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Original Article

Examination of a Chinese-made cryptococcal glucuronoxylomannan antigen test in serum and bronchoalveolar lavage fluid for diagnosing pulmonary cryptococcosis in HIV-negative patients

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KEYWORDS
Pulmonary cryptococcosis;
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HIV-Negative

Abstract *Background:* We presented the performance of a Chinese-made cryptococcal glucuronoxylomannan (GXM) antigen test using serum and bronchoalveolar lavage fluid (BALF) samples in the HIV-negative Chinese population.
Methods: Between February 2017 and January 2019, HIV-negative patients with pulmonary cryptococcosis were recruited and followed-up every three months, including completion of a chest CT examination and collection of serum and BALF samples.
Results: Here, thirty-seven confirmed and ten clinically diagnosed patients were recruited. Furthermore, samples from 174 noncryptococcosis patients that may cause false positives were also collected. The sensitivity of a lateral flow assay (LFA) for detecting cryptococcal GXM antigen in serum and BALF samples from confirmed cases was 97% and 95%, respectively, and the specificity was 98.2% and 93%, respectively, and the differences in these values between the BALF and serum samples were not significant. The serum cryptococcal GXM antigen value showed a positive correlation (r: 0.581, $p < 0.001$) with pulmonary lesion size, while the BALF value showed no correlation (r: 0.253, p: 0.13). The positivity rate of BALF was higher than that of serum when the diameter of the pulmonary lesion was small (diameter less than 20 mm). Moreover, the serum cryptococcal GXM antigen levels showed an overall decreasing trend with the decrease in pulmonary lesion size after antifungal therapy in patient follow-up.
Conclusions: The Chinese-made cryptococcal GXM antigen test has better sensitivity and

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specificity for diagnosing pulmonary cryptococcosis in the HIV-negative Chinese population, and it could be used to diagnose and to monitor this disease.

Introduction

Recently, the incidence of pulmonary cryptococcosis (PC) has been found to be increasing yearly in HIV-negative patients. Foreign epidemiological studies show that the incidence of PC increased by more than 6-fold from 1999 to 2006, reaching 38 cases per million people, and most of these cases were among HIV-negative patients.[1] The reported cases of PC in non-HIV patients increased significantly after 2000 in China.[2] Since the outbreak of cryptococcosis in Vancouver in 2000, HIV-negative cryptococcosis has been increasingly studied.[3] PC is easily misdiagnosed as tuberculosis or lung cancer because of the similar imaging findings and mild symptoms.[4] Of 589 South African miners tested for PC, 51.9% were misdiagnosed with tuberculosis. Studies show that 15% of patients in public hospitals in South Africa develop cryptococcal meningitis within 3 months after being misdiagnosed as negative for tuberculosis.[5] In summary, the incidence of PC is increasing, its imaging and clinical manifestations lack specificity and are easy to misdiagnose, and diagnosis may be missed. If not diagnosed and treated in time, disseminated cryptococcosis may develop and affect the prognosis. Therefore, early diagnosis and treatment are important for preventing the spread of Cryptococcus infection and reducing the mortality among patients with cryptococcal disease.

Currently, the diagnosis of PC mainly depends on pathological biopsy, and the methods of obtaining pathological tissues mainly include surgery, percutaneous lung puncture and tracheoscopy biopsy, which are invasive examinations. These examinations are difficult for patients because of complications, including pneumothorax and hemorrhage. Moreover, diagnostic yields of transbronchial fine needle aspiration (TBNA) and transbronchial biopsy can significantly vary depending on the size and location of the nodule.[6] Previous studies reported that chest imaging of lesions from patients with PC revealed mostly nodules. In one report about HIV-negative PC, the percentage of nodules with a diameter of less than 25 mm was as high as 79.4%, and lesions were mostly distributed below the pleura,[7] which made bronchoscopy biopsy very limited. The addition of endobronchial ultrasound guidance or electromagnetic navigation increases diagnostic yield, but the yields are still lower for lesions not more than 2.0 cm in diameter (56.3% for lesions $<$ 2.0 cm vs. 77.7% for lesions 2.0 cm).[6] Small lung lesions are an independent risk factor for pneumothorax during puncture. The reported incidence of pneumothorax after percutaneous lung biopsy ranges from 0% to 61%, with 0—13% of patients requiring intervention. Peripheral bleeding may occur in 26% of patients.[8] Moreover, the diagnostic yield of these approaches is variable because biopsies can contain nontumor elements, such as areas of fibrotic tumor stroma and/or surrounding normal lung tissue.

Compared with the above methods, the detection of cryptococcal GXM antigen has the advantages of high sensitivity and specificity and economic convenience. There are three methods for the detection of cryptococcal GXM antigen, including enzyme-linked immunosorbent assay (ELISA), latex agglutination assay (LA) and lateral flow assay (LFA). Compared with the other two methods, LFA is simple to perform, fast, economical, sensitive and specific.[9] LFA was 5-fold as sensitive than LA when cross-comparing semi-quantitative titers by serial dilution.[10] Since 2011, detection with the cryptococcal GXM antigen LFA assay has gradually replaced LA assay in some laboratories.[11] The IMMY LFA (Immuno-Mycologics, Inc., Norman, OK, USA) is a semi-quantitative test. As the sample concentration cannot be estimated, a large number of reagent strips are consumed, and the number of operation steps may be high. Compared to IMMY LFA, the Chinese-made Dynamiker LFA may detect the strength of the detection band more conveniently and effectively by using an immunoquantitative analyzer.[12] The theory is that the sensor of the immunoquantitative analyzer can directly read the gray level of the T line on the reagent strips. The gray level has been shown to be proportional to the concentration, so the value can reflect the cryptococcal GXM antigen concentration. The other advantage of the Chinese-made Dynamiker LFA is that the minimum LOD is 0.5—1.0 ng/ml, while the IMMY LFA minimum LOD is 1.0—1.5 ng/ml.

Because of the advantage of the Chinese-made cryptococcal GXM antigen test, we wanted to evaluate its performance in detecting PC in serum and BALF samples. The clinical application of the diagnosis of PC was also evaluated, which will aid in the development of more effective test methods for diagnosing and monitoring PC in the future.

Methods

Ethics

This prospective study was performed at the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. Furthermore, this study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University.

Study design

Selection and description of participants

Forty-seven PC patients were enrolled between February 2017 and January 2019. Furthermore, these PC patients were classified according to the European Organization of Research and Treatment of Cancer/Mycoses Study Group

revised definitions of 2008.[13] The diagnosis of PC was confirmed by the presence of cryptococcosis in either histopathological or cytological specimens, including transbronchial lung biopsies and CT-guided percutaneous lung biopsies. Patients positive for serum cryptococcal GXM antigen but negative in the histopathologic or cytologic examinations were defined as "clinically diagnosed." The exclusion criteria were as follows: 1) AIDS, 2) disseminated cryptococcal disease, 3) a combination of other infections, 4) patient had undergone surgical removal of a lung cryptococcal infection, 5) patient was not willing to undergo this detection procedure. The control group of this study included patients with other noncryptococcal infectious diseases, including other pulmonary fungal diseases, bacterial pneumonia, connective tissue disease, tuberculosis, lung cancer, and idiopathic pulmonary fibrosis.

Specimen collection and PC cryptococcal GXM antigen testing

Blood specimens (5 ml) were collected from enrolled patients on the day of the chest CT examination or within 7 days, and serum was separated by centrifugation at 3000 rpm for 10 min. Furthermore, BALF was collected after the patients underwent bronchoscopy tests due to the disease diagnosis. These patients were lavaged in the infected lung segment, and the bronchoscopy tip was confined to the target bronchial segment. Physiological saline was rapidly injected into the operating channel at 37 °C or room temperature at a total volume of 60—120 ml, and the injection was 20—50 ml each time. Appropriate vacuum suction was adopted, and the recovery rate should be greater than 30%. The aspirated fluid was centrifuged (3000 rpm) for 10 min, and the cell-free supernatant was tested; the pellet was cultured and used for cytologic examination.

A new Chinese-made cryptococcal GXM antigen test was used for the quantitative detection of cryptococcal GXM antigen. According to the kit instructions, 40 μl of serum or BALF was placed on the test strip. After 10 min, the value was read with an immunoquantitative analyzer. If the value was greater than 100, the above operation was repeated after the sample was diluted 10-fold.

The PC GXM antigen was tested on both serum and BALF samples using LFA according to the manufacturer's instructions. The above detection kits were provided by Dynamiker Biotechnology Co., Ltd. (Tianjin, China).

Radiological assessment

CT is commonly performed under the Chinese medical insurance system; thus, all of the patients enrolled in this study underwent CT examinations. A radiologist and a respiratory physician independently assessed each patient's CT scans. The maximum diameter of the largest pulmonary lesion was used in the subsequent analyses if the patient had multiple lesions.

Statistics

ROC curves were constructed using the cryptococcal GXM antigen values of the confirmed PC and control groups, and the area under the curve (AUC) > 0.5 was significant. Correlation significance between cryptococcal GXM antigen titers and pulmonary lesion size was calculated using Spearman's rank analysis. P-values < 0.05 indicated significant differences.

Results

Patient background

A total of 221 HIV-negative patients were recruited into this study, including those with and without PC. Among the 47 PC patients, 20 patients did not have underlying conditions, accounting for 43%, and the other 27 (57%) had one or more underlying conditions. Among the 174 patients without PC, 66 (38%) patients did not have underlying conditions, and the other 108 (62%) had multiple underlying conditions (Table 1).

Sensitivity and specificity of the Chinese-made cryptococcal GXM antigen test

Table 2 shows that the Chinese-made cryptococcal GXM antigen test had good sensitivity and specificity in the serum samples, 97% and 98.2%, respectively. The test also had good sensitivity and specificity in the BALF samples, 95% and 93%, respectively; although these numbers were slightly inferior to those for serum, the difference was not significant.

Furthermore, the area under the curve (AUC) represents the accuracy of the Chinese-made cryptococcal GXM antigen test, which was 0.9960 (95% CI 0.9896—1.002, $P < 0.0001$, optimal threshold value 1.59) and 0.9635 (95% CI 0.9080—1.018, $P < 0.0001$, optimal threshold value 0.34) for serum (red line) and BALF (blue line), respectively (Fig. 1).

Relationship between pulmonary lesion size and antigen titer of the Chinese-made cryptococcal GXM antigen test

First, the correlation between cryptococcal GXM antigen titers and pulmonary lesion size is shown (Fig. 2). There was a positive correlation between the lesion size and the antigen value in both sample types. However, the serum cryptococcal GXM antigen value showed a better correlation (r: 0.536 $p < 0.001$; Spearman's rank correlation) with lesion size than did the BALF titer (r: 0.373, p: 0.073).

Then, the correlation between the pulmonary lesion size and the rate of positive cryptococcal GXM antigen tests for all patients was determined. The rate of positive samples was higher for BALF tests than for serum tests, especially for pulmonary lesions with diameters $\leq$20 mm (Fig. 3).

Relationship between the value of the Chinese-made cryptococcal GXM antigen test and prognosis in serum samples

It was shown that there were positive correlations between the lesion size and the antigen value of the Chinese-made cryptococcal GXM antigen test. This analysis was performed to evaluate whether the improvement of illness, which was estimated via lesion absorption, has a good relationship with

Table 1 Characteristics of 221 HIV-negative patients with and without pulmonary cryptococcosis.

Characteristics	
Mean age (years)	63.2 ± 13.6
Sex (men: women)	124:97
Underlying conditions[a] (n = 221)	
Nonpulmonary cryptococcosis (n = 174)	Pulmonary cryptococcosis (n = 47)
Other pulmonary mycoses	Hypertension (7)
Pulmonary Talaromyces marneffei (3)	Hepatitis B virus (6)
Pulmonary mucormycosis(2)	Diabetes (8)
Pulmonary aspergillosis (26)	Rheumatoid arthritis (6)
Pneumonia	Coronary heart disease (1)
Bacterial pneumonia (21)	Hypoalbuminemia (1)
Cytomegaloviral pneumonitis (4)	IgA nephropathy (1)
Pulmonary tuberculosis (13)	Membranous nephropathy (2)
Lung cancer (34)	Renal insufficiency (1)
Connective tissue disease	Systemic lupus erythematosus (1)
Rheumatoid arthritis (7)	Kidney transplantation (2)
Systemic lupus erthematosus (6)	Thymus gland malignant tumor (1)
Connective tissue-related interstitial lung disease (20)	Gastric cancer (1)
Idiopathic interstitial fibrosis (11)	Nephrotic syndrome (1)
No underlying disease (66)	Pulmonary embolism (2)
	No underlying disease (20)

[a] *Some people had multiple underlying conditions.*

Table 2 Sensitivity and specificity of the Chinese-made cryptococcal GXM antigen test.

	Serum GXM test	BALF GXM test
Sensitivity (%)	97%	95%
Specificity (%)	98.2%	93.0%
Positive predictive value (%)	92.3%	95%
Negative predictive value (%)	99.4%	93%

A total of 37 confirmed pulmonary cryptococcosis and 174 noncryptococcosis patients were included in the analysis. The numbers of serum and BALF specimens from confirmed patients were 37 and 20, respectively, and from the noncryptococcosis patients were 174 and 20, respectively.

the antigen value of the Chinese-made cryptococcal GXM antigen test in serum samples. Follow-up occurred at the 0-, 3-, 6- and 9-month visits after the first test. Six patients had complete follow-up. The baseline serum cryptococcal GXM antigen test was positive for all six patients. One case became negative after 6 months (at the end of treatment). One case showed some fluctuations, and the degree of decline in antigen value was not obvious at 9 months but showed an obvious decline at 12 months after treatment. The antigen values of other patients showed a gradual decrease, accompanied by a narrowing of the lesion. In summary, the serum cryptococcal GXM antigen levels decreased when the pulmonary lesions resolved after antifungal therapy in all followed-up patients, which contributed to the indication of this test for assessing clinical response to treatment for pulmonary cryptococcus (Fig. 4).

Discussion

Here, we first evaluated the sensitivity, specificity and clinical use of the Chinese-made cryptococcal GXM antigen test. Furthermore, the test showed that there were positive correlations between lesion size and the antigen value and that the rate of positivity was higher for BALF tests than for serum tests, especially for pulmonary lesions with diameters ≤20 mm; moreover, the decrease in antigen value may indicate improvement of the disease.

The cryptococcal GXM antigen test for PC diagnosis was originally proven to be feasible,[7] and our findings for the Chinese-made cryptococcal GXM antigen test were the same. However, the Chinese-made cryptococcal GXM antigen test had better sensitivity than the other cryptococcal GXM antigen test, which was published as 97% via 73.9% in serum and 95% via 82.6% in BALF (Table 2),[7] but the specificity was slightly lower. Furthermore, previous reports showed that the sensitivity of the cryptococcal GXM antigen test was 44—100% in BALF and 92—100% in serum.[9,14,15] These results imply that the Chinese-made cryptococcal GXM antigen test is more sensitive, which can be attributed to the lower minimum LOD of this test. Furthermore, the Chinese-made cryptococcal GXM antigen test is more sensitive than ELISA,[16] which may be because the cryptococcal GXM antigen LFA test employs two monoclonal antibodies,

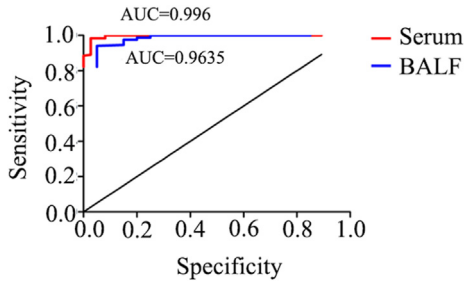

Figure 1. Receiver operating characteristic (ROC) curve for the Chinese-made cryptococcal GXM antigen test in serum and BALF. The ROC curve is plotted between the true-positive rate (sensitivity) on the y-axis and the false-positive rate (specificity) on the x-axis. The red line is serum, and the blue line is bronchoalveolar lavage fluid (BALF).

one of which binds to serotypes A, B, and C and the other to serotypes A and D.[17] In our study, we also compared the performance (sensitivity and specificity) of Chinese-made cryptococcal GXM antigen test with that from cryptococcal GXM antigen ELISA test (Dynamiker Biotechnology Co., Ltd. Tianjin, China). The specimen type used for cryptococcal GXM antigen ELISA were serum and BALF. The numbers of serum and BALF specimens from confirmed patients were 37 and 18, respectively, and from the non-cryptococcosis patients were 65 and 13, respectively, and our results demonstrated that the sensitivity of the Chinese-made cryptococcal GXM antigen test was better than that of the cryptococcal GXM antigen ELISA test 97% vs. 51% for serum samples, and 95% vs. 39% for BALF samples, which confirmed the above study.

However, there is a disadvantage of the Chinese-made cryptococcal GXM antigen test: its specificity is low (Table 1 and Fig. 1). It has been proven that there are cross-reactions between Cryptococcus and other disease markers, such as *Aspergillus* galactomannan,[18] systemic lupus erythematosus, rheumatoid factor, megagglutinemia, carbon dioxide phagocytic bacteria, acid-resistant bacilli and trichospore bacteria. Thus, samples from patients with these diseases were included in the control group in this

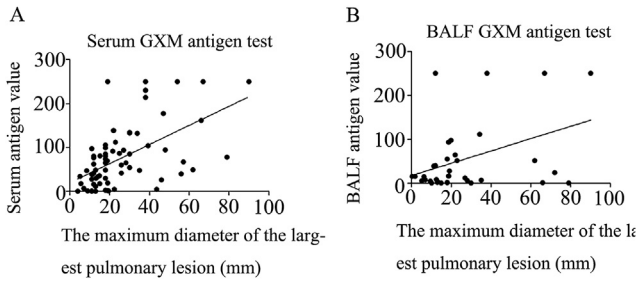

Figure 2. Correlation analysis between the GXM antigen value and the maximum diameter of the largest pulmonary lesion. A means correlation between serum GXM antigen value and the maximum diameter of the largest pulmonary lesion. B shows the correlation between the BALF GXM antigen value and the maximum diameter of the largest pulmonary lesion. The correlation significance was calculated using Spearman's rank analysis.

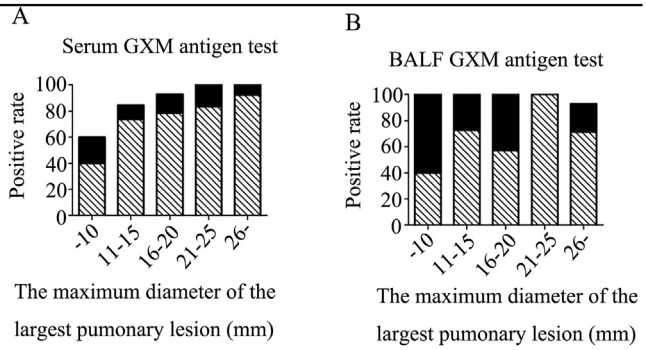

Figure 3. Correlation between the maximum diameter of the largest pulmonary lesion and the rate of positive cryptococcal GXM antigen tests (for confirmed and clinically diagnosed cases). A indicates the serum GXM antigen test, and B indicates the BALF GXM antigen test. Black boxes represent confirmed cases, and boxes with slanted lines represent clinically diagnosed cases.

study (Table 1). In the control group, there were several samples that produced false positive serum antigen test results via the Chinese-made cryptococcal GXM antigen test. Qualitative test results were not easy to identify by the naked eye, and the values read by the immunoquantitative analyzer were all less than 1. According to previous reports, when the value of LFA is classified as strong (+++), medium (++), weak (+), suspicious positive (±) and negative (−) based on the titers of the latex agglutination experiment, the sensitivity and specificity are 100% and 90%, respectively, if (+) is accepted as positive and 100% if (−) and (+) are regarded as negative results.[19] Therefore, if patients with extremely low antigen titers are encountered in the process of diagnosis and treatment, the diagnosis should be made after careful consideration to avoid misdiagnosis.

Nodules account for approximately 57% of PC cases according to statistics. A Japanese study reported that the pulmonary lesion diameter was less than 25 mm in 79.4% of HIV-negative pulmonary cryptococcosis patients. The detection rate of serum latex agglutination antigen was low, and puncture biopsy was difficult in patients whose pulmonary lesion was 16.4 ± 5.5 mm.[7] Our results show that the detection rate of BALF is higher when the pulmonary lesion diameter is less than 20 mm (Fig. 3), which is consistent with a previous report[7] and can help in the early diagnosis of pulmonary cryptococcosis. However, the correlation between BALF and pulmonary lesion diameter was worse than that of serum (Fig. 2), which may be because the volume of physiological saline and recovery rate were not certain, and the antigen concentration was diluted to different degrees.

The duration of pulmonary cryptococcosis treatment is long, so disease development should be monitored during the period of antifungal therapy. Whether the change in antigen value is related to patient prognosis is worth debating. Some researchers think antigen detection cannot be an indicator of monitoring condition because the literature shows that persistently positive antigen titers are most common in untreated patients and may remain strongly positive despite complete or partial resolution of disease,[20] and after the resection of pulmonary cryptococcosis lesions, cryptococcal GXM antigen still show positive results. Patients with PC who

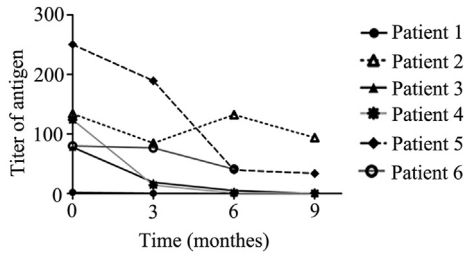

Figure 4. The dynamic change in the serum GXM antigen level was detected in patients with antigenic deficiency 6 months after treatment. A total six patients, patient one to six, are shown. This figure mainly presents the change in level of serum GXM antigen value with antifungal therapy.

could stop drug use after treatment evaluation were still positive for cryptococcal GXM antigen and remained positive for nearly 1 year after the drug was stopped.[21] This result is because dead cryptococcus continue to release capsular polysaccharide antigens, and the body clears such antigens relatively slowly. Even after several months of effective treatment, the body fluid antigen test can still be positive; therefore, whether the antigen test turns negative cannot be used as an indicator of whether cryptococcosis is cured.[22] However, although cryptococcal GXM antigen in pulmonary cryptococcosis remain detectable for as long as months following successful therapy, serum cryptococcal GXM antigen titers in pulmonary cryptococcosis patients can continuously decrease after effective therapy and remain at low levels for long periods after therapy.[21] Similarly, in our study, the results showed that the overall trend of antigen titer of cryptococcal GXM antigen was decreased, which was related to prognosis (Fig. 4). Moreover, some studies show that the antigen titer gradually decreases with PC treatment and that a continuous increase in antigen titer suggests that disseminated cryptococcosis may exist.[23] Consequently, these studies suggested that although a decrease in cryptococcal GXM antigen titers cannot be used as an indicator of cure, it can be used to monitor the efficacy of antifungal treatment. This finding may be because antifungal treatment prevented the progressive development of the disease. The number of cryptococcus decreased, and metabolism gradually eliminated dead cryptococcus. Therefore, the release of cryptococcal GXM antigen gradually decreased, and the antigen titers continued to decrease. Moreover, our data show that cryptococcal GXM antigen monitoring can be carried out at three-month intervals during the treatment process to determine the therapeutic efficacy of pulmonary cryptococcosis. However, further study is needed for confirmation to prove these findings in the future.

Conclusion

In this study, we proved for the first time that the Chinese-made cryptococcal GXM antigen test had good sensitivity, although it had slightly worse specificity than the previously reported GXM antigen test. This test can be used to assist in the diagnosis of pulmonary cryptococcosis; in particular, it can help in the early diagnosis of PC when the pulmonary lesion diameter is less than 20 mm. Furthermore, the Chinese-made cryptococcal GXM antigen test may be used to evaluate the prognosis of PC disease. However, there are also some limitations in our study, such as the small number of follow-up patients and short follow-up time. In the future, more patients could be included for longer-term follow-up.

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

1. Galanis E, Macdougall L, Kidd S, Morshed M, British Columbia *Cryptococcus gattii* Working Group. Epidemiology of cryptococcus gattii, British Columbia, Canada, 1999—2007. *Emerg Infect Dis* 2010;**16**:251—7. https://doi.org/10.3201/eid1602.090900.
2. Zhejiang Medical Association Society of Respiratory Diseases. Expert consensus on diagnosis and treatment of pulmonary crytococcosis. *Chin J Clin Infect Dis* 2017;**10**:321—6. https://doi.org/10.3760/cma.j.issn.1674-2397.2017.05.001.
3. Kidd SE, Hagen F, Tscharke RL, et al. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). *Proc Natl Acad Sci USA* 2004; **101**:17258—63. https://doi.org/10.1073/pnas.0402981101.
4. Pawar S, Ganakumar V, Jha S, et al. Pulmonary cryptococcoma masquerading as lung cancer. *J Assoc Phys India* 2016;**64**:66—8.
5. Jarvis JN, Wainwright H, Harrison TS, Rebe K, Meintjes G. Pulmonary cryptococcosis misdiagnosed as smear-negative pulmonary tuberculosis with fatal consequences. *Int J Infect Dis* 2010;**14**(Suppl 3):e310—2. https://doi.org/10.1016/j.ijid.2010.02.2255.
6. Hariri LP, Villiger M, Applegate MB, et al. Seeing beyond the bronchoscope to increase the diagnostic yield of bronchoscopic biopsy. *Am J Respir Crit Care Med* 2013;**187**:125—9. https://doi.org/10.1164/rccm.201208-1483OE.
7. Oshima K, Takazono T, Saijo T, et al. Examination of *cryptococcal* glucuronoxylomannan antigen in bronchoalveolar lavage fluid for diagnosing pulmonary cryptococcosis in HIV-negative patients. *Med Mycol* 2018;**56**:88—94. https://doi.org/10.1093/mmy/myx010.
8. Covey AM, Gandhi R, Brody LA, Getrajdman G, Thaler HT, Brown KT. Factors associated with pneumothorax and pneumothorax requiring treatment after percutaneous lung biopsy in 443 consecutive patients. *J Vasc Intervent Radiol* 2004;**15**:479—83. https://doi.org/10.1097/01.rvi.0000124951.24134.50.
9. Jitmuang A, Panackal AA, Williamson PR, Bennett JE, Dekker JP, Zelazny AM. Performance of the *cryptococcal* antigen lateral flow assay in non-HIV-related cryptococcosis. *J Clin Microbiol* 2016;**54**:460—3. https://doi.org/10.1128/JCM.02223-15.

10. Rajasingham R, Meya DB, Boulware DR. Integrating *cryptococcal* antigen screening and pre-emptive treatment into routine HIV care. *J Acquir Immune Defic Syndr* 2012;**59**: e85—91. https://doi.org/10.1097/QAI.0b013e31824c837e.
11. Lindsley MD, Mekha N, Baggett HC, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis* 2011;**53**:321—5. https://doi.org/10.1093/cid/cir379.
12. Kim S, Park JK. Development of a test strip reader for a lateral flow membrane-based immunochromatographic assay. *Biotechnol Bioproc E* 2004;**9**(2):127—31. https://doi.org/10.1007/BF02932995.
13. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) consensus group. *Clin Infect Dis* 2008;**46**:1813—21. https://doi.org/10.1086/588660.
14. Baughman RP, Rhodes JC, Dohn MN, Henderson H, Frame PT. Detection of *cryptococcal* antigen in bronchoalveolar lavage fluid: a prospective study of diagnostic utility. *Am Rev Respir Dis* 1992; **145**:1226—9. https://doi.org/10.1164/ajrccm/145.5.1226.
15. Senghor Y, Guitard J, Angoulvant A, Hennequin C. Cryptococcal antigen detection in broncho-alveolar lavage fluid. *Med Mycol* 2018;**56**:774—7. https://doi.org/10.1093/mmy/myx092.
16. Panackal AA, Dekker JP, Proschan M, Beri A, Williamson PR. Enzyme immunoassay versus latex agglutination cryptococcal antigen assays in adults with non-HIV-related cryptococcosis. *J Clin Microbiol* 2014;**52**(12):4356—8. https://doi.org/10.1128/JCM.02017-14.
17. Vidal JE, Boulware DR. Lateral flow assay for *cryptococcal* antigen: an important advance to improve the continuum of HIV care and reduce *cryptococcal* meningitis-related mortality. *Rev Inst Med Trop Sao Paulo* 2015;**57**(Suppl 19):38—45. https://doi.org/10.1590/S0036-46652015000700008.
18. Dalle F, Charles PE, Blanc K, et al. *Cryptococcus neoformans* galactoxylomannan contains an epitope(s) that is cross-reactive with *Aspergillus* galactomannan. *J Clin Microbiol* 2005;**43**:2929—31. https://doi.org/10.1128/JCM.43.6.2929-2931.2005.
19. Datcu Raluca, Björndóttir María Krístin, Mahler Stine Steen, et al. *Evaluation of the new dynamiker cryptococcal antigen lateral flow assay (LFA) in comparison with IMMY LFA and meridian latex agglutination test*. Madrid: European Congress of Clinical Microbiology; 2018.
20. Fisher JF, Valencia-Rey PA, Davis WB. Pulmonary cryptococcosis in the immunocompetent patient-many questions, some answers. *Open Forum Infect Dis* 2016;**3**:ofw167. https://doi.org/10.1093/ofid/ofw167.
21. Kohno S, Kakeya H, Izumikawa K, et al. Clinical features of pulmonary cryptococcosis in non-HIV patients in Japan. *J Infect Chemother* 2015;**21**:23—30. https://doi.org/10.1016/j.jiac.2014.08.025.
22. Lu H, Zhou Y, Yin Y, et al. Cryptococcal antigen test revisited: significance for cryptococcal meningitis therapy monitoring in a tertiary Chinese hospital. *J Clin Microbiol* 2005;**43**(6): 2989—90. https://doi.org/10.1128/JCM.43.6.2989-2990.2005.
23. Hung MS, Tsai YH, Lee CH, Yang CT. Pulmonary cryptococcosis: clinical, radiographical and serological markers of dissemination. *Respirology* 2008;**13**:247—51. https://doi.org/10.1111/j.1440-1843.2007.01202.x.