

*Prikaz slučaja /
Case report*

MICROSCOPIC FINDING OF LUNG YEAST
INFECTION – CRYPTOCOCCOSIS OR
SOMETHING ELSE?
MIKROSKOPSKA SLIKA PLUĆNE
INFEKCIJE KVASNICAMA –
KRIPTOKOKOZA ILI NEŠTO DRUGO?

Correspondence to:

Prof. dr **Dušan Lalošević**,
Pasterov zavod
Novi Sad, Hajduk Veljka 1
e-mail: dusan.lalosevic@gmail.com
Mob: 064/1370912

Nikola Gardić^{1,3}, Aleksandra Lovrenski^{1,3}, Dejan
Miljković^{1,3}, Milorad Bijelović³, Dejan Vučković^{1,3},
Goran Đenadić⁴, Dušan Lalošević^{1,2}

¹ Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

² Pasteur Institute Novi Sad

³ Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica,
Serbia

⁴ General Hospital „Đorđe Joanović“, Clinical Pathology Department,
Dr Vase Savića br. 5, 23101 Zrenjanin.

Key words

fungal infections; lung cryptococcosis;
differential diagnosis; imprint cytology

Ključne reči

gljivične infekcije; kriptokokoza pluća;
diferencijalna dijagnoza; citologija otiska

Abstract

Introduction: Fungal infections are becoming a major public health problem in an era of global increase in the number of immunocompromised patients. Radiological manifestations of this disease include a wide range of differential diagnoses, including malignant diseases. **Case report:** We present the case of a patient who underwent for surgical treatment as a therapeutic procedure for radiologically verified lung mass. Imprint smear stained with Diff-Quik, GMS and PAS, as well as in histological samples stained H&E, GMS, and PAS showed necrotizing granulomatous inflammation with presence of rounded/oval shaped, ranging from 2-15 µm fungi which were GMS and PAS positive. Based on cytological and histological analysis fungi belongs to the *Cryptococcus neoformans* species. **Conclusion:** Fungal lung infections are one of the differential diagnoses of lung lesions that are suspicious of malignancy. For such lesions, the method of choice for diagnosis is histological verification. Imprint cytology smears are a helpful tool in demonstrating granulomatous inflammation and identifying organisms. It is necessary to perform microbiological cultural and molecular identification of the causative agent in order to confirm the morphological diagnosis.

INTRODUCTION

With an increased number of immunocompromised patients, fungal infections are becoming a significant threat to public health. The most common causes of pulmonary fungal diseases are *Aspergillus*, *Cryptococcus*, *Pneumocystis*, and endemic fungi.⁽¹⁾ These organisms are rarely found in healthy individuals but can cause life-threatening conditions in immunocompromised patients. In this group of patients, we include patients who are being treated for AIDS, malignant diseases, patients after transplant procedures, or patients who are on immunosuppressive therapy for any other reason.⁽²⁾ The signs and symptoms of this disease in immunocompetent patients are quite nonspecific. The chest X-ray findings in immunocompromised patients usually indicate diffuse lung infiltration. But in a regular

host, the findings can be a lung mass that sometimes mimics lung cancer. So, the correct diagnosis of a lung mass is necessary for an appropriate treatment.⁽³⁾ So, this work aimed to present the morphological aspects of lung cryptococcosis.

CASE REPORT

We present the case of a patient who underwent diagnostic and therapeutic thoracotomy to clarify the etiology of radiologically verified lung mass. Atypical resection was performed. Macroscopically, two nodules with a diameter of 0.5 cm and 1 cm were found in the lung tissue, from which imprint cytology was performed. Imprint smears were stained with Diff-Quik, Grocott-Gomori methenamine silver (GMS), and Periodic Acid-Schiff (PAS) stain. In cytology smear, a group of epithelioid cells focally formed granulomas between which the fungi were found.

On Diff-Quik stained smears, fungi are achromatic round shaped, ranging from 2-15 μm (Figure 1a). Described organisms are positive on PAS and GMS staining (Figure 1b-d). Histological analysis of specimens sampled from described nodules showed large areas of distorted lung architecture due to necrotic foci surrounded with palisaded histiocytes, epithelioid cells, lymphocytes, and giant multinucleated cells (Figure 2). In H&E-stained sections, pale gray to light

blue pleomorphic yeast-like cells were found between histiocytes and necrotic debris (Figure 3). Like in imprint smears, fungi were positive on PAS and GMS staining (Figure 4-6). In one of the sections stained with Grocott, one large blood vessel was filled with fibrin masses mixed with blood cells and fungi (Figures 5 and 6). Based on the histological and cytological findings, fungi belongs to the *Cryptococcus neoformans* species.

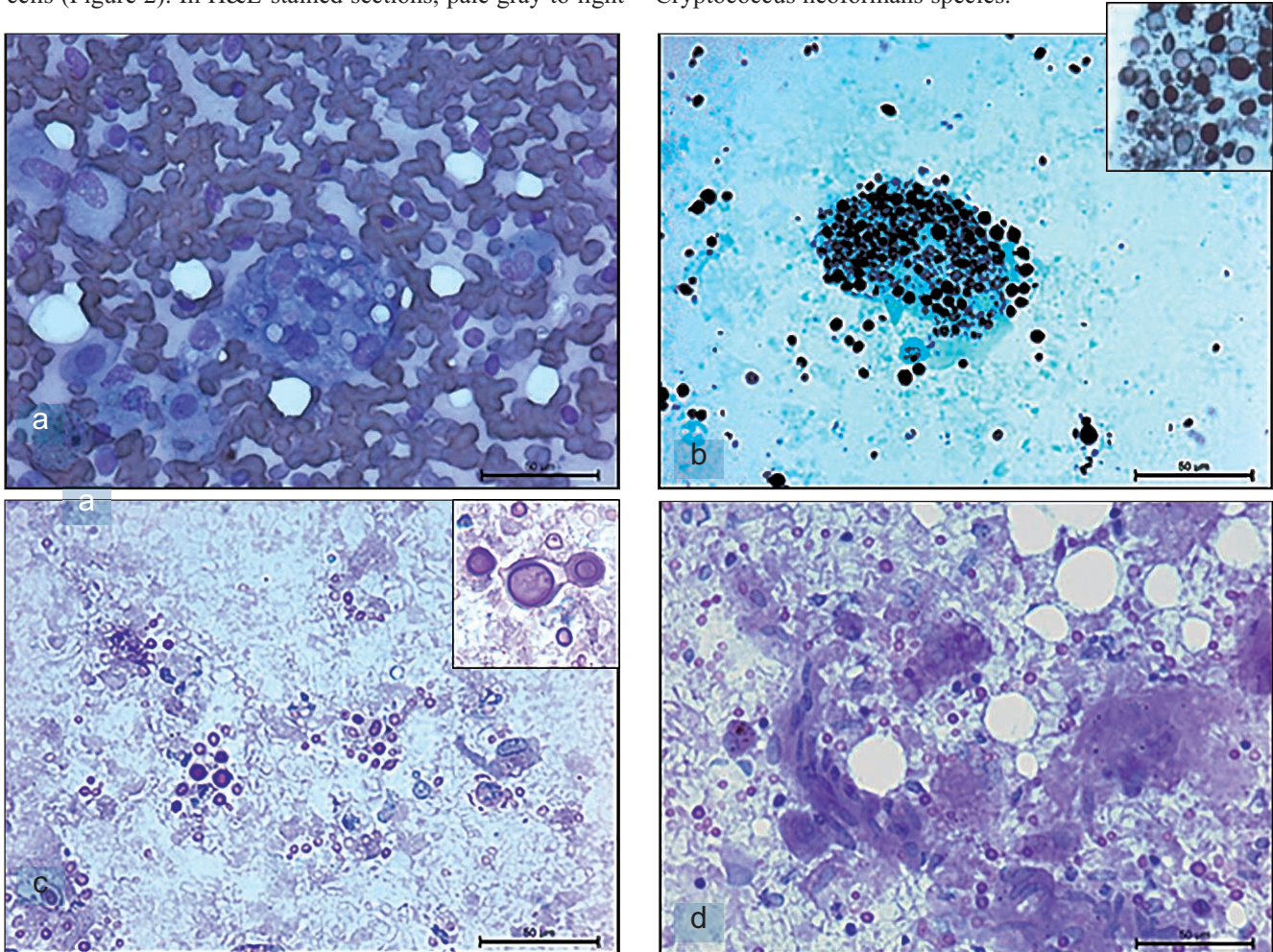


Figure 1. Imprint cytology. a – Fungi within the cytoplasm of the giant multinucleated cell (arrow), Diff Quik, x400; b and c – PAS and GMS positive fungi, x400; d – granulomas formed by epithelioid cells, macrophages, and lymphocytes with fungi, PAS, x400.

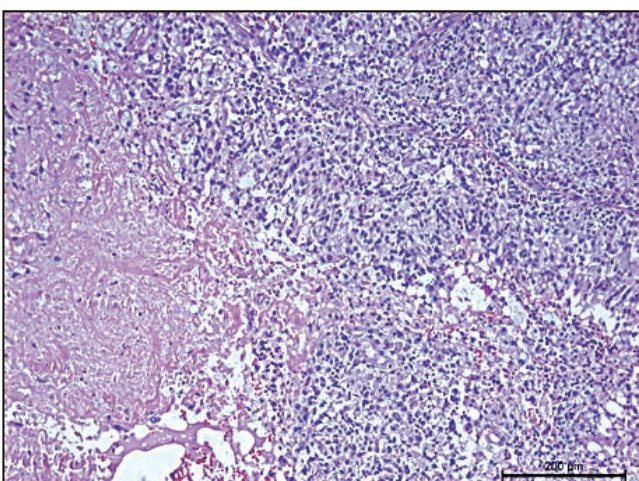


Figure 2. Areas of necrotic tissue surrounded by palisaded histiocytes, epithelioid cells, and lymphocytes, H&E, x100.

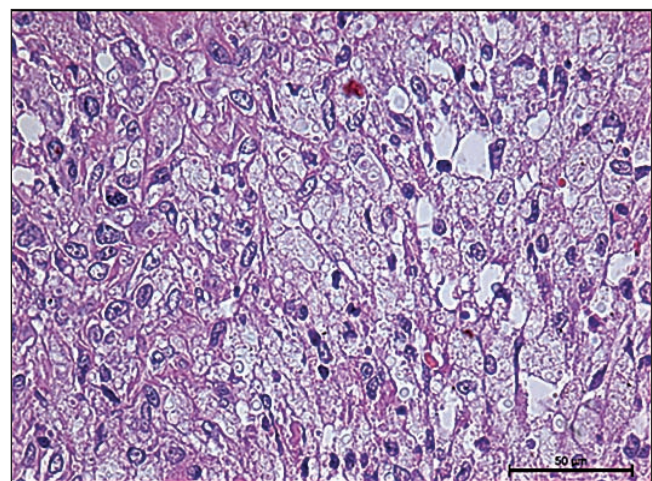


Figure 3. Pale gray to light blue, pleomorphic yeast-like cells, H&E, x400.

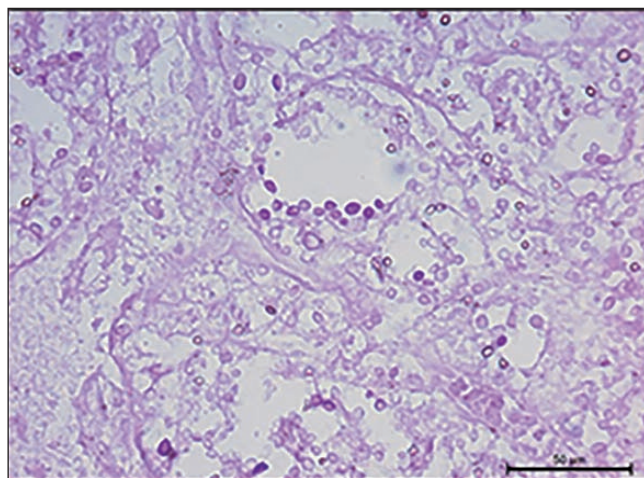


Figure 4. Fungi found in necrotic debris positive on PAS, 400.

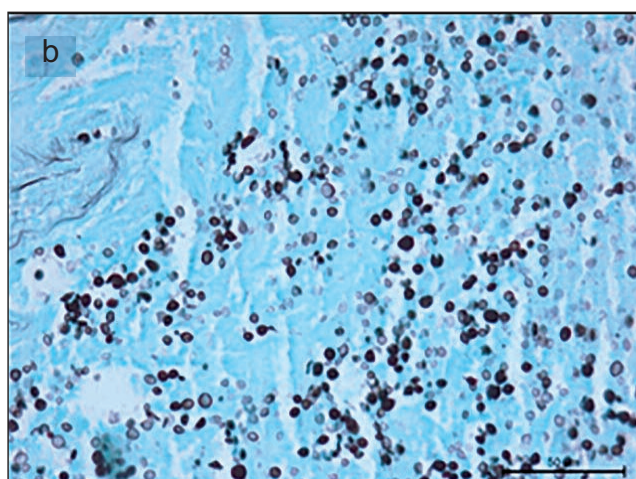
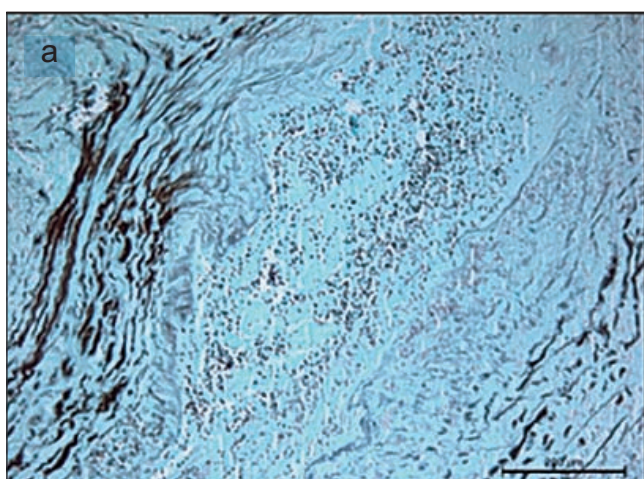


Figure 5. Blood clots within the blood vessel (a) filled with masses mixed with blood cells and fungi (b), GMS, x100, and x400.

DISCUSSION

Cryptococcus neoformans was first isolated from peach juice by *Sanfelice* in 1894 in Italy and was named *Saccharomyces neoformans*. In the same year, *Busse* provided the first description of a case of cryptococcosis and isolated a yeast culture from a sarcoma-like lesion in the infected young woman's tibia. *Busse* called the fungus *Saccharomyces* while naming the disease *Sacharomycosis hominis*. Confusion about the identity of the cryptococcosis agent persisted until *Benham* performed comprehensive studies with clinical *Cryptococcus* strains and concluded that all strains from human infections belonged to one species with two varieties based on serological differences.⁽⁴⁾

Cryptococcosis, an invasive mycosis caused by basidiomycetous yeasts of the *Cryptococcus neoformans* or *Cryptococcus gattii* species complexes, is a cause of significant morbidity and mortality. From over the 30 widely distributed species identified, only two *Cryptococcus* species (*C. neoformans* and *C. gattii*) cause most of the human infections.⁽⁵⁾

The development of large granulomatous lesions following infection with *C. neoformans* var. *gattii* is consistent with the induction of an immune response, but it fails to eliminate the organism or its antigens efficiently.⁽⁶⁾

The mainstay of diagnosis relies on histopathology, culture-based methods, and adjunctive cryptococcal antigen (CrAg) testing, which confers high specificity and at least moderate sensitivity in pulmonary disease.⁽⁵⁾ Nowadays, fungal infection diagnostics predominantly rely on serological rather than histological examination.⁽⁷⁾ However, many cases in the literature demonstrate lung lesions suspicious of malignancy. Since we can also expect a complication with a fungal infection in these patients, histopathological analysis and confirmation of the diagnosis is necessary.⁽⁸⁾ Although, in our case, the open surgical method was the method of choice for the removal of the undiagnosed lung mass, the literature suggests that the percutaneous or trans-bronchial biopsy is the optimal and less invasive specimen type to establish the diagnosis in the case when resection is contraindicated.⁽⁹⁾

From the histochemical methods, we used the GMS and

PAS stain, while the literature, in addition to mentioned stains, recommends the India ink method for proving cryptococcal infection in cerebrospinal fluid.⁽¹⁰⁾ On H&E and Diff Quik stain, fungi can simulate artifacts like pollen grain contamination surrounded by clear space.⁽¹¹⁾ More specific stains like Fontana-Masson stain and mucicarmine could be used to identify *Cryptococcus* spp.⁽¹²⁾

Many studies investigated different therapy options for patients with *Cryptococcus* infections. Only three classes of antifungal drugs have been considered for this purpose: the polyenes (amphotericin B), the azoles (ketoconazole, itraconazole, fluconazole, voriconazole), and a pyrimidine-derived drug (5-flucytosine).⁽¹³⁾

CONCLUSION

Fungal lung infections are one of the differential diagnoses of lung lesions that are suspicious of malignancy. For such lesions, the method of choice for diagnosis is histological verification. Imprint cytology smears are a helpful tool in demonstrating granulomatous inflammation and identifying organisms.

Differential morphological diagnosis of yeast infection in human biptic samples must include fungal genera *Candida*, *Blastomyces*, *Histoplasma*, *Coccidioides*, *Cryptococcus*, and very rare, *Saccharomyces*.

Sažetak

Uvod: Gljivične infekcije postaju veliki problem u javnom zdravlju u eri globalnog povećanja broja imunokompromitovanih pacijenata. Radiološka manifestacije ove bolesti obuhvataju širok spektar diferencijalnih dijagnoza uključujući i maligne bolesti. **Prikaz slučaja:** Prikazujemo slučaj pacijenta koji je podvrgnut hirurškom lečenju u cilju tretmana radiološki uočene promene u plućima. U otisnutim citološkim razmazima bojenim metodom Diff-Quik, GMS i PAS i histološkim uzorcima bojenim metodama H&E, GMS i PAS prisutna je histološka slika nekrotične granulomatozne upale sa prisutnim gljivicama, okruglog do ovalnog oblika, prečnika od 2-15 µm koje su GMS i PAS pozitivne. Na osnovu citološke i histološke morfološke slike gljivice pripadaju specijesu *Cryptococcus neoformans*. **Zaključak:** Gljivične infekcije pluća su jedna od diferencijalnih dijagnoza radiološki verifikovanih plućnih promena koje su suspektne maligne. Metoda izbora za takve lezije je histološka verifikacija dijagnoze. Citologija otiska se pokazala kao korisna metoda u dijagnostici granulomatoznih upala i identifikaciji organizama. Potrebno je uraditi mikrobiološku kulturelnu i molekularnu identifikaciju uzročnika radi potvrde morfološke dijagnoze.

LITERATURA

1. Li Z, Lu G, Meng G. Pathogenic Fungal Infection in the Lung. *Front Immunol.* 2019 Jul 3;10:1524. doi: 10.3389/fimmu.2019.01524. PMID: 31333658;

2. José RJ, Brown JS. Opportunistic and fungal infections of the lung. *Medicine (Abingdon).* 2012 Jun;40(6):335-339. doi: 10.1016/j.mpmed.2012.03.013. Epub 2012 May 18. PMID: 32288572;

3. Kanjanapradit K, Kosjerina Z, Tanomkiat W, Keeratichananont W, Panthuwong S. Pulmonary Cryptococcosis Presenting With Lung Mass: Report of 7 Cases and Review of Literature. *Clin Med Insights Pathol.* 2017 Aug 4;10:1179555717722962. doi: 10.1177/1179555717722962. PMID: 28814908;

4. Howard-Jones AR, Sparks R, Pham D, Halliday C, Beardsley J, Chen SC. Pulmonary Cryptococcosis. *J Fungi (Basel).* 2022 Oct 31;8(11):1156. doi: 10.3390/jof8111156. PMID: 36354923;

5. Torda A, Kumar RK, Jones PD. The pathology of human and murine pulmonary infection with *Cryptococcus neoformans* var. *gattii*. *Pathology.* 2001 Nov;33(4):475-8. doi: 10.1080/00313020120083197. PMID: 11827415.

6. Zhang Y, Li N, Zhang Y, Li H, Chen X, Wang S, Zhang X, Zhang R, Xu J, Shi J, Yung RC. Clinical analysis of 76 patients pathologically diagnosed with pulmonary cryptococcosis. *Eur Respir J.* 2012 Nov;40(5):1191-200. doi: 10.1183/09031936.00168011. Epub 2012 Mar 9. Erratum in: *Eur Respir J.* 2013 Jan;41(1):252. PMID: 22408204.

7. Ohshimo S, Guzman J, Costabel U, Bonella F. Differential diagnosis of granulomatous lung disease: clues and pitfalls: Number 4 in the Series „Pathology for the clinician” Edited by Peter Dorfmueller and Alberto Cavazza. *Eur Respir Rev.* 2017 Aug 9;26(145):170012. doi: 10.1183/16000617.0012-2017. PMID: 28794143;

8. Xin Z, Li B, Xue W, Lin W, Zhao Q, Zhang X. Pulmonary cryptococcosis mimicking lung cancer: 3 case report. *Int J Surg Case Rep.* 2022 May;94:106973. doi: 10.1016/j.ijscr.2022.106973. Epub 2022 Apr 1. PMID: 35658271;

9. Zhang Y, Li N, Zhang Y, Li H, Chen X, Wang S, Zhang X, Zhang R, Xu J, Shi J, Yung RC. Clinical analysis of 76 patients pathologically diagnosed with pulmonary cryptococcosis. *Eur Respir J.* 2012 Nov;40(5):1191-200. doi: 10.1183/09031936.00168011. Epub 2012 Mar 9. Erratum in: *Eur Respir J.* 2013 Jan;41(1):252. PMID: 22408204.

10. Chisale MR, Salema D, Sinyiza F, Mkwaila J, Kamudumuli P, Lee HY. A comparative evaluation of three methods for the rapid diagnosis of cryptococcal meningitis (CM) among HIV-infected patients in Northern Malawi. *Malawi Med J.* 2020 Mar;32(1):3-7. doi: 10.4314/mmj.v32i1.2. PMID: 32733652; PMCID: PMC7366160.

11. Chatterjee S. Artefacts in histopathology. *J Oral Maxillofac Pathol.* 2014 Sep;18(Suppl 1):S111-6. doi: 10.4103/0973-029X.141346. PMID: 25364159;

12. Lazcano O, Speights VO Jr, Bilbao J, Becker J, Diaz J. Combined Fontana-Masson-mucin staining of *Cryptococcus neoformans*. *Arch Pathol Lab Med.* 1991 Nov;115(11):1145-9. PMID: 1720948.

13. Aguiar PADF, Pedrosa RDS, Borges AS, Moreira TA, Araújo LB, Röder DVDB. The epidemiology of cryptococcosis and the characterization of *Cryptococcus neoformans* isolated in a Brazilian University Hospital. *Rev Inst Med Trop Sao Paulo.* 2017 Apr 13;59:e13. doi: 10.1590/S1678-9946201759013. PMID: 28423088; PMCID: PMC5398185.