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CLINICAL CASE

Tomography guided biopsy for the diagnosis of pediatric pulmonary *Cryptococcosis*

Punción Biopsia guiada por tomografía para el diagnóstico de *Cryptococcosis* pulmonar pediátrica

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What do we know about the subject matter of this study??

Pulmonary cryptococcosis is a lung infection caused by the opportunistic fungus *Cryptococcus neoformans*. It is a condition rarely suspected and underdiagnosed in immunocompetent children.

What does this study contribute to what is already known??

In immunocompetent patients with nonspecific clinical presentations, epidemiological history such as rural origin and contact with bird droppings, combined with imaging, can guide the diagnosis of pulmonary cryptococcosis. Etiological confirmation is essential, for which various techniques such as fine-needle aspiration biopsy are relied upon.

Abstract

Pulmonary cryptococcosis is a lung infection caused by the *Cryptococcus* yeast. It is rare in pediatrics, especially in immunocompetent children. The diagnosis of pulmonary cryptococcosis can be challenging due to the low specificity of symptoms, low index of suspicion, and limited diagnostic resources. **Objective:** To describe a clinical case of pulmonary cryptococcosis in an immunocompetent adolescent, detailing the diagnostic approach. **Clinical Case:** A 15-year-old patient, previously healthy, from a rural town, who consulted due to cough and a 1-month rib stitch pain, without fever or associated respiratory difficulty, with two images of condensation in the left lung on the chest x-ray. In the Computed Tomography, the images showed a nodular appearance. Due to suspicion of neoplastic pathology, a Positron Emission Tomography was performed, which showed hypermetabolic nodular lesions. The tomographic characteristics could correspond to fungal or granulomatous involvement. Considering the images and epidemiological risk factors such as rural origin and contact with bird droppings, the possibility of a mycosis was considered. A lung needle biopsy was performed under tomographic guidance. *Cryptococcus neoformans* was identified in the microbiology laboratory culture. The patient received treatment with itraconazole and fluconazole with good clinical and imaging response after 10 months of therapy and follow-up. **Conclusion:** In immunocompetent patients with a nonspecific clinical presentation, images can guide the diagnosis of pulmonary cryptococcosis, and an etiological search is essential to confirm it. In our case, the CT-guided needle biopsy was of great diagnostic utility.

Keywords:
Cryptococcosis;
Cryptococcus neoformans;
PET-CT Scan;
Fine Needle Biopsy;
Infectious Disease;
Mycoses;
Pneumonology

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EDITORIAL IKU

Introduction

Cryptococcosis is a systemic mycosis that occurs mostly in adults aged 20 to 50 years and immunocompromised individuals but is increasingly found in immunocompetent hosts[1]. It appears to be less common in children, and there is limited literature on this disease in this population. The reason for this apparent lower frequency may be due to differences in exposure to the fungus and the host's immune response. The incidence in adults is approximately between 1% and 5% of the population, while in children, it is less than 1%[2]. It is caused by species complexes of *Cryptococcus neoformans* and *Cryptococcus gattii*. *Cryptococcus* is an encapsulated yeast widely distributed worldwide, found in tree hollows, flowering plants, and bird droppings[3].

Some *Cryptococcus* spp. are pathogenic to humans and generally cause meningitis and respiratory tract infections. The mode of entry is usually through the inhalation of spores and dry yeasts that can reach the lower respiratory tract, triggering a response depending on the host's immune status[4]. Normally, immunocompetent individuals resolve the infection, although it may persist as a granuloma and, depending on immunity and associated risk factors, can reactivate and cause progressive disseminated infection. In patients with human immunodeficiency virus (HIV) with significant alteration of helper T-lymphocyte immunity, the infection can spread beyond the lungs[4]. This pathogen-host relationship allows it to manifest as asymptomatic colonization to disseminated disease, mimicking other pathologies, making detection difficult, and requiring different diagnostic tools to obtain the etiology.

The most common infection is central nervous system (CNS) involvement, followed by disseminated and pulmonary cryptococcosis[5-12]. Meningoencephalitis can present with clear cerebrospinal fluid (CSF) and be confused with a herpes infection, delaying its treatment, and potentially resulting in severe irreversible sequelae[5].

There are various reports of cases in South America, highlighting the higher frequency in patients with HIV and meningeal involvement[5-9]. The largest paper on disseminated cryptococcosis in previously healthy children describes 52 patients in 20 years in China, with the lung as the most frequently affected organ, in 96% of cases[10]. This does not mean that they have pulmonary cryptococcosis but rather that they usually have some type of lung involvement. An Australian series describes 22 pediatric patients with cryptococcosis where 82% had meningitis, being in the same percentage, apparently immunocompetent[11]. In India, in 6 years of follow-up of 5420 specimens processed due to suspected cryptococcosis, they had 21 episodes (0.39%) in 15 pediatric patients of which 10 were apparently immunocompetent[12]. Until 2022, 125 studies in English identified a total of 1134 cases of pediatric cryptococcosis worldwide[12].

The diagnosis of pulmonary cryptococcosis can be challenging due to the low specificity of symptoms, low index of suspicion, and limited diagnostic resources. As symptoms are nonspecific, they are likely to cause delays in diagnosis and treatment, resulting in increased disease dissemination. The host's immune condition is a determining factor. It is very rare for immunocompromised patients to present asymptomatic.

The objective of our work is to describe a clinical case of pulmonary cryptococcosis in an immunocompetent adolescent, with special emphasis on the tools used in the diagnostic approach.

Clinical Case

A 15-year-old patient, previously healthy, with no medical history of note, and complete vaccination scheme for his age. Originally from a rural area in the Province of Buenos Aires, Argentina. His father worked in the fields. They had dogs and chickens at home. Among eucalyptus groves, there was a shed where seed bags were stored, and there were usually pigeons.

The patient presented with a one-month history of productive cough and side stitch pain, with no fever or associated respiratory difficulty. Initially, a chest X-ray (Figure 1) showed two consolidation images in the left lung base. A chest CT scan was performed, revealing nodular consolidation in the left base (Figure 2). With suspicion of pneumonia, antibiotic treatment with Amoxicillin was initiated, and the patient was referred to our institution.

The CT scan images were interpreted as pulmonary nodules and a positron emission tomography (PET) was performed with 18-fluorodeoxyglucose to measure metabolic activity and identify glucose uptake in tissues through the standardized uptake value (SUV). Neoplastic cells, as well as inflammatory and infectious processes, show increased metabolism in this study[13]. The exam revealed multiple lung lesions in the left lower lobe with increased metabolism according to SUV ranging from 10.88 to 13.53 (Figure 3). No lesions were found in the CNS, abdomen, or pelvis. The tomographic characteristics could correspond to fungal or granulomatous involvement.

Complete laboratory tests were within normal range (complete blood count, liver function, renal function, electrolyte panel, coagulation tests), with negative acute phase reactants. Abdominal, kidney, tes-

ticular, and thyroid ultrasounds were performed without pathological findings. In the context of studying lung nodules, tuberculosis screening was performed, with no evidence of close contact for this disease; purified protein derivative (PPD) intradermal reaction resulted negative (0 mm); serial sputum bacilloscopies by Ziehl-Neelsen staining and culture were negative.

Given the images and epidemiological risk factors such as rural origin and contact with bird droppings, the possibility of mycosis was considered. Bronchoalveolar lavage was performed, from which samples were collected for microbiological study. The culture was negative for bacteria, fungi, and mycobacteria. Serological tests for *Aspergillus* in bronchoalveolar lavage were negative, as well as PCR for *Histoplasma*.

A CT-guided lung biopsy was performed. Direct mycological examination and Giemsa staining showed yeasts. Culture of the biopsy was positive for *Cryptococcus neoformans* variety *grubii* (Figure 4) sensitive to fluconazole (minimum inhibitory concentration 4ug/ml) and itraconazole (minimum inhibitory concentration 0.06ug/ml). Pathological anatomy showed a pyogranulomatous inflammatory process with the presence of multinucleated giant cells and round structures that stained positively with periodic acid-Schiff (PAS) staining, which could correspond to a deep mycosis, without neoplastic cells.

As the patient had no history of immunocompromise, an immunological study was indicated showing a lymphocyte count (B cells, CD3, CD4, CD8, and NK cells) within normal limits, as well as quantification of Immunoglobulins IgG, IgA, IgM, and IgE, and determination of complement C3, C4, and CH-50. The HIV test by Elisa method was negative.

Due to elevated cryptococcal antigen titers in serum (1:128), a central nervous system CT scan was performed, with normal results. Lumbar puncture showed normal physical-chemical results, negative India ink staining, cryptococcal antigen detection in CSF, and negative culture. Serum and CSF antigen analysis was performed at the *Hospital de Infecciosas Francisco Javier Muñiz*, in Buenos Aires, and analysis was done by latex agglutination test.

Treatment with Itraconazole 200mg per day was initiated for 4 weeks, until a definitive diagnosis was obtained, followed by 10 months with fluconazole 200mg per day. There were no adverse effects to the treatment.

At 6 weeks of treatment initiation, a metabolic reassessment by PET of the lung lesions showed that the nodular opacities were smaller with SUV metabolism of 3.01 to 5.19. No new foci were present (Figure 5). The patient had resolution of the initial symptoms and remained clinically asymptomatic.

At the end of the 10-month treatment, a chest X-ray

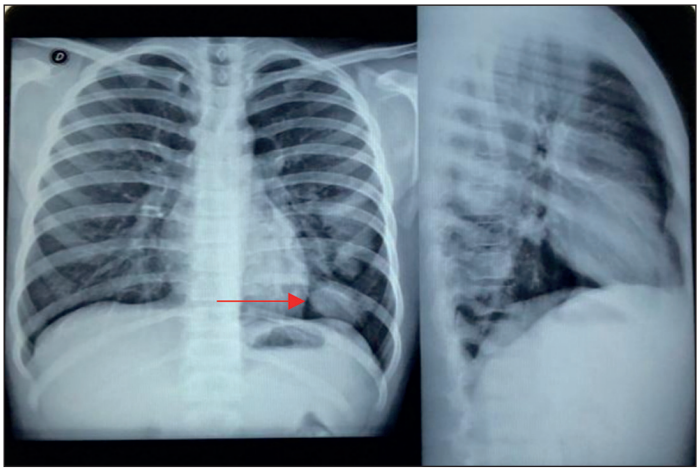

Figure 1. Initial Chest X-ray: Nodular images at the left lower lung lobe.

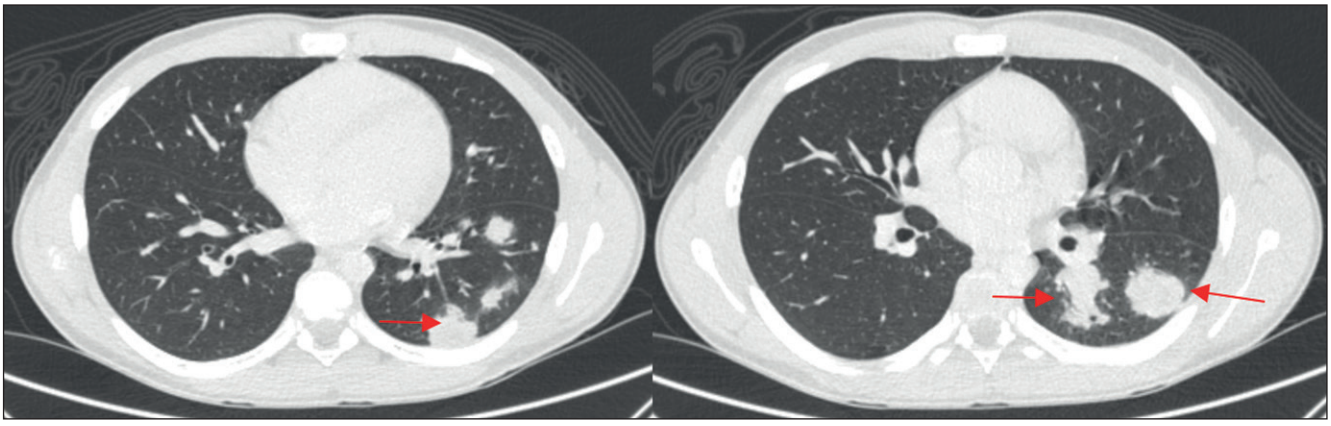

Figure 2. Initial CT scan: Multiple lung lesions of nodular appearance with lobulated borders in the left lower lung lobe.

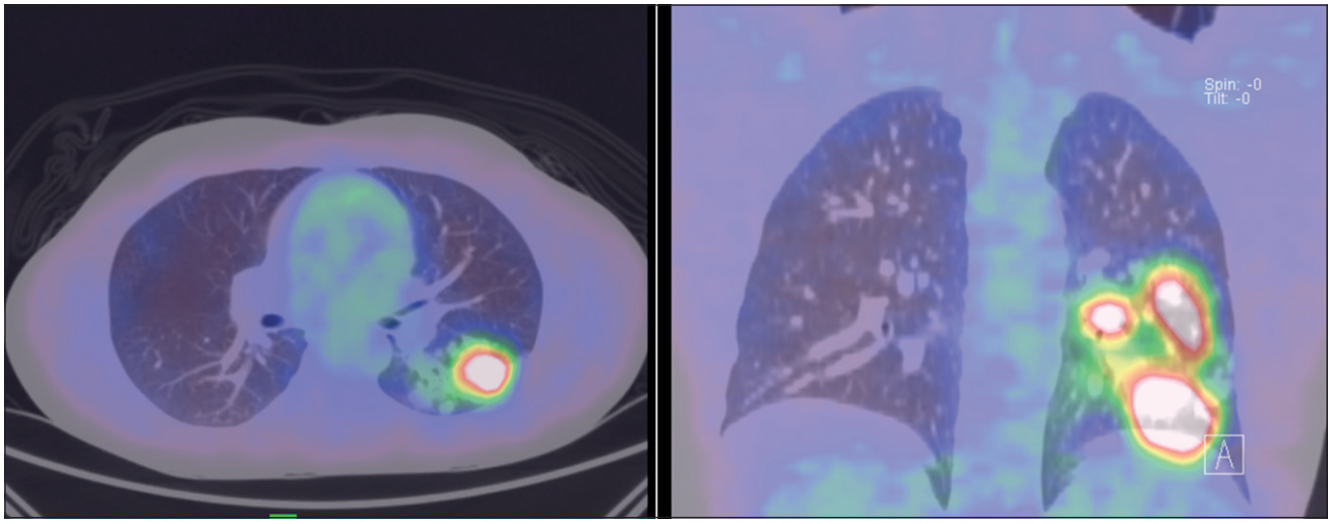

Figure 3. Positron Emission Tomography (PET-CT): Hypermetabolic lung lesions in the left lower lobe, which, due to the tomographic characteristics, could correspond to fungal or granulomatous involvement.

showed no pathological findings, and antigen search in blood was negative.

Discussion

Cryptococcosis in pediatrics is a rare disease. In children without HIV, the incidence is around 0.85%, and in children with HIV, it ranges from 5% to 10%[14]. Patients with HIV and solid organ transplant recipients are those who suffer from this disease the most[15].

Pulmonary cryptococcosis usually occurs with non-specific symptoms such as cough, dyspnea, fever, and chest pain. It can be totally asymptomatic or present with respiratory failure depending on localized, disseminated, and/or pleural parenchymal involvement. Lung lesions can progress, spontaneously regress, or remain stable for extended periods[15].

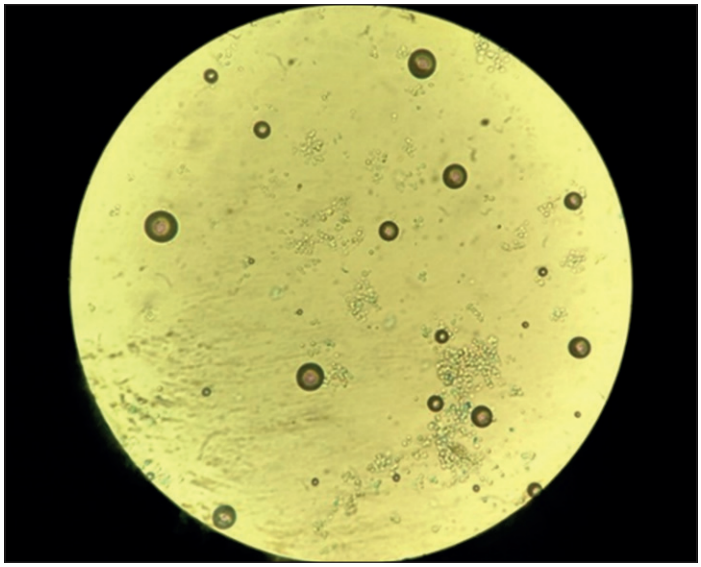

Figure 4. Culture from lung puncture with a fine needle, in which cryptococcus yeasts are observed at 37ªC.

Radiological images of pulmonary cryptococcosis may be similar to other infections caused by bacteria, fungi, parasites, mycobacteria, or viruses. They can also be confused with tumor processes, inflammatory reactions, or abscesses[16]. The most frequent tomographic findings are peripheral lung nodules. Nodules can be single or multiple, with calcified areas, with a tree-in-bud pattern, adenopathies, and pleural effusion[16]. PET is mostly performed to identify tumors since neoplastic cells have increased metabolism, but it also serves to identify inflammatory and infectious processes, as in this case, with a sensitivity of 77-92%[13]. Lung nodules can test positive for F-fluorodeoxyglucose uptake similar to lung cancer[13, 17].

Immunocompromised patients present a wide range of abnormal images on chest X-rays and CT scans, including single or multiple nodules, segmental consolidation, cavitatory lesions, bilateral pneumonia, mass pattern, or diffuse miliary pattern.

Laboratory methods used to confirm the infection include culture, direct microscopy, histopathology, serology, and molecular detection. Once pulmonary cryptococcosis is diagnosed, a lumbar puncture is recommended to study CSF in all patients. If the patient with pulmonary disease is immunocompetent, asymptomatic, and has negative or very low cryptococcal antigens, lumbar puncture could be omitted. In our case, the patient had high serum cryptococcal antigen titers,

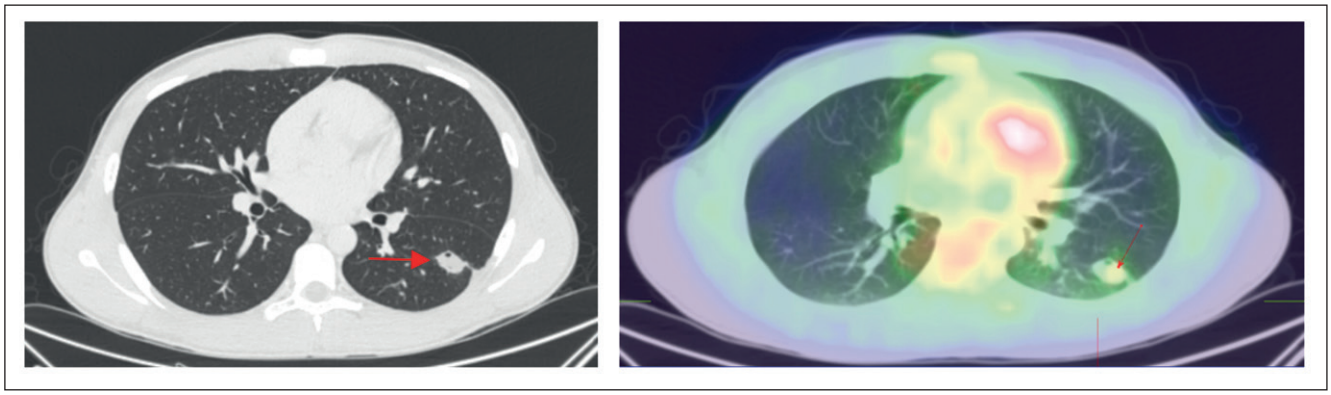

Figure 5. Positron Emission Tomography (PET-CT) after 6 weeks of treatment, where the reduction of the images is evident, with less metabolism.

so a lumbar puncture was performed, showing normal results.

A lung biopsy is the best diagnostic option when sputum and bronchoalveolar lavage samples are negative or unavailable. It can be percutaneous, transbronchial, thoracoscopic, or open, depending on the availability, experience, and location of the lesions. In our case, the patient had accessible peripheral nodules for percutaneous biopsy, performed under tomographic guidance, collecting a good sample for pathological anatomy and culture.

The 2010 IDSA guidelines for the management of cryptococcal meningitis in non-HIV and non-transplanted pediatric patients recommend induction therapy with amphotericin B (0.7-1mg/kg/d) plus flucytosine (100mg/kg/d every 6 hours orally) for 2 weeks followed by consolidation with fluconazole (6mg/kg/d) for a minimum of 8 weeks and then maintenance therapy with fluconazole (3mg/kg/d) for at least 6 to 12 months. For pediatric pulmonary cryptococcosis, fluconazole 6 to 12mg/kg/day orally for 8 to 12 months is recommended. Persistent positive cryptococcal antigen is not a criterion for continuing treatment.

In severely ill non-immunocompromised adults with pulmonary disease, treatment similar to CNS infection is recommended. If fluconazole is not available or contraindicated, itraconazole 200mg every 12 hours orally, voriconazole 200mg every 12 hours orally, and posaconazole 400mg every 12 hours orally are accepted as alternatives. Surgery may be considered for diagnosis or if radiological abnormalities and symptoms persist despite antifungal treatment. In non-immunocompromised patients with pulmonary cryptococcosis, consider lumbar puncture to rule out asymptomatic CNS involvement. However, in normal hosts with asymptomatic lung nodule or pulmonary infiltrate, no CNS symptoms, and negative or very low serum cryptococcal antigen, lumbar puncture could be avoided[18].

In this case, treatment was initiated with itraconazole because initially, pulmonary histoplasmosis could not be ruled out. On the same day as the biopsy, microbiology confirmed the presence of yeasts on direct examination, but histoplasma was also a possibility based on appearance. Then, with the diagnostic confirmation and *Cryptococcus* sensitivity, having ruled out CNS involvement, antifungal treatment was changed to fluconazole, completing 10 months of treatment.

Immunity should be studied in every patient with cryptococcosis. Follow-up should include laboratory tests, liver profile, checking that plasma levels are in the therapeutic range and follow-up on the evolution of imaging tests[19].

Conclusion

Pulmonary cryptococcosis is a condition rarely suspected in immunocompetent patients. Imaging can guide the diagnosis, but the etiological study is essential to confirm it, with CT-guided biopsy being very useful for collecting samples for pathological anatomy and culture. Confirming the diagnosis allows appropriate treatment for each situation.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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