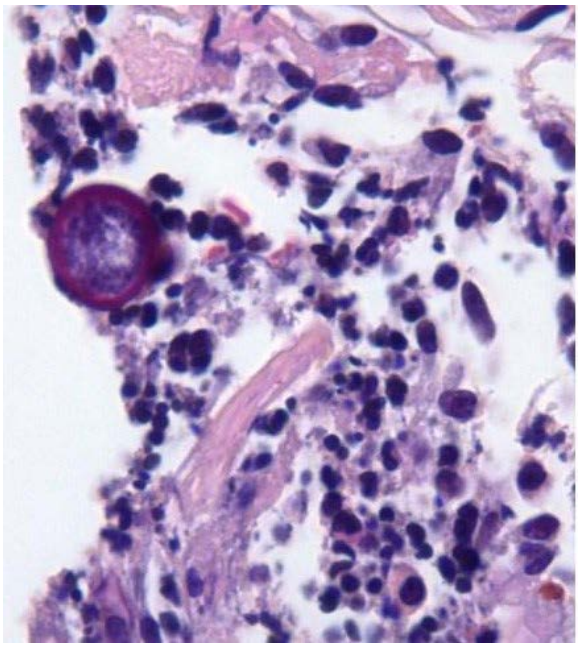

Figure 1. *Core biopsy of the lung demonstrating spherical yeast with a thick eosinophilic wall containing white-purple intra-cystic deposits. Surrounding intra-alveolar interstitial inflammatory infiltrate composed of predominantly neutrophils, eosinophils, and scattered lymphocytes. (Hematoxylin and eosin, 1000x).*

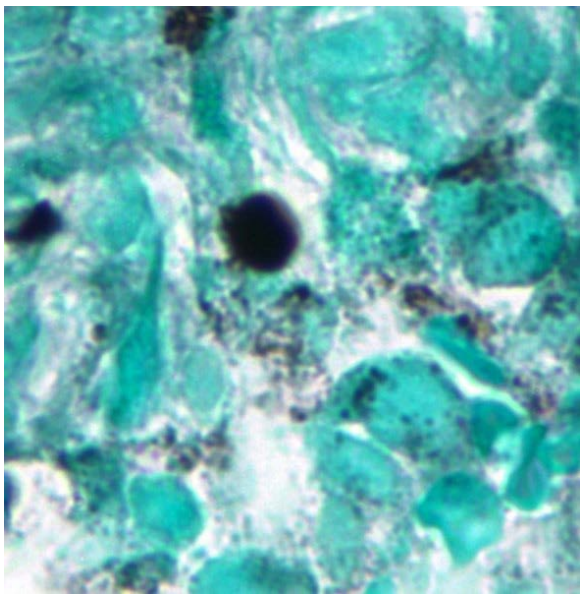

Figure 2. *Spherical yeast with frayed, wooly border. (Gomori's methenamine silver, 1000x).*

locally invasive or systemic fungal infection. The remaining tissue biopsy showed no fibrinoid deposits, granulomas, foamy histiocytes, vascular abnormalities, other infectious organisms, or any foreign body material. Although the patient has not had any chemotherapy at the time of biopsy, the presence of yeast within the tissue suggested an immunocompromised state. The yeast measured up to 35 microns in diameter and was found within the inflammatory infiltrate in both H&E **(Figure 1)** and methenamine silver-stained sections **(Figure 2)**. The rounded yeast forms were filled with pale to bluish, irregularly shaped structures. The wall was thick and densely eosinophilic on H&E, with a peculiar wooly border on Gomori's methenamine silver stain, which is better appreciated on Lactophenol cotton blue stain **(Figure 4)**. Using available immunohistochemical stains to narrow down the differential diagnoses, polyclonal antibodies directed against specific exoantigens of *Coccidioides, Blastomyces,* and *Cryptococcus* were performed. The results showed negative immunoreactivity to both *Coccidioides* and *Cryptococcus* but the *Blastomyces* immunostain was reactive, outlining the yeast wall **(Figure 5)**.

Mycology Studies. Aerobic, anaerobic, and mycobacteria tissue culture studies were all negative, but a mold was noted after seven days on Saboraud's dextrose agar plate incubated at 25°C. The preliminary identification of the mold was based on microscopic morphology prepared by taking a portion of the colony and mounting it in Lactophenol cotton blue and identifying the characteristic sporangioles emanating from a round vesicle **(Figure 3)**. Subsequently, a wrinkled white-yellow colony grew on Saboraud's dextrose agar after six days of incubation at 35-37°C in 5-7% CO_2.

Case presentation continued. Subsequent to the radiologic imaging studies and the diagnostic report from Microbiology, the patient was seen by a pulmonologist and an infectious disease specialist. Both recommended follow-up of the lung nodules by an interval non-contrast chest CT scan. Antifungal therapy was not recommended because *C. recurvatus* is considered non-pathogenic and treatment is considered if the patient is symptomatic. Once the fungus was categorized by Microbiology, confirmatory tissue immunohistochemistry revealed reactivity for Rhizomucor. Our initial diagnosis of large yeast consistent with *Blastomyces* had to be amended, and a subsequent surgical report was issued noting the fungus in the order Mucorales and referring to the microbiology report for species identification. On hospital day 19, the patient had a successful laparoscopic gastrojejunostomy and anastomosis to bypass the gastric outlet obstruction. On hospital day 24, a follow-up chest CT scan revealed interval resolution of the patchy ground-glass opacities further supporting the self-limited nature of infection with this organism. The patient was discharged on hospital day 26 and was scheduled to begin adjuvant chemotherapy.

Discussion

Cokeromyces recurvatus is a dimorphic fungus with unclear pathogenic potential. It belongs to the class Zygomycetes in the order Mucorales. This order of fungi includes more commonly recognized genus names like *Mucor*, *Rhizomucor*, and *Rhizopus*. *C. recurvatus* has been isolated only in North America and grows on the excreta of animals including lizards, rabbits, squirrels, and mice [1]. It is a large, thick-walled, spherical yeast that ranges from 30 to 90 microns in diameter on histologic sections and can morphologically mimic other fungi [2]. The fungus is thought to be acquired once the airborne fungal spores in the environment are inhaled and colonize the patient [1].

The mycology studies in our case initially revealed an aseptate mold with ribbon-like hyphae which was converted to a yeast form by inoculating a Saboraud's dextrose agar plate and incubating it at 37°C in 5-7% CO_2, confirming the dimorphic nature of this fungus [1-2,4]. A "mariner's wheel" pattern of budding large yeast was identified after six days, which is a characteristic finding in *Paracoccidioides brasiliensis* but is also seen with other fungi [2,3]. *P. brasiliensis* is primarily distributed in South America and Brazil and has a unique clinical presentation with chronic granulomatous disease of mucous membranes, skin, and pulmonary system. A definitive diagnosis with fungal polymerase chain reaction and DNA sequencing was not carried out because culture of the fungus was readily available, the lung nodules and opacities were self-limiting and resolved on their own supporting a non-pathogenic fungus, and the management of the patient would not change with results from this specific DNA testing.

Extensive review of the English literature through Medline search revealed 10 previously reported cases of *C. recurvatus* isolated from human sources. The anatomic sites where the organism was found include the cervix, vagina, genitourinary tract, pleural and peritoneal surfaces, gastrointestinal tract, and lungs. The organisms at these locations were predominantly colonizers or contaminants and showed no tissue invasion. Odronic et al. report two cases of *Cokeromyces recurvatus*

Figure 3. *Sporangiophores (not seen in this image) are 100-500 microns in length and terminate in a round vesicle (center of the image) with overlying recurving stalks. Each stalk holds spherical sporangioles (8-15 microns in diameter) containing sporangiospores. (Lactophenol cotton blue, 400x).*

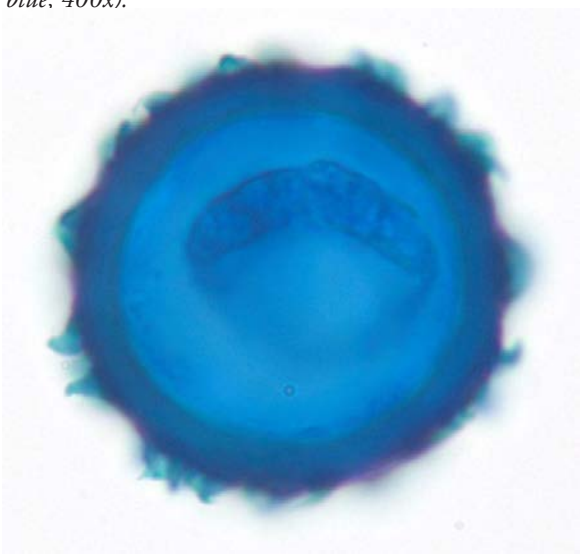

Figure 4. *Round yeast (25-65 microns in diameter) with multiple radially arranged sporangioles creating a wooly border. (Lactophenol cotton blue, 400x).*

in liquid-based Papanicolaou tests in two healthy, asymptomatic women [3]. Axelrod et al. report the fungus in multiple urine samples in a 72-year old male with chronic cystitis [11]. McGough et al. reported isolating *C. recurvatus* from a 14-year old young woman with vaginitis [12]. These three patients were treated with antifungal agents including

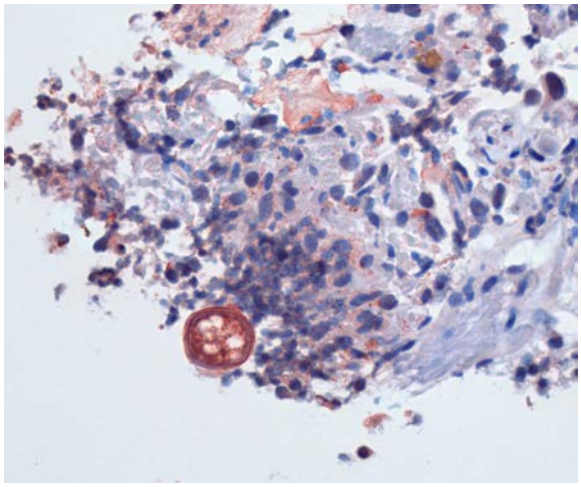

Figure 5. *Yeast wall and intra-cystic inclusions highlighted by immunostain for Blastomyces (Immunoperoxidase stain for Blastomyces, 400x).*

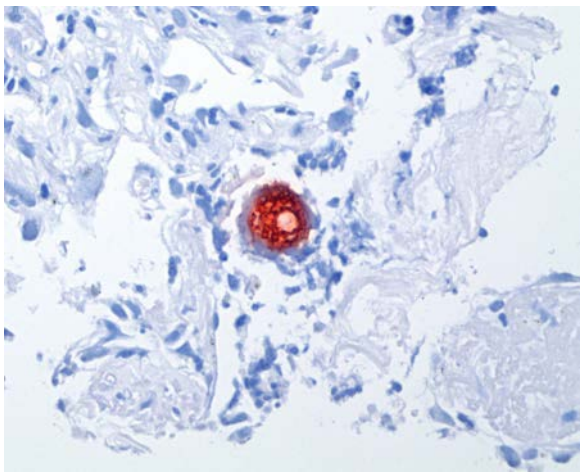

Figure 6. *C. recurvatus showing immunoreactivity with Rhizomucor monoclonal antibody, highlighting the intracystic inclusions. (Immunoperoxidase stain for Rhizomucor, 400x).*

amphotericin B or miconazole with subsequent resolution of their symptoms. In our case, the lung involvement by the yeast following the episode of vomiting and aspiration supports the theory of *C. recurvatus* being a colonizer of the gastrointestinal tract.

Although more commonly reported as a colonizer with symptoms heavily attributed to other organisms that are simultaneously identified, the pathogenic potential of this organism is illustrated in a prior case report in which the identification of *C. recurvatuswas* associated with a fatal pneumonia [1-3,5-8]. This occurred in a patient who had undergone stem cell transplantation, had graft versus host disease, and was on steroid therapy. The chest radiograph in this case report demonstrated lower lobe pneumonia unlike the non-specific nodular pattern with ground-glass opacities which was present in our case. In this case of fatal pneumonia from invasive *C. recurvatus*, the histological sections of the lung revealed prominent intra-alveolar neutrophilic exudate, areas of hemorrhage and necrosis, and multiple yeastlike forms of the organism [8]. No histiocytic or granulomatous inflammatory component was noted, and no angioinvasion was identified [8]. Other reported cases where *C. recurvatus* acted like a pathogen in immunosuppressed patients include the report by Alvarez et al. of a 46-year old patient with watery diarrhea one-month post-allogeneic bone marrow transplantation [6]. Tsai et al. also reported a case of a patient with diarrhea one-month after bone marrow transplantation for myeloma [13].

Upon review of the reported cases in the literature, patients who were colonized by *C. recurvatus* were treated when they were symptomatic or immunosuppressed. In the two cases of diarrhea post-bone marrow transplantation, tissue invasion was demonstrated, and both patients were treated with nystatin with subsequent recovery [11,13]. In our case, the patient was asymptomatic and although tissue invasion was suggested by the presence of necrosis, the fungal infection was self-limited and resolved on its own without antifungal therapy. Thus, the fungus appears to colonize tissue in healthy individuals and may invade tissue in patients with underlying debilitating conditions such as malignancy and immunosuppression.

With the pressing need to expedite the identification of the fungus, immunohistochemical studies to further investigate the differential diagnoses of large yeasts were performed. Immunostains using polyclonal antibodies to *Blastomyces*, *Coccidioides*, and *Cryptoccocus* were applied in which immunoreactivity to Blastomyces was seen, with negative

reactivity to both *Cryptococcus* and *Coccidioides*. Cases of cross-reactivity of culture-proven *C. recurvatus* with polyclonal antibodies to *Coccidioides* and *Blastomyces* were previously reported, highlighting the potential misinterpretations of using immunohistochemistry alone to make a positive fungal identification [9,10]. After obtaining the results from mycology studies, immunohistochemistry using a monoclonal antibody against *Rhizomucor* was performed, and it highlighted the yeast in the tissue **(Figure 5)**. Although the stain uses a monoclonal antibody, the positive reactivity is expected since the antibody is order-specific, and *C. recurvatus* belongs to the order Mucorales.

Given the lack of demand for fungal-specific immunohistochemistry, the cost of making the antibody, and the perishable nature of the antibody, it is not surprising that companies have decreased and even stopped production. Although at the time of testing our lab had all the fungal antibodies performed in this case, they are no longer a resource that is available to us. However, the lack of the availability of the fungal antibodies will not necessarily compromise patient care if the patient sample is correctly triaged upfront. This includes a sample for culture and histologic studies including cytology. In our case, the immunohistochemistry was pointing us toward a diagnosis of Blastomycosis and it wasn't until the culture grew *C. recurvatus* that a correct diagnosis was made and supported by an immunostain for *Rhizomucor*.

Review of the literature also reveals other ancillary studies that can speciate the organism in formalin-fixed paraffin embedded tissue. These include fungal polymerase chain reaction and DNA sequencing [3]. These nucleic acid-based tests rely on matching the exact nucleic acid sequence of the organism to sequences available in a GenBank sequence database which can more accurately characterize the organism [3]. Although the molecular methods, including DNA sequencing are subject to contamination and potential errors, these tests are particularly helpful when a specimen was not submitted for culture.

We demonstrate the importance of procuring tissue for fungal culture to accompany the corresponding histologic sample as the appropriate triage protocol to provide efficient patient care and minimize the number of tests ordered. We also want to emphasize the importance of communication, as the patient's clinical history, signs, and symptoms (provided to the Microbiology laboratory by the physician) swayed us from our initial impression of Blastomycosis due to a positive immunohistochemistry.

This case illustrates the need to have a broader differential diagnoses when yeast-like structures are encountered in histologic sections as well as the potential significance of recognizing yeast morphology in tissue, interpreting immunohistochemistry for fungi, and obtaining good clinical history. *C. recurvatus* in histologic tissue sections may present as large yeasts which vary in size, showing irregularly shaped intra-cystic structures (unlike the spherules in *Coccidiodes sp.* or the central multiple nuclei in *Blastomyces sp.*); thick, eosinophilic wall with wooly border on the Gomori's methenamine silver stain (unlike the sharply demarcated double cell wall in other large yeasts); show immunohistochemical reactivity to *Rhizomucor* monoclonal antibody; and produce a prominent neutrophilic and/or mixed neutrophilic and eosinophilic host reaction.

Note: The immunohistochemical markers for the identification of *Coccidioides*, *Blastomyces,* and *Cryptococcus* were polyclonal antibodies, and those for *Rhizomucor* were monoclonal antibodies. To the best of our knowledge, these antibodies are currently not commercially available, so obtaining tissue for culture, histologic assessment, and potentially DNA sequencing are more significant in being able to make a correct diagnosis.

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