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UNUSUAL MORPHOLOGIES OF *Cryptococcus* spp. IN TISSUE SPECIMENS: REPORT OF 10 CASES

Alexandra Flávia GAZZONI(1), Flávio de Mattos OLIVEIRA(2), Emily Ferreira SALLES(3), Emilio MAYAYO(1,4), Josep GUARRO(5), Javier CAPILLA(5) & Luiz Carlos SEVERO(2,6,7)

SUMMARY

Ten cases of cryptococcosis due to unusual microscopic forms of *Cryptococcus* sp. observed over a twenty-eight year period (1981-2009) are presented. The most important clinicopathological and laboratory data are tabulated. The uncommon forms of cryptococcal cells given are: structures resembling germ tube (one case), chains of budding yeasts (one case), pseudohyphae (two cases) and nonencapsulated yeast-like organisms (eight cases). The diagnosis was based on the histopathological findings. The causative organism was isolated and identified in seven cases; five were due to *C. neoformans*, and two to *C. gattii*. In addition, the importance of using staining histochemical techniques - Grocott's silver stain (GMS), Mayer's mucicarmine stain (MM) and Fontana-Masson stain (FM) - in the diagnosis of cryptococcosis is argued.

KEYWORDS: Cryptococcosis; Pseudohyphae; Chains of budding yeasts; Germ tube-like structures; Nonencapsulated yeast-like organisms.

INTRODUCTION

In clinical specimens, *Cryptococcus* species are usually identified as spherical-to-oval yeast cells, range from 4-20 µm in diameter, and are surrounded by a mucopolysaccharide capsule, which is a major virulence factor[5,14]. Single or multiple budding cells with a narrow base are usually observed[14]. In addition to these classical aspects, *Cryptococcus* may also be present in unusual forms, which include pseudohyphae[1,7], chains of budding yeasts[7,27], structures resembling germ tubes[7] and poorly encapsulated cells[8].

Histopathological identification of the cryptococcosis is based on the micromorphological and staining features of the cryptococcal cells, and include histochemical techniques of hematoxylin and eosin (HE), and Grocott's silver stain (GMS), as well as special histochemical techniques such as Mayer's mucicarmine method (MM), which stains the capsule magenta, and Fontana-Masson procedure (FM), which stains fungal melanin reddish-brown[7,8].

In this study, we highlight the unusual micromorphological forms of the *Cryptococcus* species in tissue specimens. It also emphasizes the use of histochemical techniques in the diagnosis of cryptococcosis.

MATERIAL AND METHODS

Through database analysis, we retrospectively reviewed all cases of cryptococcal infections diagnosed between January 1981 and May 2009 at the Mycology Laboratory of Santa Casa Complexo Hospitalar (Porto Alegre, RS), in Southern Brazil.

Clinical-epidemiological and laboratory records of the cases diagnosed by histopathological examination were reviewed, and we were primarily interested in collecting data such as: sex, age, underlying diseases, titers of cryptococcal antigens (CrAg) in sera, urine and cerebrospinal fluid (CSF) and species of *Cryptococcus* recovered in cultures.

***Cultures*:** The identification was confirmed by: (a) colony morphology - by isolation of yeast colonies with white mucoid aspect (depending on the capsule thickness) after cultivation on fungal media (within 48-72 h), namely Sabouraud's (SAB) at 25 °C, and brain-heart infusion (BHI) agar at 35 °C; (b) microscopy morphology - by demonstration of spherical-to-oval encapsulated yeast cells and budding on a narrow base. After identity of an isolate had been established, such as *Cryptococcus*, we proceeded to determine its species status. Canavanine-glycine-broothymol blue (CGB) agar was successfully used for this purpose. In one to five days, isolates of *C. gattii* cause the CGB medium to turn blue, whereas those of *C. neoformans* do not.

***CrAg detection*:** For serological diagnosis, latex agglutination for cryptococcal polysaccharide antigens was perfomed a specific and sensitive alternative for rapid diagnosis. In this study, the commercial kit IMMY test was used, which has a vital component in Detacher Enzyme (DE), Pronase®. DE eliminates the rheumatoid factor, which can produce false positives.

(1) Pathologic Anatomy Unit, Medical School, Rovira i Virgili University, Reus, Spain.
(2) Mycology Laboratory, Santa Casa-Complexo Hospitalar, Porto Alegre, RS, Brazil.
(3) Department of Pathology, Santa Casa-Complexo Hospitalar, Porto Alegre, RS, Brazil.
(4) Joan XXIII University Hospital, Rovira i Virgili University, Tarragona, Spain.
(5) Microbiology Unit, Medical School, Rovira i Virgili University, Reus, Spain.
(6) Researcher 1B from CNPq, Brazil
(7) Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
Correspondence to: Prof. Dr. Luiz Carlos Severo. Laboratório de Micologia/Hospital Santa Rita, Santa Casa Complexo Hospitalar. Rua Profº Annes Dias 285, 90020-090 Porto Alegre, RS, Brasil. Phone: +55.51.32148410. E-mail: severo@santacasa.tche.br; severo@pesquisador.cnpq.br

Histopathological studies: Fragments obtained through biopsy or surgical excision were fixed in formalin, embedded in paraffin and, after sectioned, stained for evaluation. The histopathological examination was made on routine HE preparations to determine organ involvement by fungal pathogen and to determine details of tissue response. In all cases, tissue sections stained with selective staining such as GMS, MM and FM were examined.

RESULTS

Between 1981 and 2009, 925 patients with cryptococcosis were diagnosed in our mycology laboratory, with 33 cases being established through histopathological diagnosis.

In a total of 10 out of these 33 cases unusual microscopic forms of *Cryptococcus* were observed. Gender distribution showed a predominance of males (70% - 7/10), and the mean age was 42.4 years old (range, 23 - 60 years). Table 1 summarizes the clinical and pathological data for the 10 patients. With the exception of three immunocompetent patients, the remaining patients had the following underlying diseases: AIDS (three cases), kidney and lung transplantation (two cases and one case, respectively), and lymphoma (one case).

Cryptococcal antigen detection: CrAg test of patients showed titres ranging from 1:5112 to 1:1048576. In six cases (60%), CrAg test was negative.

Identification of cultures: *Cryptococcus* isolated from these patients was identified as species *neoformans* in five cases (50%). *Cryptococcus gattii* was recovered from an immunocompetent patient and from a patient with AIDS. In three cases, the identification from cultures - on fungal media, namely SAB, BHI and CGB - was not performed due to biopsy specimens being in formalin fixation.

Histopathological findings: Histologically, *Cryptococcus* was found in multiple organs (lungs, brain, liver, cervical and axillary lymph nodes and left axillary region). The lungs were the organs most often involved (cases 1-3, 5 and 8) (50% - 5/10).

In cases 4, 6, 9 and 10, HE staining followed by microscopic examination showed usual rounded refractile bodies with a surrounding halo, accompanied by minimal, predominantly lymphatic infiltration. These paucireactive patterns were associated with complete replacement of host tissue by extracellular yeasts and mucin (Fig. 1A). The cases 1-3, 5, 7 and 8 were characterized by a marked granulomatous response composed predominantly of histiocytes, multinucleated giant cells, and lymphocytes in which cryptococci were predominantly intracellular (phagocytosis). These reactive patterns were associated with fibrosis (Fig. 1B).

In cases 4 and 6, staining with GMS revealed black organisms on a light green background - numerous spherical-to-oval yeasts were surrounded by a narrow, circumferential, refractile zone of negative staining (halos) (Fig. 2A).

Several uncommon micromorphologies of *Cryptococcus* were identified. These included germ tube-like structures (one case), chains of budding yeasts (one case), pseudohyphae (two cases) and nonencapsulated yeast-like organisms (eight cases).

Nonencapsulated yeast-like organisms were observed in cases 1-3, 5 and 7-10. The numerous fungal cells were stained strongly with GMS, although encapsulation was not evident (pericellular clear zone absent) (Fig. 2B).

Germ tube-like structures were seen in case 6 (Fig. 2C). However, these possessed a constriction at their base at the point of attachment to the adjacent yeasts. Chains of budding yeasts were also observed in this case (Fig. 2D). In spite of having a pseudohyphae-like appearance, the individual cells did not possess the characteristic elongation of typical pseudohyphae.

In cases 4 and 6, the MM staining strongly stained the capsules with a deep magenta color, consistent with the presence of mucopolysaccharides. Although many of the yeasts were arranged singly, some demonstrated narrow-based budding, and a few clusters of yeasts with multiple budding were seen. In these cases, pseudohyphae were seen that varied in size. In several instances, these elongated structures, consistent with pseudohyphae, were seen in continuity with a chain of budding

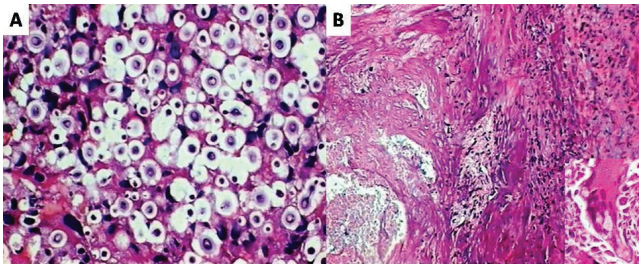

Fig. 1 - A, Left axillary tumor biopsy with paucireactive cryptococcosis. Numerous yeast-like organisms are surrounded by clear halos representing gelatinous capsules stained with HE (case 4) (x 10). B, Lung biopsy with reactive cryptococcosis. Granulomatous inflammation with necrosis, fibroblastic activity and foreign-body giant-cells (case 3) (x10).

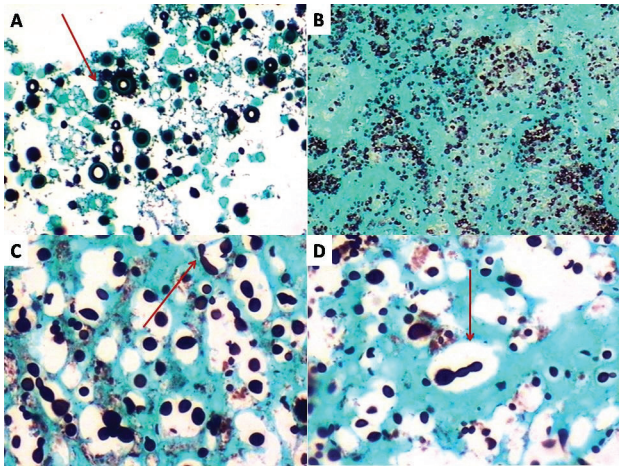

Fig. 2 - A, Left axillary tumor biopsy stained with GMS stain. Capsulated morphology of yeast-like fungal bodies; some structures exhibit narrow-based budding (case 4) (x10). B, Pulmonary cryptococcosis. Nonencapsulated fungal morphologies are observed. Fungal cell wall is stained in black (case 2) (x10). C, Cervical lymph node tissue. Germ tube-like structure; constriction at the base is evident (case 6) (x 10). D, Cervical lymph node tissue. Budding yeasts arranged in chains (case 6) (x 10).

Table 1
Clinicopathologic summary of cases which presented unusual morphologies of ***Cryptococcus*** species in tissue specimens at Mycology Laboratory, Complexo Hospitalar/Santa Casa

Cases [Ref.]	Sex, Age	Underlying diseases	Tissue site (type of specimens)	Histologic pattern	Grocott's silver stain	Mayer's mucicarmine stain	Fontana-Masson stain	Cr Ag titers	Identification of culture
1 [8]	F, 42	Immunocompetent patient	Lung	Reactive	Nonencapsulated yeast-like within multinucleated giant cells	Negative	Positive	Negative	NA
2	F, 40	Immunocompetent patient	Lung	Reactive	Nonencapsulated yeast-like	Negative	Positive	Negative	NA
3	M, 59	Lung transplant	Lung	Reactive	Nonencapsulated yeast-like within multinucleated giant cells	Negative	Positive	Negative	NA
4	M, 56	Immunocompetent patient	Left axillary region	Paucireactive	Spherical-to-oval yeasts-like with pericellular clear halos Pseudohyphae	Fungal cell with magenta capsule Chains of budding yeasts	Positive Classical aspects of *Cryptococcus*	1:5112 [a]	*C. gattii* [c]
5	M, 60	Lymphoma	Lung	Reactive	Nonencapsulated yeast-like	Negative	Positive	NA	*C. neoformans* [d]
6 [7]	M, 29	AIDS	Cervical lymph node	Paucireactive	Spherical-to-oval yeast-like with pericellular clear halos Germ tube-like structures Pseudohyphae Chains of budding yeasts	Fungal cell with magenta capsule, Pseudohyphae Chains of budding yeasts	Positive Classical aspects of *Cryptococcus*	1:1.048.576[a] 1:4096 [b]	*C. neoformans* [d]
			Tongue biopsy	Paucireactive	Spherical-to-oval yeast-like with pericellular clear halos	Fungal cell with magenta capsule	Positive Classical aspects of *Cryptococcus*		
7	M, 40	Kidney transplant	Skin	Reactive	Nonencapsulated yeast-like	Negative	Positive	Negative	*C. neoformans* [d]
8	M, 42	Kidney transplant	Lung	Reactive	Nonencapsulated yeast-like Pseudohyphae	Negative	Positive	Negative	*C. neoformans* [d]
9	M, 23	AIDS	Liver	Paucireactive	Nonencapsulated yeast-like	Negative	Positive	Negative	*C. neoformans* [d]
10	M, 10	AIDS	Axillary lymph node	Paucireactive	Nonencapsulated yeast-like in the intercellular spaces	Negative	Positive	1: 4096 [a]	*C. gattii* [c]
			Brain	Paucireactive	Nonencapsulated yeast-like	Negative	Positive		

[a]: serum; AIDS: acquired immunodeficiency syndrome; [b]: urine; in 1 to 5 days, *Cryptococcus gattii* [c] isolates turn the CGB medium blue, whereas those of *Cryptococcus neoformans* [d] do not; C: *Cryptococcus*; Cr Ag: Cryptococcal antigen; NA: not available.

yeasts (Fig. 3A). In other instances, several contiguous pseudohyphae demonstrated prominent branching. In cases 1-3, 5 and 7-10, the usual magenta color of the capsule after MM staining was absent (Fig. 3B).

When stained by FM, the black color in the cell wall of the organisms was disclosed. All the cases were positive - patients 4 and 6 presented classical micromorphological aspects in this yeast (Fig. 3C), while patients 1-3, 5 and 7-10 revealed a capsule-deficient form, confirming infection by *Cryptococcus* species (Fig. 3D).

DISCUSSION

As observed by others[4], male patients predominated in our study. In the AIDS era, the relationship of cryptococcosis with gender distribution

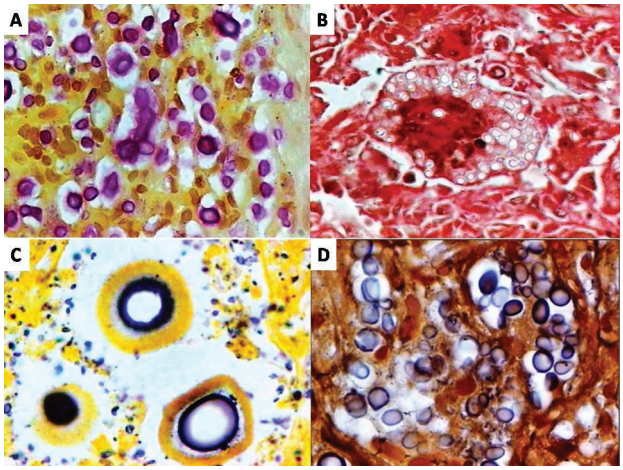

Fig. 3 - A, Cervical lymph node biopsy. Pseudohyphae demonstrating branching and numerous conventional yeast cells with the magenta capsules stained by Mayer's mucicarmine stain (case 6) (x 10). B, Lung tissue biopsy. The staining for capsular material is negative (case 8) (x 10). C, Left axillary tumor biopsy. Classical encapsulated fungal body of *Cryptococcus* (case 4) (x 100). D, Lung tissue biopsy. This special stain pattern is a characteristic of *Cryptococcus*, especially for the capsule deficient form. The black color in the walls of the organisms is disclosed by Fontana-Masson stain (case 1) (x100).

correlated to that of HIV infection[17]. Thus, the predominance of male patients reflects the predisposing conditions. The majority of patients were suffering from AIDS, supporting previous epidemiological studies which had reinforced that HIV infection was the main risk factor for cryptococcosis[4]. Consequently, diagnosis of cryptococcosis in patients with unknown predisposition always suggests an examination for HIV infection. The association with neoplastic and lymphoproliferative disorders[16], and with organ transplantations[26], has been well established.

The lungs are invariably the portal of entry, by inhalation from an environmental source, and also the initial site of infection for *Cryptococcus* spp. [5,18]. The infection can then spread via the bloodstream[16]. In these conditions, all areas of the body can be infected, including liver, lymph nodes, kidneys, adrenal glands and most notably the central nervous system (CNS)[18]. According to the largest reported study of cryptococcosis in the AIDS era, five major sites can be involved that are particularly important in the diagnosis and management of cryptococcosis - the lungs, CNS, skin, prostate and eyes[16].

Definitive diagnosis of cryptococcosis requires specific histopathological examination, detection of the cryptococcal antigen in body fluids by latex agglutination and identification of the yeast from cultures[8,28]. Characteristic cryptococcal morphology does not pose any difficulty in recognizing the fungus[7,8]. However, unusual forms can give rise to a diagnostic dilemma[23]. Recognition of variants by morphology examination is important in the laboratory confirmation of the disease[7,25]. In the current series, it is worthy of note that the cryptococcal cells had aberrant morphologies in about 1/3 of our patients whose diagnosis was made histologically. Here, we have also highlighted that formalin fixation causes death of fungal agent preventing its growth on culture media.

Polysaccharide capsules are known to be a major cryptococcal virulence factor[16]. The loss of capsule material elicits an intense inflammatory response that includes early suppuration, phagocytosis and granuloma formation[8,20,24]. Previous reports described cryptococcosis caused nonencapsulated forms as extremely rare, and with no apparent incidence in humans[3,9]. In contrast, considering all of the features described above, a final diagnosis of capsule-deficient cryptococcosis was made in 80% (8/10) of our cases. The application of FM staining to the nonencapsulated forms of *Cryptococcus* was first reported by KWON-CHUNG *et al.*[13], which identifies melanin pigments in the cell wall and selectively stains these yeasts in tissue specimens[8]. In our present series, an FM stain was an alternative diagnostic technique for the differentiation of the pathogenic fungal species, especially for distinguishing cryptococcal infection from the other fungal infections that can mimic it with their similar size and shape, tissue response and negative mucicarmine staining. In addition, detecting false-negative CrAg results is strongly associated to these poorly encapsulated cryptococcal cells as these strains do not produce enough polysaccharide antigens to be detected by the CrAg test. It is of note that patients with pulmonary disease without dissemination may have false-negative serum titres, since yeasts would not yet have spread from the lungs.

As previously noted, the appearance of *Cryptococcus* sp., a budding yeast cell, and the absence of pseudohyphae have been used as a point of differential diagnosis between cryptococcosis and candidiasis in several recent reviews that have discussed the histopathology of cryptococcal infection[7,10]. Other reports describe the identification of variant forms having pseudohyphae[2,19], germ-tube like structures and hyphae-forming structures[6], all identified in an India ink preparation of CSF. In a review of prior necropsy specimens, a case was discovered in which germ tubes and hyphae were observed in brain tissues[15]. Rare germinating forms of *Cryptococcus* spp. have also been reported in a subcutaneous, erythematous nodule[17]. In 1971, a murine model study demonstrated hyphae-formation in 11 strains of this yeast[21,22]. Interestingly, BAVA *et al.*[2] concluded that the pseudohyphae should be considered a virulence factor and that great immunological deficit and further clinical evolution will better enable the fungal cells to produce atypical forms instead of the conventional yeast forms. In retrospect, KWON-CHUNG[12] reported that the hyphal forms represented attempts of *Cryptococcus* to produce its sexual teleomorph, *F. neoformans*. However, some authors believe that although *Cryptococcus* rarely occurs in short chains resembling pseudohyphae, neither pseudohyphae nor true hyphae are presented[12].

Our current series demonstrates the existence of the uncommon features such as pseudohyphae formation, chains of budding yeasts and structures resembling germ tubes during cryptococcal infection in humans. Awareness that *Cryptococcus* can form these features highlights the importance of special stains, such as mucicarmine[7]. In cases 4 and 6, mucicarmine strongly stained the capsules of the individual yeasts, there were chains of budding yeasts, pseudohyphae and germ tube-like structures; all of which proved critical in histopathological diagnosis of the organisms as *Cryptococcus* rather than as a *Candida*-type species[7,27]. In addition, these histopathological findings, together with cryptococcal antigen detection by latex agglutination in sera and urine, and identification from the cultures, confirmed the *Cryptococcus* species infection in these two cases.

In summary, the current series emphasizes the importance of recognizing that cryptococcal cells do not always show typical encapsulated morphology, but may also be present as nonencapsulated yeast-like forms, pseudohyphae or germ tube-like structures, because

generally this is not extensively described in the literature. In such instances, the association of HE and GMS preparations with MM and FM stains is useful for micromorphological diagnosis of cryptococcosis, as well as for identification of the uncommon forms of *Cryptococcus* species.

RESUMO

Histopatologia, sorologia e cultivo no diagnóstico da criptococose

A criptococose é a mais comum infecção fúngica oportunística observada em pacientes com síndrome da imunodeficiência adquirida (AIDS). Relatamos 13 casos da infecção baseados no diagnóstico histopatológico, sorológico e cultivo. Foram analisadas: a epidemiologia, as técnicas histoquímicas básicas de hematoxilina-eosina (HE) e coloração pela prata (GMS), bem como as técnicas histoquímicas especiais de mucicarmim de Mayer (MM) e Fontana-Masson (FM), o teste do antígeno criptocóccico (CrAg) e o isolamento em cultivos em ágar-Sabouraud (SAB), ágar infusão de cérebro-coração (BHI) e meio com canavanina azul de bromotimol (CGB). Em quatro casos, resultados tintoriais insatisfatórios pela coloração de MM associados a títulos negativos pelo teste do CrAg, a coloração de FM confirmou a infecção pelo *Cryptococcus* deficiente de cápsula. Oito isolados foram identificados: seis casos apresentaram a infecção por *Cryptococcus neoformans* e dois casos apresentaram a infecção por *Cryptococcus gattii*.

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REFERENCES

1. Alfonso F, Gallo L, Winkler B, Suhrland MJ. Fine needle aspiration cytology of peripheral lymph node cryptococcosis. A report of three cases. Acta Cytol. 1994;38:459-62.

2. Bava J, Solari R, Isla G, Troncoso A. Atypical forms of *Cryptococcus neoformans* in CSF of an AIDS patient. J Infect Dev Ctries. 2008;2:403-5.

3. Cheon WS, Eom KS, Yoo BK, Jang SH, Bahn JW, Kim DG, *et al*. A case of pulmonary cryptococcosis by capsule-deficient *Cryptococcus neoformans*. Korean J Intern Med. 2006;21:83-7.

4. Dromer F, Mathoulin-Pelissier S, Fontanet A, Ronin O, Dupont B, Lortholary O, *et al*. Epidemiology of HIV-associated cryptococcosis in France (1985-2001): comparison of the pre- and post-HAART eras. AIDS. 2004;18**:**555-62.

5. Feldmesser M, Kress Y, Casadevall A. Dynamic changes in the morphology of C*ryptococcus neoformans* during murine pulmonary infection. Microbiology. 2001;147:2355-65.

6. Freed ER, Duma RJ, Shadomy HJ, Utz JP. Meningoencephalitis due to hyphae-forming *Cryptococcus neoformans*. Am J Clin Pathol. 1971;55**:**30-3.

7. Gazzoni AF, Severo CB, Barra MB, Severo LC. Atypical micromorphology and uncommon location of cryptococcosis: a histopathologic study using special histochemical techniques (one case report). Mycopathologia. 2009;167:197-202.

8. Gazzoni AF, Pegas KL, Severo LC. Histopathological techniques for diagnosing cryptococcosis due to capsule-deficient *Cryptococcus*: case report. Rev Soc Bras Med Trop. 2008;41:76-8.

9. Kanazawa M, Ishii M, Sato Y, Kitamura K, Oshiro H, Inayama Y. Capsule-deficient meningeal cryptococcosis. Acta Cytol. 2008;52:266-8.

10. Kimura M, Furuta T, Teramura K, Satou T. Pseudohyphae of *Cryptococcus* in a cytologic specimen. Acta Cytol. 1998;42:817-8.

11. Kwon-Chung KJ. A new genus, *filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia. 1975;67:1197-200.

12. Kwon-Chung KJ, Bennett JE. Medical Mycology. Philadelphia: Lea & Febiger; 1992.

13. Kwon-Chung KJ, Hill WB, Bennett JE. New, special stain for histopathological diagnosis of cryptococcosis. J Clin Microbiol. 1981;13:383-7.

14. Lin X. *Cryptococcus neoformans*: morphogenesis, infection, and evolution. Infect Genet Evol. 2009;9:401-16.

15. Lurie HI, Shadomy HJ. Morphological variations of a hypha-forming strain of *Cryptococcus neoformans* (Coward strain) in tissues of mice. Sabouraudia. 1971;9:10-4.

16. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS – 100 years after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev. 1995;8:515-48.

17. Moore M. Cryptococcosis with cutaneous manifestations. Four cases with a review of published reports. J Invest Dermatol. 1957;28:159-82.

18. Moretti ML, Resende MR, Lazéra MS, Colombo AL, Shikanai-Yasuda MA *et al*. Consenso em criptococose - 2008. Rev Soc Bras Med Trop. 2008;41:524-44

19**.** Paschoal RC, Melhem MSC, Guelli T, Szeszs MW. Células atípicas de C*ryptococcus neoformans* var. *neoformans* observadas em líquido cefalorraquidiano. Rev Inst Adolfo Lutz. 2007;66:78-80.

20. Schwartz DA. Characterization of the biological activity of *Cryptococcus* infections in surgical pathology. Ann Clin Lab Sci. 1988;18:388-97.

21. Shadomy HJ, Utz JP. Preliminary studies on a hyphaforming mutant of C*ryptococcus neoformans*. Mycologia. 1966;58:383-90.

22. Shadomy HJ, Lurie HI. Histopathological observations in experimental cryptococcosis caused by a hypha-producing strain of *Cryptococcus neoformans* (Coward strain) in mice. Sabouraudia. 1971;9:6-9.

23. Shashikala, Kanungo R, Srinivasan S, Mathew R, Kannan M. Unusual morphological forms of *Cryptococcus neoformans* in cerebrospinal fluid. Indian J Med Microbiol. 2004;22:188-90.

24. Shibuya K, Coulson WF, Wollman JS, Wakayamam M, Ando T, Oharaseki T, *et al*. Histopathology of cryptococcosis and other fungal infections in patients with acquired immunodeficiency syndrome**.** Int J Infect Dis. 2001;5:78-85.

25. Sugiura Y, Homma M, Yamamoto T.Difficulty in diagnosing chronic meningitis caused by capsule-deficient *Cryptococcus neoformans*. J Neurol Neurosurg Psychiatry. 2005;76:1460-1.

26. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: evidence-based envolving trends. Clin Infect Dis. 2009;48:1566-76.

27. Williamson JD, Silverman JF, Mallak CT, Christie JD. Atypical cytomorphologic appearance of *Cryptococcus neoformans*. A report of five cases. Acta Cytol. 1996;40:363-70.

28. Xavier MO, Oliveira FM, Severo LC. Laboratory diagnosis of pulmonary mycoses. J Bras Pneumol. 2009;35:907-19.

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