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Original Article

Cryptococcal disease in patients with or without human immunodeficiency virus: clinical presentation and monitoring of serum cryptococcal antigen titers

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Background and purpose: *Cryptococcus neoformans* is an encapsulated pathogenic yeast that causes a wide range of clinical manifestations. The serum cryptococcal latex agglutination test is a simple, rapid, and reliable diagnostic test for cryptococcosis. This study was performed to assess the clinical relevance of serum cryptococcal antigen (CRAG) titer in patients with cryptococcosis with or without human immunodeficiency virus (HIV).
Methods: From January 1999 to December 2007, 45 patients with a diagnosis of cryptococcosis made by culture and/or histopathology were enrolled in this retrospective study. Ten patients had HIV and 35 were not infected.
Results: Patients with HIV were more likely to have central nervous system (CNS) involvement than patients without HIV (100% vs 37.1%; $p = 0.0005$), higher serum CRAG titers (median, 1:1024 vs 1:64; $p < 0.05$), higher positive cerebrospinal fluid (CSF) CRAG (100% vs 37.1%; $p = 0.0005$), and higher CRAG titers in the CSF (median, 1:1024 vs 1:32; $p < 0.001$). Patients without HIV were more likely to have pulmonary involvement (62.9% vs 0%; $p = 0.0005$) and no underlying disease at diagnosis (42.9% vs 0%; $p = 0.011$). Serum CRAG titers among patients without HIV with CNS or pulmonary cryptococcosis declined during treatment and no relapse was noted when serum CRAG titers were ≤1:8 at the end of treatment.
Conclusion: Serum CRAG titer can be used to monitor disease activity during treatment for CNS and pulmonary cryptococcosis in patients without HIV.

Key words: Antigens; Cryptococcosis; *Cryptococcus neoformans*; HIV

Introduction

Cryptococcus neoformans is a ubiquitous pathogenic encapsulated yeast that causes infections ranging from asymptomatic pulmonary colonization to life-threatening meningitis [1]. *C. neoformans* occurs both among apparently immunocompetent hosts without underlying disease and immunocompromised hosts [2]. The 2 most common sites of cryptococcosis are the lungs and central nervous system (CNS). Following infection of tissues, the polysaccharide capsule of *C. neoformans*, a determinant of fungal pathogenicity, markedly increases in size and is released into the body fluids of infected individuals [3]. Hence, its detection in the serum and cerebrospinal fluid (CSF) serves as an important diagnostic marker. Since 1963 [4], the latex agglutination (LA) test, which detects cryptococcal polysaccharide antigens using latex particles coated with antibody, has been a simple, rapid, and reliable serodiagnostic method for detecting cryptococcosis [4]. The test is effective for patients both with and without human immunodeficiency virus (HIV) infection [5-7].

Studies that have assessed the use of serum cryptococcal antigen (CRAG) titers in patients with acquired immunodeficiency syndrome (AIDS) have concluded that persistent high titers suggest a poor outcome [8,9].

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Powderly et al found serum or CSF CRAG declined after antifungal treatment in patients with AIDS-associated cryptococcal meningitis, but did not find any correlation with changes in CSF or serum CRAG and the outcome of cryptococcal meningitis [10]. Aberg et al found similar conclusions [11]. Before the introduction of highly active antiretroviral therapy (HAART), Zuger et al found that CSF CRAG titer >1:8 at the end of treatment was associated with relapse in AIDS-related cryptococcal meningitis [12]. In the study by Sheng et al, performed in the HAART era, only 1 of 7 patients with AIDS whose serum CRAG titer was >1:8 successfully discontinued fluconazole as secondary prophylaxis for cryptococcosis [13].

The role of the CRAG test in patients without HIV has also been demonstrated. Pappas et al found CSF CRAG declined after antifungal therapy among non-HIV–infected patients with cryptococcal meningitis [14]. Chang et al found changes in serum CRAG titers reliably reflected disease progression and response to therapy among non-HIV–infected patients with pulmonary cryptococcosis [15]. Dismukes et al suggested CSF or serum CRAG titer ≤1:8 as a treatment endpoint for non-HIV–infected patients with cryptococcal meningitis [16].

This study was performed to compare the clinical presentations of cryptococcosis between patients with and without HIV and to correlate serum CRAG titers with clinical outcome during follow-up.

Methods

Patients

A retrospective review of the medical records of patients with cryptococcosis admitted to the Tri-Service General Hospital, Taipei, Taiwan, from January 1999 to December 2007 was done. Forty five patients were identified by review of mycology laboratory records, and histopathologic and serologic results. Standard case report forms were used to collect demographic data, associated conditions, presenting symptoms, serum and CSF CRAG titers, treatment, and outcomes.

Case definition

Cryptococcal meningitis was defined as isolation of *C. neoformans* from CSF culture, positive CSF CRAG titer, or positive results of CSF India ink studies, and consistent clinical features of meningitis. Pulmonary cryptococcosis was diagnosed based on radiographic characteristics, sputum cultures, and cytologic or histologic identification of *C. neoformans* obtained by biopsy or bronchoalveolar lavage. HIV was defined according to the Centers for Disease Control and Prevention (CDC) 1993 revised classification system [17].

Laboratory investigation

CRAG titers of the serum and CSF specimens were measured by means of a commercially available LA test, Immy Latex-Crypto Antigen (Immuno-Mycologics, Norman, OK, USA), by following the manufacturer's instructions. CD4+ cell count and plasma viral load were checked in patients with HIV.

All patients with cryptococcosis underwent serum and CSF testing for antigen titers. All patients with pulmonary cryptococcosis underwent lumbar puncture to exclude CNS involvement. The definition of a positive titer was defined as a titer ≥1:8. The definition of a positive rate of serum or CSF antigenemia was the number of patients with a positive serum or CSF titer divided by the number of patients who underwent serum or CSF testing for antigen titers.

Treatment and outcome evaluation

Patients with cryptococcosis were treated according to the practice guidelines for the management of cryptococcal disease [18]. Patients with HIV received amphotericin B 0.7-1.0 mg/kg/day followed by fluconazole. Patients without HIV with CNS cryptococcosis received amphotericin B 0.3-0.5 mg/kg/day followed by fluconazole. The treatment for pulmonary cryptococcosis in patients without HIV depended on the host immune status. The treatment duration was individualized, and was at the discretion of the treating physician.

A patient was considered to have died of active disease during therapy if cultures from the affected site were positive at death or if clinical deterioration in the affected system had progressed despite therapy. A patient was considered to have died of other causes during therapy if CRAG declined and cultures were negative at the time of death, or if clinical improvement in the affected system was evident before death. Relapse was defined as clinical, mycologic, serologic, or radiographic evidence of recurrence after discontinuation of antifungal therapy. Clinical outcome was assessed on 31 December 2007, or at the date of death or loss to follow-up. Observation duration was defined as the time from cessation of antifungal therapy until 31 December 2007, or the date of death or the last follow-up visit.

Follow-up studies

Twenty nine patients (5 with HIV and 24 without HIV) had serial CRAG tests done at 2 months, 3 months, and the end of treatment during follow-up; 13 patients with CNS cryptococcosis and 16 with pulmonary cryptococcosis underwent serial antigen follow-up studies.

Statistical analysis

Continuous variables were analyzed by Student *t* test. Categorical variables were compared using the chi-squared test. A significant difference was considered to be present for *p* values <0.05.

Results

Demographics and clinical data

During the 9-year study period from January 1999 to December 2007, 45 patients with cryptococcosis were enrolled in the study. The temporal trend over the years is shown in Fig. 1. The median age was 54 years (range, 16-85 years). Thirty one patients (68.9%) were men and 14 (31.1%) were women. CNS and pulmonary cryptococcosis accounted for 23 (51.1%) and 22 (49.9%) patients, respectively. Fifteen patients (33.3%) had no underlying disease at the time of diagnosis of cryptococcosis. Other predisposing conditions were AIDS (n = 10; 22.2%), malignancy (n = 5; 11.1%), diabetes mellitus (n = 5; 11.1%), systemic lupus erythematosus (SLE; n = 4; 8.9%), cerebral infarction (n = 3; 6.7%), cirrhosis of the liver (n = 1; 2.2%), hyperthyroidism (n = 1; 2.2%), chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis (n = 1; 2.2%). The most common symptoms were headache (n = 24; 53.3%), nausea and/or vomiting (n = 18; 40.0%), cough (n = 15; 33.3%), fever (n = 10; 22.2%), malaise (n = 9; 20.0%), and dyspnea (n = 4; 8.9%). Serum and CSF CRAG test positivity occurred in 36 (80.0%) and 23 (51.1%) patients, respectively.

Comparison of the demographics and clinical outcomes of patients with cryptococcosis with and without HIV are shown in Table 1. Patients with HIV were more likely to have CNS involvement than patients without HIV (100% vs 37.1%; $p = 0.0005$), symptoms of headache (100% vs 40.0%; $p = 0.0008$) and nausea and/or vomiting (80.0% vs 28.6%; $p = 0.003$), higher positive rate of CSF CRAG (100% vs 37.1%; $p = 0.0005$); and higher serum CRAG titers (median, 1:1024 vs 1:64; $p < 0.05$) and CRAG titers of the CSF specimen (median, 1:1024 vs 1:32; $p < 0.001$). Patients without HIV were more likely to have pulmonary involvement (62.9% vs 0%; $p = 0.0005$) and no underlying disease at diagnosis (42.9% vs 0%; $p = 0.011$).

The demographics and clinical outcomes of patients without HIV with cryptococcosis are shown in Table 2. The positive rate of serum CRAG of patients with CNS cryptococcosis or pulmonary cryptococcosis were 92.3% and 90.9%, respectively. No statistical difference was noted in the serum CRAG titers between the 2 groups (median, 1:64 vs 1:32; $p = 0.34$).

Mortality related to all causes was 26.7% (n = 12), and the rate of death due to cryptococcosis was 8.9% (n = 4). Other causes of death included septic shock (n = 4), out-of-hospital cardiac death (n = 2), intracerebral hemorrhage (n = 1), and hepatocellular carcinoma (n = 1). The cause-specific mortality was 20.0% among patients with HIV, 7.7% among patients without HIV with CNS cryptococcosis, and 4.5% among patients without HIV with pulmonary cryptococcosis.

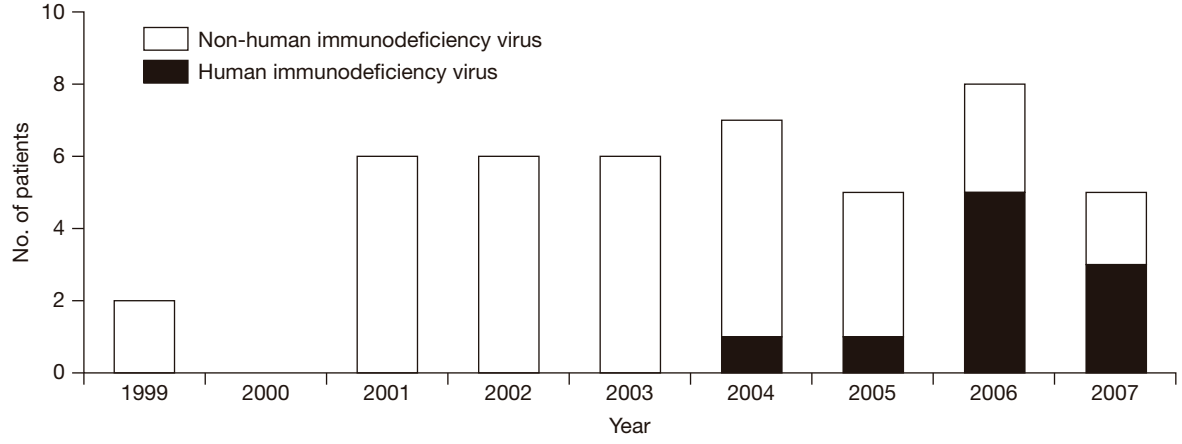

Fig. 1. Temporal trends of cryptococcosis from 1999 to 2007.

Table 1. Demographics and clinical outcomes of cryptococcosis in patients with and without human immunodeficiency virus.

Variable	HIV-positive (n = 10) No. (%)	HIV-negative (n = 35) No. (%)	*p*
Age (years) [median (range)]	45 (20-63)	57 (16-85)	0.23
Men	9 (90.0)	22 (62.9)	0.1
Site of infection			
Central nervous system	10 (100)	13 (37.1)	0.0005[a]
Lungs	0 (0)	22 (62.9)	0.0005[a]
Predisposing conditions			
Systemic lupus erythematosus	0 (0)	4 (11.4)	0.26
Malignancy	0 (0)	5 (14.3)	0.2
Cerebral infarction	0 (0)	3 (23.1)	0.34
Diabetes mellitus	0 (0)	5 (14.3)	0.2
Cirrhosis	0 (0)	1 (2.9)	0.59
None	0 (0)	15 (42.9)	0.011[a]
Presenting symptoms			
Headache	10 (100)	14 (40.0)	0.0008[a]
Malaise	4 (40.0)	5 (14.3)	0.07
Nausea or vomiting	8 (80.0)	10 (28.6)	0.003[a]
Fever	5 (50.0)	5 (14.3)	0.017[a]
Dyspnea	1 (10.0)	3 (8.6)	0.89
Cough	2 (20.0)	13 (37.1)	0.31
Positive serum CRAG titer	8 (80.0)	28 (80.0)	1
Serum CRAG titer [median (range)]	1024 (0-8192)	64 (0-2048)	<0.05[a]
Positive cerebrospinal fluid	10 (100)	13 (37.1)	0.0005[a]
CRAG titer			
Cerebrospinal fluid [median (range)]	1024 (1024-16,384)	32 (2-1024)	<0.001[a]
Mortality	5 (50.0)	7 (20.0)	0.06

[a] $p < 0.05$.

Abbreviation: CRAG = cryptococcal antigen; HIV = human immunodeficiency virus.

Table 2. Comparison of clinical characteristics and outcome of central nervous system and pulmonary cryptococcosis in patients without human immunodeficiency virus.

Variable	Central nervous system cryptococcosis (n = 13) No. (%)	Pulmonary cryptococcosis (n = 22) No. (%)	*p*
Age (years) [median (range)]	58 (16-85)	56 (20-82)	0.68
Men	7 (53.8)	15 (68.2)	0.4
Predisposing conditions			
Systemic lupus erythematosus	2 (15.4)	2 (9.1)	0.57
Malignancy	2 (15.4)	3 (13.6)	0.89
Cerebral infarction	3 (23.1)	0 (0)	0.02[a]
Diabetes mellitus	2 (15.4)	3 (13.6)	0.89
Cirrhosis	0 (0)	1 (4.5)	0.45
None	4 (30.8)	11 (50.0)	0.27
Positive serum CRAG titer	12 (92.3)	20 (90.9)	0.89
Serum CRAG titer [median (range)]	32 (0-512)	64 (0-2048)	0.34
Treatment			
No treatment	0 (0)	5 (22.7)	NA
Antifungal drugs	13 (100)	16 (72.7)	NA
Mortality	3 (23.1)	4 (18.2)	0.73

[a] $p < 0.05$.

Abbreviations: CRAG = cryptococcal antigen; NA = not applicable.

Follow-up studies

Of the 29 patients who had serial CRAG tests during follow-up, 3 patients with HIV died within 2 months of diagnosis and follow-up serum CRAG titers of 2 patients with HIV were not available. The median CD4+ count for the 5 patients with HIV was 154 cells/μL (range, 120-721 cells/μL) at the end of the study, and the median plasma HIV RNA load was 130 copies/mL (range, undetectable-500 copies/mL). All 5 patients continued maintenance therapy for recurrent meningitis (n = 2), persistent neurological symptoms (n = 1), fluconazole-resistant *C. neoformans* infection (n = 1; voriconazole treatment duration, ≤6 months), and CD4 count <100 cells/μL after HAART (n = 1). The serum CRAG titer at diagnosis, and 2 and 3 months for the HIV-positive patients are shown in Fig. 2.

Follow-up serum CRAG titers of 11 patients without HIV were not available. The serum CRAG titers at diagnosis, 2 and 3 months, and at the end of treatment for patients without HIV with CNS cryptococcosis and pulmonary cryptococcosis (n = 24) are shown in Fig. 3 and Fig. 4. Serum CRAG titers declined during treatment.

The median treatment duration of the 8 patients without HIV with CNS cryptococcosis was 16 weeks (range, 12-78 weeks). Six patients had serum CRAG titers of ≤1:8 and 2 had titers of >1:8 at the end of treatment. After a median duration of observation of 43.5 months (range, 8-55 months), 1 of the 2 patients with titers >1:8 had a relapse; this patient had SLE and cryptococcal meningitis with pulmonary involvement (patient 5 in Fig. 3). She received amphotericin B plus fluconazole for 78 weeks to treat the initial infection, and her serum CRAG titer was 1:64 at the end of treatment. She relapsed 3 years later, when her serum CRAG titer was 1:256.

The median treatment duration for the 16 patients with pulmonary cryptococcosis was 12 weeks (range, 12-24 weeks). The serum CRAG titers at the end of treatment for 14 patients were ≤1:8 and for 2 were >1:8. After a median duration of observation of 38.5 months (range, 5-64 months), 1 of the 2 patients with a titer >1:8 had a relapse; this patient was a 63-year-old woman with type 2 diabetes and hypertensive cardiovascular disease (patient 2 in Fig. 4). She received fluconazole for 12 weeks to treat the initial infection, and her serum CRAG titer was 1:16 at the end of treatment. She relapsed 5 years later, when her serum CRAG titer was 1:32. The patients without HIV with serum CRAG >1:8 had a higher relapse rate than those with serum CRAG ≤1:8 (50% vs 0%; $p = 0.0009$) [Table 3].

Discussion

In this study, HIV-infected patients with cryptococcosis had more CNS involvement and a higher fungal load than those without HIV. Serum CRAG titers at the end of treatment for patients without HIV with CNS or pulmonary cryptococcosis declined with time

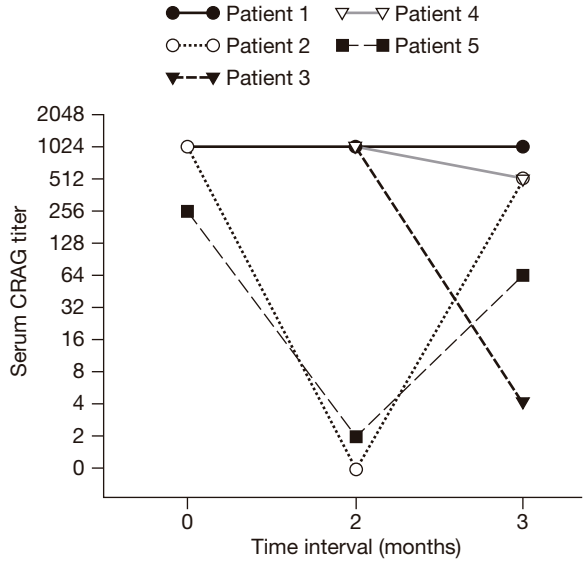

Fig. 2. Serial serum cryptococcal antigen titer changes in patients with human immunodeficiency virus during follow-up. Abbreviation: CRAG = cryptococcal antigen.

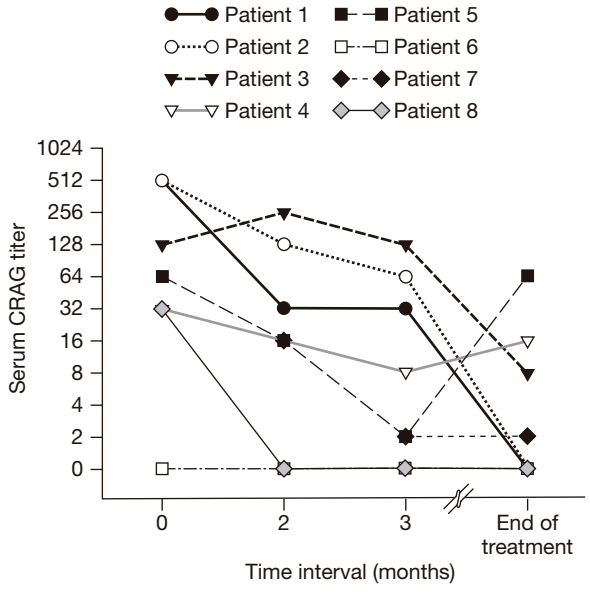

Fig. 3. Serial serum cryptococcal antigen titer changes in patients without human immunodeficiency virus with central nervous system cryptococcosis during follow-up. Abbreviation: CRAG = cryptococcal antigen.

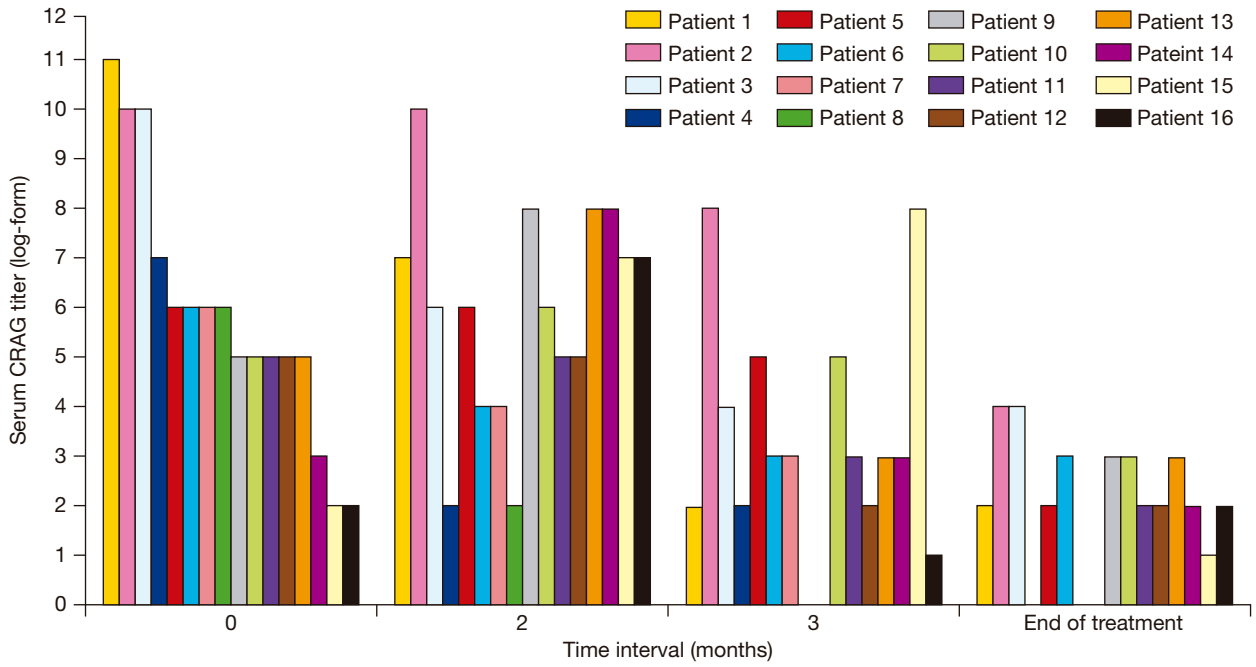

Fig. 4. Serial serum cryptococcal antigen titer changes in patients without human immunodeficiency virus with pulmonary cryptococcosis during follow-up.
Abbreviation: CRAG = cryptococcal antigen.

Table 3. Relapse rate according to serum cryptococcal antigen titer at the end of treatment in patients without human immunodeficiency virus.

Variable	Serum CRAG >1:8 (n = 4) No. (%)	Serum CRAG ≤1:8 (n = 20) No. (%)	*p*
Relapse	2 (50)	0 (0)	0.0009[a]

[a] $p < 0.05$; chi-squared test.
Abbreviation: CRAG = cryptococcal antigen.

following antifungal treatment. Relapse occurred in 2 patients without HIV who had serum CRAG titers >1:8 at the end of treatment.

Limited data comparing serum CRAG among patients with CNS and pulmonary cryptococcosis are available. In the study by Pappas et al [14], the positive rate of the serum CRAG test was 86% and 56% for patients with CNS and pulmonary cryptococcosis, respectively. This study found that the positive rate for serum CRAG for patients with CNS and pulmonary cryptococcosis was 92.3% and 90.9%, respectively, with no statistically significant difference noted in the median serum CRAG titers between the 2 groups. Therefore, it is useful to screen high-risk patients by measuring serum CRAG to help with the diagnosis of cryptococcosis.

Chuck and Sande suggested that follow-up monitoring of serum CRAG titers was not useful for the management of patients with AIDS-related cryptococcal disease [8]. However, Chang et al [15] found that serial serum CRAG titers correlated with serial radiographic changes in patients with pulmonary cryptococcosis, and Pappas et al [14] also found serial CSF CRAG titers correlated with CNS cryptococcosis during treatment. This study showed that serum CRAG titer in CNS or pulmonary cryptococcosis in patients without HIV declined during treatment. However, the results of the changes in serum CRAG titers among patients with HIV was limited by the small number of patients.

Since the introduction of HAART, several studies have shown a favorable outcome after discontinuation of secondary prophylaxis for cryptococcosis among patients with AIDS who respond to HAART [19-21]. In the study by Sheng et al [13], no relapse of cryptococcosis was detected in 7 patients following discontinuation of secondary prophylaxis. The serum CRAG titers at discontinuation of antifungal prophylaxis was undetectable in 3 patients, while those of the other 4

patients were 2, 2, 8, and 16. In this study, 5 patients continued secondary prophylaxis. Therefore, no conclusions can be drawn on the role of serum CRAG in predicting relapse among patients with AIDS after the end of treatment. In the study by Pappas et al, relapse after cessation of therapy occurred in 4% of patients without HIV [14]. In this study, serum CRAG titer >1:8 at the end of treatment was associated with relapse in patients without HIV.

In conclusion, the site of involvement of cryptococcosis differs between patients with and without HIV. The serum CRAG titers were significantly higher among patients with HIV than among those without HIV. Serum CRAG test can be used to monitor disease activity during treatment for CNS and pulmonary cryptococcosis in patients without HIV.

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