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CASE REPORT

Cryptococcal Meningitis in a Patient with Sjogren's Syndrome: a Case Report

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SUMMARY

Background: **Cryptococcal meningitis (CM) is a life-threatening infectious disease and causes high morbidity and mortality. No information about Cryptococcal meningitis in populations with Sjogren's syndrome (SS) was available.**
Methods: **This report details the first case of Cryptococcal meningitis in a 75-year-old female patient with 10-years history of Sjogren's syndrome.**
Results: **Detailed findings of *C. neoformans* from CSF examinations, including routine examination, India ink stain, immunological test, culturing, mass spectrum analysis and molecular biology identification were all delineated in this case, which facilitated understanding of detection methods in *C. neoformans* infection. The etiological exploration was initiated from a positive finding of yeast cells in routine examination of unstained CSF in the present case. Morphology description of *C. neoformans* in unstained CSF was depicted for the first time.**
Conclusions: **Clinicians should consider the possible complication of Cryptococcal meningitis when patients with Sjogren's syndrome show neurological symptoms. Importance of screening yeast cells from unstained CSF for routine examination was emphasized, which may reduce errors in cell counting and trigger further etiological exploration of *C. neoformans* infection in laboratory and clinical practice.**
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KEY WORDS

cryptococcal meningitis, *Cryptococcus neoformans*, Sjogren's syndrome

INTRODUCTION

Cryptococcal meningitis (CM), caused by cryptococcus neoformans (*C. neoformans*) infection, is a life-threatening infectious disease, mainly affecting immunocompromised patients and causing high morbidity and mortality, which is typically observed in patients with later stages of acquired immunodeficiency syndrome (AIDS) [1]. Sjogren's syndrome (SS) is the second most common autoimmune rheumatic disease characterized by

lymphocytic infiltration of exocrine glands and other organs in association with the production of autoantibodies in the blood [2]. To our knowledge, no case of CM in populations with SS has been described. Herein, we report the first case of CM in a 75-year-old woman with 10-years history of SS. Detailed information on clinical manifestations, laboratory profiles and outcome were clarified. Especially, a series of test results for *C. neoformans* in cerebrospinal fluid (CSF) specimen, including morphologic observation in wet mount and India ink stain, 1ateral flow immunoassay (LFA), culturing, mass spectrum analysis and molecular identification were all delineated in this case. Intriguingly, the etio logical exploration was initiated from a positive finding of yeast cells in routine examination of unstained CSF in the present case.

CASE PRESENTATION

A 75-year-old woman was admitted to the Department of Rheumatology with complaints of intermittent fever for half a year and worsening in the last 2 months and consciousness disorders for 1 week. She had a 10-year history of SS and had been treated with regular oral prednisone. A series of medical examinations showed multiple organ involvement, including pulmonary interstitial disease, heart dysfunction, and thrombocytopenia with platelets 27 x 10^9/L (reference interval 125 - 350 x 10^9/L) and a high titer of spotted antinuclear antibody (ANA > 1:1,280). This prompted a lumbar puncture to be performed, and CSF was sent to the Department of Laboratory for further examinations.

A biochemistry test of CSF showed obviously low glucose (0.24 mmol/L), low chloride (104.2 mmol/L), and high protein (0.875 g/L). Appearance of CSF was colorless and turbid. Cell counting of CSF was performed in a Neubauer chamber. Unexpectedly, many budding yeast cells, which were spherical in different sizes with or without spores and obviously different from blood cells, aroused technician's attention. They had thick wall and transparent cytoplasm with reflective particles, which were highly suspected of *C. neoformans* and led to further investigations (Figure 1A). Some encapsulated yeasts were observed in the following India Ink stain (Figure 1B). Eliminating the interference of yeast cells, a total white blood cells of 20 × 10^6/L and red blood cells of 120 × 10^6/L were detected in CSF. The cryptococcal antigen (CrAg) was detected by lateral flow immunoassay (LFA), which provided the earliest proof of *C. neoformans* infection. Culturing was performed on concentrated CSF specimen and smooth, translucent and creamy colonies were formed on Sabouraud dextrose agar after culturing at 37°C for 72 hours (Figure 1C). The Microflex LT/SH mass spectrometer (Bruker, Germany) identified the strain as *Cryptococcus neoformans var. grubii*. The CSF sample was sent to Shanghai Sangon Biotech Co., Ltd. (Shanghai, China) for molecular identification. A 18S rRNA sequence analysis was performed with the following primers: NS1F (5′-GTAGTCATATGCTTGTCTC-3′) and NS6R (5′-GCATCACAGA CCTGTTATTGC CTC-3′). The BLAST results confirmed that the isolate was *C. neoformans*, and it had high homology with published *C. Gattii* gene (GenBank: KF036677.1) (Figure 2). Diagnosis of complication of CM in SS was confirmed based on the molecular identification. Amphotericin B (2.0 mg/kg/day) and flucytosine (100 mg/kg/day) was administrated. However, *C. neoformans* was cultured from the blood two days later which indicated the occurrence of cryptococcal fungemia. Unfortunately, the patient deteriorated and died of heart failure five days after diagnosis of CM.

DISCUSSION

Differences in cryptococcosis are mainly dependent on the host immune condition. Prolonged steroid therapy is another major risk factor for developing CM [3], which may explain why CM is possible to be concurrent with SS. A majority of patients with CM present with signs and symptoms of meningitis, such as headache, neck stiffness, and fever. The presence of cryptococcemia is the most significant prognostic factor in CM [4], which eventually led to a poor prognosis in our case.

Early diagnosis and timely intervention are keys to reducing mortality from CM. Traditionally, the India ink stain was widely used for the rapid detection of C. neoformans from CSF by identifying the encapsulated yeast. However, according to WHO guidelines, a rapid cryptococcal antigen (CrAg) assay, preferably lateral-flow assay (LFA), in CSF, serum, plasma or whole blood is preferred to the India ink test, based on the higher sensitivity and specificity, and depending less on the health provider's skills [5]. Semiskilled health care workers without laboratory training can perform LFA in clinics or at the patients' bedsides [1], thus LFA can be one of the most attractive diagnostic tests ever developed in clinical microbiology [3]. CSF culturing is considered the gold standard for diagnosis of CM, but requires a prolonged time to obtain definitive results, up to 1 - 2 weeks in some settings [1]. Our results also support the use of mass spectrometer for the rapid identification of subspecies of C. neoformans within a clinical setting. Molecular biological assays have been proven to be much more specific than conventional yeast identification methods, which are usually performed in research settings [6]. Molecular identification targeting 18S rRNA sequence promoted the definitive diagnosis of CM in our case.

Intriguingly, the earliest suspicion of C. neoformans originated from a positive finding of budding yeast cells in routine examination of unstained CSF. Morphologic description of C. neoformans mostly focuses on characteristics in India ink stain, and those in unstained CSF have never been described in literature. Typical characteristics of C. neoformans under the microscope in un-

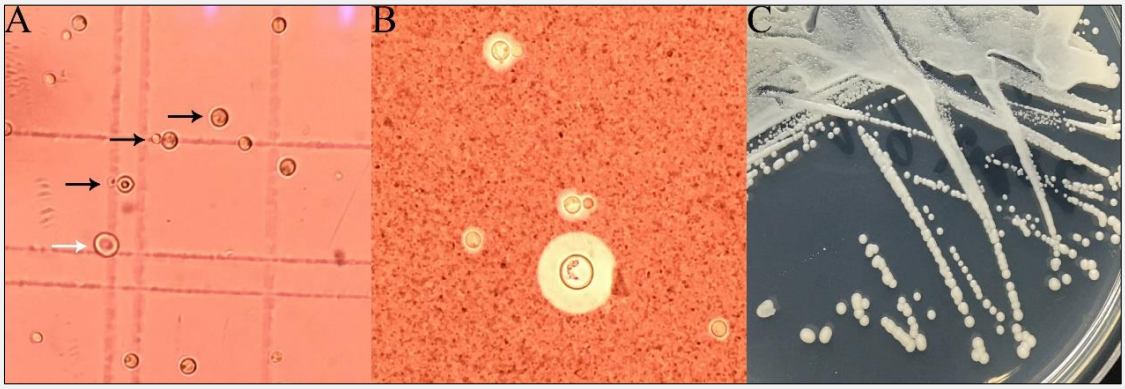

Figure 1. A. Wet mount preparation of CSF displayed variably sized, spherical yeast cells (black arrow) (the white arrow refers to a red blood cell) (x 400). B. Encapsulated *C. neoformans* were detected in India ink preparation (x 400). C. Colonies of *C. neoformans* appeared smooth, translucent, and creamy on Sabouraud dextrose agar.

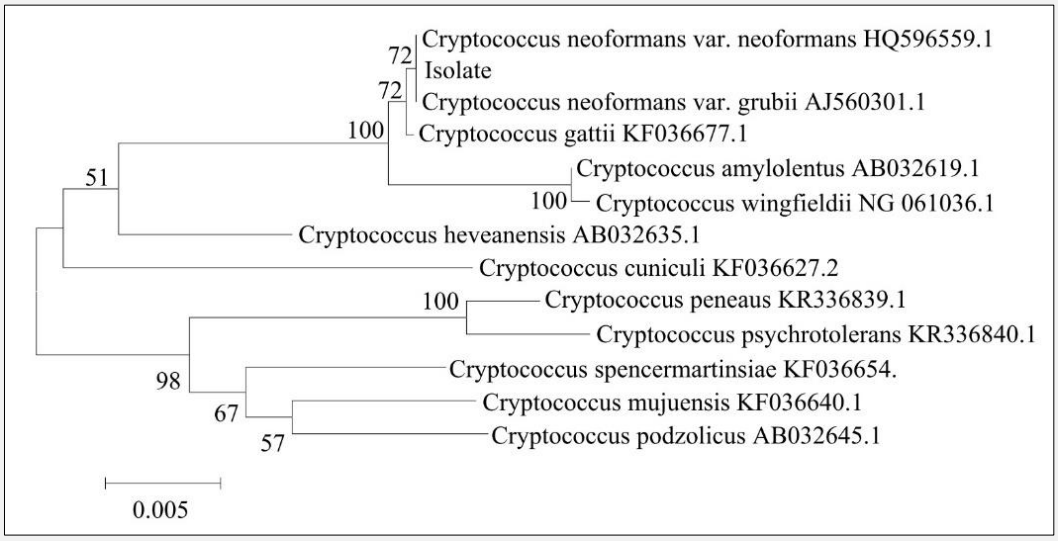

Figure 2. The phylogenetic tree of 18S rDNA sequence in the case and related species.

stained CSF were depicted vividly in our case. CSF samples were sent to laboratories more routinely for cell count than India ink stain. The importance of screening yeast cells in routine test of unstained CSF samples has never been discussed in previous literature, which may be vital for reducing errors in cell counting and providing first-hand tentative detection of C. neoformans in some settings. Another interesting question is whether Gram stain should be used as a routine method for detecting C. neoformans infection. A suggestive viewpoint was that particular attention should be paid to the Gram stain for the starburst pattern produced by Cryptococcus, as this may be seen more often in Gram stain than in India ink [6]. If so, the chances of detecting C. neoformans will greatly increase because of the widest application of Gram stain in clinical microbiology. It is

noteworthy that C. neoformans infections can present initially as a pulmonary infection; disseminated cryptococcosis may include skin lesions, endocarditis, heaptitis, renal infection, and pleural effusion [6], which should remind technicians to pay attention to detection of C. neoformans in various specimens from the affected tissues.

CONCLUSION

In summary, we report the first case of CM in a patient with SS, which suggests clinicians should consider the possible complication of CM when patients with SS show neurological symptoms. Detailed findings from CSF examinations, including routine examination, Indian ink stain, immunological test, culture, mass spectrometer, and molecular biology were all delineated in this case, which facilitated understanding of detecting methods in C. neoformans infection. The earliest suspicion of C. neoformans originated from a positive finding of budding yeast cells in routine examination of unstained CSF. The importance of screening yeast cells in routine test of unstained CSF was emphasized which may reduce errors in cell counting and trigger further etiological exploration of C. neoformans infection in laboratory and clinical practice.

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Additional File:
18S rRNA sequence analysis (DOCX 57 kb).

Declaration of Interest:
The authors declare that they have no competing interests.

References:

1. Rajasingham R, Wake RM, Beyene T, Katende A, Letang E, Boulware DR. Cryptococcal Meningitis Diagnostics and Screening in the Era of Point-of-Care Laboratory Testing. J Clin Microbiol 2019 Jan 2;57(1):e01238-18 (PMID: 30257903).

2. Vivino FB. Sjogren's syndrome: Clinical aspects. Clin Immunol 2017 Sep;182:48-54 (PMID: 28428095).

3. Temfack E, Boyer-Chammard T, Lawrence D, et al. New Insights Into Cryptococcus Spp. Biology and Cryptococcal Meningitis. Curr Neurol Neurosci Rep 2019 Oct 31;19(10):81 (PMID: 31673881).

4. Tsai WC, Lien CY, Lee JJ, et al.The clinical characteristics and therapeutic outcomes of cryptococcal meningitis in elderly patients: a hospital-based study. BMC Geriatr 2019 Mar 25;19(1):91(PMID: 30909914).

5. Guidelines for The Diagnosis, Prevention and Management of Crytococcal Disease in HIV-Infected Adults, Adolescents and Children: Supplement to the 2016 Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Geneva: World Health Organization; 2018 Mar (PMID: 30285342).

6. Hall GS . Bailey & Scott's Diagnostic Microbiology, 13th Edn. Laboratory Medicine(4):e138-e139. ISBN: 978-0-323-08330-0 (https://doi.org/10.1309/LM5JC0PH0OGGBSZZ).

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