

# High Prevalence of Cryptococcal Infection Among HIV-Infected Patients Hospitalized With Pneumonia in Thailand

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**Background.** Cryptococcal meningitis (CM) is a major cause of death among HIV-infected patients. Cryptococcal antigenemia (CrAg+) in the absence of CM can represent early-stage cryptococcosis during which antifungal treatment might improve outcomes. However, patients without meningitis are rarely tested for cryptococcal infection. We evaluated *Cryptococcus* species as a cause of acute respiratory infection in hospitalized patients in Thailand and evaluated clinical characteristics associated with CrAg+.

**Methods.** We tested banked serum samples from 704 human immunodeficiency virus (HIV)–infected and 730 HIV-uninfected patients hospitalized with acute respiratory infection from 2004 through 2009 in 2 rural provinces in Thailand for the presence of CrAg+. Retrospective chart reviews were conducted for CrAg+ patients to distinguish meningeal and nonmeningeal cryptococcosis and to identify clinical characteristics associated with CrAg+ in patients with and without evidence of CM.

**Results.** CrAg+ was found in 92 HIV-infected patients (13.1%); only tuberculosis (19.3%) and rhinovirus (16.5%) were identified more frequently. No HIV-uninfected patients were CrAg+. Of 70 CrAg+ patients with medical charts available, 37 (52.9%) had no evidence of past or existing CM at hospitalization; 30 of those patients (42.9% of all CrAg+) had neither past nor existing CM, nor any alternate etiology of infection identified. Dyspnea was more frequent among CrAg+ patients without CM than among CrAg– patients ( $P = .0002$ ).

**Conclusions.** *Cryptococcus* species were the most common pathogens detected in HIV-infected patients hospitalized with acute respiratory infection in Thailand. Few clinical differences were found between antigenemic and nonantigenemic HIV-infected patients. Health care providers in Thailand should evaluate HIV-infected patients hospitalized with acute respiratory infection for cryptococcal antigenemia, even in the absence of meningitis.

*Cryptococcus* species are pathogenic fungi that cause disease in human immunodeficiency virus (HIV)–infected persons worldwide. Although early cryptococcal

infection can sometimes present as pneumonia, it is often asymptomatic [1, 2], and cryptococcal testing is typically not performed until patients present with cryptococcal meningitis (CM) [3], at which point acute mortality can range from 20% to 50% [4–13]. However, cryptococcal antigenemia, considered to be diagnostic for infection, can be detected a median of 3 weeks before clinical evidence of CM [9]; antigenemia can also persist for several months after clinical resolution of infection [14, 15]. Early detection and treatment of cryptococcal infections before patients progress to meningitis may improve outcomes [7, 16].

Received 9 August 2011; accepted 28 October 2011; electronically published 23 December 2011.

Presented in part: Infectious Diseases Society of America Meeting, October 2010, Vancouver, British Columbia. Abstract LB-22.

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**Clinical Infectious Diseases** 2012;54(5):e43–50

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2011.

DOI: 10.1093/cid/cir903

In Thailand, cryptococcosis is well-recognized as a public health problem among persons with AIDS [17, 18]. Early in Thailand's AIDS epidemic, cryptococcosis was among the top 2 opportunistic infections among patients with AIDS, with approximately 20% developing CM [19]. More recently, 2 studies in Southeast Asia found that *Cryptococcus* species were the second and third most commonly isolated blood pathogens from HIV-infected persons [20, 21]. Another study suggested that approximately 10% of HIV-infected Thai patients might have asymptomatic cryptococcosis [22].

Since 2003, we have been conducting a pneumonia etiologic study among patients hospitalized with acute respiratory infection (ARI) in 2 rural provinces in Thailand [23]. Testing of specimens collected from these patients during 2003–2005 identified a pathogen in only 42.5% of ARI episodes [24]. However, fungal etiologies of infection were not sought. In 2010, we tested these specimens for evidence of *Cryptococcus* infection.

## METHODS

### Study Enrollment Procedures

The pneumonia etiologic study was performed in 2 rural, primarily agrarian provinces in Thailand: Nakhon Phanom (bordering Laos; 2005 population, 730 659 [25]) and Sa Kaeo (bordering Cambodia; population, 521 634 [25]). All 8 acute care hospitals in Sa Kaeo (beginning in September 2003) and all 12 hospitals in Nakhon Phanom (beginning in January 2005) participated. Eligible patients included persons admitted to these hospitals with ARI, defined as evidence of active infection (body temperature  $<35.5^{\circ}\text{C}$  or  $>38.2^{\circ}\text{C}$  documented within 24 hours after admission, history of fever or chills, or abnormal white blood cell count or differential) and documented respiratory tract illness (abnormal breath sounds during physical examination or presence of tachypnea, cough, sputum production, or dyspnea). After enrollment, blood, urine, and nasopharyngeal swab specimens were collected by study nurses. During 2003–2005, a second blood specimen was collected 3 weeks after enrollment for serologic testing. Leftover serum specimens were stored at  $-70^{\circ}\text{C}$  at the Thailand National Institute of Health (Thai-NIH) for future testing.

During 2003–2007, eligibility was limited to patients with ARI for whom the clinician obtained a chest radiograph within 48 hours after hospital admission (regardless of results) [24]. Beginning in January 2008, every other patient hospitalized with ARI was considered for enrollment, regardless of whether chest radiography was performed.

The study was approved by a Centers for Disease Control and Prevention Institutional Review Board (protocol no. 3754) and the Ethical Review Committee of the Thailand Ministry of Public Health. All patients or their guardians provided informed

consent for specimen collection and use of specimens for research purposes.

### Laboratory Testing

Clinical specimens were collected as part of routine clinical care. Initially, sputum was collected at the discretion of the physician for acid-fast bacilli staining; beginning in October 2008, sputum samples for acid-fast bacilli staining were sought from all enrolled patients. Blood cultures were performed as clinically indicated with use of the BactT/ALERT 3D system (bioMérieux); blood was inoculated into a culture bottle optimized for standard aerobic growth and, when sufficient volume was available, a second bottle for enhanced growth of mycobacteria and other fastidious pathogens [26].

Nasopharyngeal, blood, and urine specimens were tested for respiratory pathogens, including rhinovirus, coronavirus, influenza A and B, respiratory syncytial virus, human parainfluenzaviruses 1, 2, and 3, *Chlamydia pneumoniae*, human metapneumovirus, adenovirus, and *Streptococcus pneumoniae*, at the Centers for Disease Control and Prevention during 2003–2005 and at the Thai-NIH during 2005–2009. Testing methods are described elsewhere. Not all tests were performed during all years, and not all pathogens were included in testing during all years; however, testing for 5 pathogens (influenza A and B, respiratory syncytial virus, adenovirus, and *S. pneumoniae*) was conducted during all study years.

### Patient Selection for *Cryptococcus* Testing

*Cryptococcus* antigen testing was performed using the Premier Cryptococcal Antigen Enzyme Immunoassay (Meridian Bioscience) or, for 1 sample, the Cryptococcal Antigen Latex Agglutination System (Meridian) during 2010 at the Thai-NIH on a subset of stored serum samples collected from patients in the pneumonia etiology study at enrollment and maintained at the Thai-NIH. Serum specimens from all HIV-infected patients (defined as patients who tested positive for HIV at study enrollment or who had documented evidence of HIV infection in their medical chart) enrolled from 1 January 2004 through 31 December 2009 were included in this analysis, regardless of whether another pathogen was identified during the initial testing. *Cryptococcus* testing was also performed on serum samples from a randomly selected subset of HIV-uninfected patients who enrolled in the study during 2009 and who were negative for all other aforementioned pathogens.

### Data Collection

Epidemiologic, clinical, and laboratory data were collected from the patients' medical records during hospitalization by surveillance officers. Chest radiographs were reviewed by an independent panel of radiologists in Bangkok, as described elsewhere [27].

To determine whether patients with ARI had symptoms consistent with CM or a recent history of CM and to identify

factors associated with cryptococcal antigenemia, additional inpatient and outpatient medical record reviews were conducted for all antigenemic patients. Only a subset of patients had full medical records available. Patients were defined as having evidence of existing CM if they had a discharge diagnosis of CM or were given amphotericin B during their admission. Because cryptococcal antigenemia can persist for long periods after infection [14, 15], documented past-year episodes of CM were also recorded. Because fluconazole is provided to HIV-infected patients for cryptococcal prophylaxis in Thailand, and because fluconazole is also used to treat candidiasis, we did not include its provision during hospitalization as evidence for existing CM.

### Data Analysis

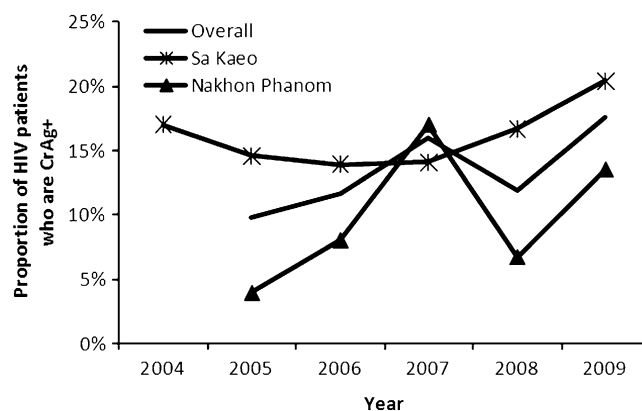
Data were analyzed using SAS software, version 9.2 (SAS Institute). Nonparametrically distributed continuous variables were analyzed using the Wilcoxon Mann–Whitney test. Two-sided *P* values are reported;  $\chi^2$  analyses are reported, except when Fisher exact test provided a more appropriate estimate of association.

## RESULTS

### Patient Selection and Samples

A total of 761 HIV-infected study patients enrolled during 2004–2009 were included in the analysis. Of the 2817 HIV-uninfected patients enrolled during 2009, 1884 (67.1%) had no other pathogen identified from any specimen; 762 of these patients were randomly selected for this analysis. Residual stored serum samples for cryptococcal antigen testing were available from 704 HIV-infected patients (92.5%) and 730 HIV-uninfected patients (95.8%).

Most patients were in the age group 19–49 years (Table 1). HIV-infected patients were younger than HIV-uninfected



**Figure 1.** Prevalence of cryptococcal antigenemia among human immunodeficiency virus–infected patients with acute respiratory infection, 2004–2009, Thailand. Study enrollment in Nakhon Phanom began in 2005. Abbreviations: CrAg, cryptococcal antigenemia; HIV, human immunodeficiency virus.

patients ( $P < .0001$ ) and were more likely to be from Sa Kaeo than from Nakhon Phanom ( $P < .0001$ ). Similar proportions of patients in each group were male (Table 1).

### Prevalence of Cryptococcal Antigenemia

All serum specimens from HIV-uninfected patients were negative for cryptococcal antigen (CrAg–). Of the 704 serum samples from HIV-infected patients, 92 (13.1%) tested positive for cryptococcal antigen (CrAg+) (Table 2). Although a higher proportion of HIV-infected patients from Sa Kaeo than patients from Nakhon Phanom were CrAg+ (15.7% vs 9.0%, respectively;  $P = .01$ ) (Table 2), within-year differences in the proportion positive were not statistically significant (Figure 1). A general upward trend in prevalence of CrAg+ over time, which was not statistically significant, was observed in both provinces (Figure 1).

**Table 1. Demographic Characteristics of Study Participants Selected for Cryptococcal Testing**

Characteristic	All Participants (N = 1434)	HIV-Infected Patients (n = 704) <sup>a</sup>	HIV-Uninfected Participants (n = 730) <sup>b</sup>	<i>P</i> Value
Province				
Sa Kaeo	678 (47.3)	426 (60.5)	252 (34.5)	<.0001
Nakhon Phanom	756 (52.7)	278 (39.4)	478 (65.5)	
Age, mean, median (range)				
<5 years	34.9, 34.0 (0–102)	33.5, 34.0 (0–92)	36.2, 40.0 (0–102)	<.0001
5–18 years	247 (17.2)	42 (6.0)	205 (28.1)	
19–49 years	162 (11.3)	59 (8.4)	103 (14.1)	
≥50 years	656 (45.8)	531 (75.4)	125 (17.1)	
Male sex	369 (25.7)	72 (10.2)	297 (40.7)	
Male sex	774 (54.0)	380 (54.0)	394 (54.0)	>.999

Data are No. (%) of patients unless otherwise indicated.

Abbreviation: HIV, human immunodeficiency virus.

<sup>a</sup> HIV-infected patients, 2004–2009.

<sup>b</sup> HIV-uninfected patients, 2009 only.

**Table 2. Demographic Characteristics, Health History, and Hospitalization Characteristics of HIV-Infected Patients Enrolled in the Pneumonia Etiology Study, 2004–2009**

Characteristic	HIV-Infected Patients (n = 704)		P Value
	CrAg+ (n = 92)	CrAg- (n = 612)	
Province			
Sa Kaeo	67 (72.8)	359 (58.7)	.01
Nakhon Phanom	25 (27.1)	253 (41.3)	
Age, mean, median (range)			
<5 years	0 (0.0)	35 (5.8)	.38
5–18 years	3 (3.3)	56 (9.2)	
19–49 years	80 (88.9)	451 (74.3)	
≥50	7 (7.8)	65 (10.7)	
Male sex	50 (54.4)	330 (53.9)	>.999
Initial patient presentation			
Temperature ≥38°C	38 (41.8)	290 (47.6)	.30
Temperature <35.5°C	0 (0.0)	13 (2.1)	.39
WBC count >11 000 cells/μL	14 (16.1)	166 (29.5)	.01
WBC count <3000 cells/μL	7 (8.1)	31 (5.5)	.35
Mean (median) WBC count, cells/μL	7778 (7060)	9956 (8600)	.002
Cough	54 (93.1)	534 (87.8)	.29
Sputum production	33 (35.9)	321 (53.0)	.002
Hemoptysis	1 (1.1)	20 (3.3)	.50
Chest pain	6 (24.0)	72 (12.0)	.08
Dyspnea	38 (77.6)	302 (50.0)	.0002
Tachypnea	30 (33.3)	230 (37.8)	.41
Chest indraw	1 (1.3)	14 (2.9)	.71
Abnormal breath sounds	34 (38.2)	279 (46.2)	.16
Rhonchi	12 (35.3)	104 (36.9)	.86
Wheezing	6 (17.7)	83 (29.4)	.15
Rales/crepitation	19 (57.6)	157 (55.7)	.84
Chest radiography performed	84 (92.3)	558 (92.2)	.98
Abnormal chest radiographic findings (per clinician's judgement)	29/50 (58.0)	250/388 (64.4)	.37
Chest radiographic reading completed by radiologist panel	41	301	
Probable pneumonia	38 (92.7)	277 (92.0)	>.999
Alveolar infiltrate	15 (39.5)	119 (43.0)	.69
Interstitial infiltrate	24 (63.2)	196 (70.8)	.34
Consolidation	8 (21.1)	79 (28.5)	.33
Hospital course			
Duration of hospitalization, d, mean, median (range)	8.9, 7.0 (0–36.0)	7.7, 6.0 (0–61.0)	.05
Intubated	4 (4.4)	24 (4.0)	.77
Given oxygen	38 (42.2)	258 (42.5)	>.999
Died before discharge	9 (10.0)	44 (7.3)	.36

Data are presented as No. (%) of all patients with information for the given variable.

Abbreviations: CrAg, cryptococcal antigenemia; WBC, white blood cell.

Because none of the HIV-uninfected patients was CrAg+, all subsequent analyses included only HIV-infected patients.

#### Factors Associated With Cryptococcal Antigenemia Among HIV-Infected Patients

Of the 92 CrAg+ patients, 67 (72.8%) were from Sa Kaeo and 25 (27.1%) were from Nakhon Phanom (Table 2). No differences

were noted in age or sex between CrAg+ and CrAg- patients. CrAg+ prevalence was highest in the 19–49-year age group, at 15.1% (Table 2).

Clinical and laboratory characteristics of HIV-infected patients at hospitalization are summarized in Table 2. CrAg+ patients were less likely than CrAg- patients to present with a white blood cell count >11 000 cells/μL (16.1% vs 29.5%;

**Table 3. Pathogens Identified From Serology, Polymerase Chain Reaction, and Culture Testing of Specimens From All HIV-Infected Patients, and Those With Cryptococcal Antigenemia**

Pathogens Detected	Proportion (%) of All HIV-Infected Patients (n = 704)	Proportion (%) of CrAg+ (n = 92)
Any pathogen	147/704 (20.9)	19/92 (20.7)
Tuberculosis <sup>a</sup>	52/270 (19.3)	5/34 (15.7)
Rhinovirus <sup>b</sup>	28/170 (16.5)	6/20 (30.0)
Coronavirus <sup>b</sup>	6/111 (5.4)	2/17 (11.8)
<i>Streptococcus pneumoniae</i> <sup>c</sup>	24/575 (4.2)	4/79 (5.1)
Influenza A or B <sup>b,d</sup>	29/703 (4.1)	2/92 (2.2)
RSV <sup>b,d,e</sup>	19/703 (2.7)	4/92 (4.4)
HPIV 1, 2, or 3 <sup>b,d,e</sup>	12/484 (2.5)	1/61 (1.6)
<i>Chlamydia pneumoniae</i> <sup>b,d</sup>	3/171 (1.8)	1/20 (5.0)
HMPV <sup>b,d,e</sup>	5/484 (1.0)	0/61 (0.0)
Adenovirus <sup>b,d,e</sup>	9/703 (0.6)	2/92 (2.2)

Not all tests were performed on all specimens. testing methods are described in Olsen et al. [24]

Abbreviations: CrAg, cryptococcal antigenemia; HIV, human immunodeficiency virus; HMPV, human metapneumovirus; HPIV, human parainfluenzavirus; RSV, respiratory syncytial virus.

<sup>a</sup> Acid-fast bacillus sputum smear.

<sup>b</sup> Polymerase chain reaction.

<sup>c</sup> Rapid test.

<sup>d</sup> Serology.

<sup>e</sup> Culture.

$P < .01$ ) or sputum production (35.9% vs 53.0%;  $P = .002$ ) and more likely to present with dyspnea (77.6% vs 50.0%;  $P = .0002$ ). Mean and median white blood cell counts were significantly lower in CrAg+ patients than in CrAg- patients ( $P = .002$ ), and CrAg+ patients experienced longer hospital stays (8.9 vs 7.7 days;  $P = .05$ ). Nine CrAg+ patients (10.0%) and 44 CrAg- patients (7.3%) died before hospital discharge (Table 2); 3 of the 6 CrAg+ patients with documented causes of death in their medical records died of CM.

Eighty-four CrAg+ patients (92.3%) and 558 CrAg- patients (92.2%) had chest radiography performed. Of the patients with radiographic evidence of pneumonia, no differences were identified in chest radiographic findings between CrAg+ and CrAg- patients (Table 2).

#### Other Pathogens Among HIV-Infected and CrAg+ Patients

Among all HIV-infected patients, the most common pathogens detected by any method were tuberculosis (19.3%), rhinovirus (16.5%), and *Cryptococcus* species (as detected by CrAg+, 13.2%) (Tables 3 and 4). On the basis of testing of nasopharyngeal swab, urine, and serum samples, the most frequently detected pathogens among CrAg+ patients were rhinovirus (30.0% of 20 tested), coronavirus (11.8% of 17 tested), and *S. pneumoniae* (5.1% of 79 tested). Nineteen CrAg+ patients

**Table 4. Pathogens Identified From Blood Culture From All HIV-Infected Patients and Those With Cryptococcal Antigenemia**

Process or Pathogen	No. (%) of All HIV-Infected Patients	No. (%) of CrAg+ Patients (n = 92)
Blood submitted for culture	325	42
Any pathogen identified from blood culture	66 (20.3)	20 (48.0)
<i>Cryptococcus</i> species	18 (5.5)	17 <sup>a</sup> (40.0)
<i>Mycobacterium tuberculosis</i>	12 (3.7)	0 (0.0)
Other mycobacteria	9 (2.8)	0 (0.0)
<i>Salmonella enterica</i>	7 (2.2)	0 (0.0)
Other <i>Salmonella</i>	5 (1.5)	1 (4.7)
<i>Penicillium</i> species	4 (1.2)	0 (0.0)
<i>Histoplasma capsulatum</i>	3 (0.9)	1 (4.7)
<i>Pseudomonas aeruginosa</i>	2 (0.6)	0 (0.0)
Other <sup>b</sup>	5 (1.5)	1 (4.7)

Abbreviations: CrAg, cryptococcal antigenemia; HIV, human immunodeficiency virus.

<sup>a</sup> Retested the culture-positive CrAg- serum sample with latex agglutination test and confirmed as positive for CrAg+.

<sup>b</sup> Includes *Staphylococcus aureus*, *Burkholderia pseudomallei*, *Escherichia coli*, *Klebsiella pneumoniae*, and unspecified *Acinetobacter* and *Pseudomonas* species.

(20.7%) had any coinfection detected from these specimens (Table 3).

Blood culture was performed for 42 CrAg+ patients (45.7%); pathogens were identified from 20 (48.0%) of these patients. The most frequently identified pathogen from blood culture was *Cryptococcus* species, found in 17 patients. Only 3 CrAg+ patients (7.1%) had another pathogen identified from blood culture (Table 4).

There were no statistically significant differences in the prevalence of coinfections between CrAg+ and CrAg- patients with HIV infection (data not shown).

#### Opportunistic Infections and Treatment History in CrAg+ Patients

Inpatient and/or outpatient medical records were available for 70 CrAg+ patients (76.1%): 45 (67.2%) from Sa Kaeo and all 25 from Nakhon Phanom. Of these patients, 57 (81.4%) had at least 1 documented opportunistic infection during the past year, including 20 *Pneumocystis* pneumonia cases, 27 tuberculosis cases, and 11 candidiasis cases. CD4 cell counts from the past 6 months were available for 18 CrAg+ patients, for whom the median CD4 cell count was 66 cells/ $\mu$ L (range, 6–291 cells/ $\mu$ L).

Twenty-seven CrAg+ patients (38.6%) had evidence of CM during their study admission; 6 (8.6%) more had evidence of CM during the past year but not during the study admission. Patients with *Cryptococcus* species isolated from blood cultures were more likely to have evidence of CM during their study admission (66.7% vs 28.6%;  $P = .02$ ) or during the past year



(77.8% vs 38.1%;  $P = .01$ ) than those without *Cryptococcus* isolated from blood cultures. Of the 27 patients with CM during admission, 12 (44.0%) received both amphotericin B and fluconazole during their hospitalization, 10 (37.0%) received amphotericin B only, 2 (7.4%) received fluconazole only, and 3 (10.7%) received no documented antifungal drugs. Eleven patients (40.7%) provided information at admission about their fluconazole status; all 11 (100%) reported that they were already taking fluconazole, possibly as prophylaxis or for past undocumented episodes of CM.

Thirty-seven CrAg+ patients (52.9%) had no evidence of CM during admission or the past year; of these, 18 (48.6%) were given fluconazole during admission and 1 (2.7%) was given itraconazole, either for initiation of prophylaxis, continuation of undocumented prophylaxis, treatment of suspected (but undocumented) cryptococcal infection, or for candidiasis (4 of the patients had a documented history of candidiasis). An additional 18 (94.7%) of 19 patients without evidence of CM during admission or the year before admission reported that they were already taking fluconazole at the time of admission; of 5 with a recorded CD4 cell count, all had CD4 cell counts  $<100$  cells/ $\mu$ L, the threshold below which fluconazole prophylaxis is recommended in Thailand.

Thirty patients (42.9%) had neither any evidence of CM during admission or the past year, nor any alternate etiology of infection identified during diagnostic testing. Six (20%) of those 30, however, did have a discharge diagnosis of *Pneumocystis jirovecii* pneumonia (typically determined empirically in this setting), for which testing was not performed during the pneumonia etiologic study.

#### **Factors Associated With CrAg+ Among Patients Without Existing or Past-Year CM**

When clinical and chest radiographic characteristics were compared between CrAg+ patients without evidence of CM or a history of CM and CrAg- patients, only dyspnea remained statistically significantly different between the 2 groups (73.2% among nonmeningitic CrAg+ patients, compared with 49.9% among CrAg- patients;  $P = .002$ ). The mean white blood cell count among CrAg+ CM-negative patients was 8387 cells/ $\mu$ L (median, 7300 cells/ $\mu$ L), which was not statistically significantly different from that in CrAg- patients.

## **DISCUSSION**

Cryptococcal infection has long been one of the most significant threats to the health and survival of persons with AIDS. Excluding rhinovirus, for which pathogenicity is uncertain [28], we found that cryptococcal infection was the second most common infection among HIV-infected patients hospitalized with ARI in Thailand. Cryptococcal antigenemia was not found in any HIV-uninfected patients, suggesting that *Cryptococcus* might rarely, if

ever, be an etiologic agent of ARI in immunocompetent patients in Thailand. Of interest, cryptococcal antigenemia was more frequent among HIV-infected patients in the eastern province of Sa Kaeo, compared with the northeastern province of Nakhon Phanom; the reasons for this are unknown.

Few clinical differences were found between antigenemic and nonantigenemic HIV-infected patients, suggesting that signs and symptoms alone are insufficient criteria to determine which hospitalized patients with ARI warrant *Cryptococcus* testing. Some respiratory signs and symptoms, such as dyspnea and sputum production, were different between patients with versus without cryptococcal antigenemia; however, these characteristics are subjective and, on an individual level, unlikely to be useful diagnostically. Total white blood cell counts, which were significantly lower among antigenemic than nonantigenemic patients, were not different when patients with evidence of CM were excluded from the antigenemic group. This suggests that antigenemic patients with CM had more advanced HIV infection than did antigenemic patients without CM. HIV-infected patients hospitalized with ARI and without clinically evident CM may represent a group for whom CrAg testing would lead to an earlier diagnosis of cryptococcal infection, when treatment might improve outcomes.

Although some antigenemic patients had a history of CM that might have explained their antigenemia, the majority (53%) had no evidence of existing or prior CM. A high proportion (43%) of all CrAg+ patients had no evidence of any CM nor any detectable coinfections, suggesting that *Cryptococcus* species were a possible etiologic agent of their respiratory illness. Cryptococcal antigenemia, particularly at high titers, has been shown to be a sensitive and specific marker for future CM and a prognostic factor increasing the risk of death in certain populations [29]. Meya et al [16] demonstrated that asymptomatic antigenemic patients who are given fluconazole have improved survival over asymptomatic antigenemic patients who are not given fluconazole. For CrAg+ patients without overt signs and symptoms of cryptococcal infection, a window of opportunity for treatment might have existed to administer amphotericin B or high-dose fluconazole to reduce their risk of developing CM [7, 16, 22, 29–31]. Blood culture positive for *Cryptococcus*, as shown in our study, was associated with CrAg+ among patients in whom a clinical suspicion of CM already existed but was less frequent among patients without symptoms of CM. These data strongly point to a role for cryptococcal antigen testing among HIV-infected patients hospitalized with acute respiratory infection in Thailand. A highly sensitive and specific point-of-care test, such as the lateral flow assay recently described by Lindsley et al [32], could facilitate such rapid testing in this population.

Because of the high frequency of cryptococcosis in Thailand during the 1990s and evidence that primary fluconazole

prophylaxis could reduce cryptococcal disease and mortality [33], in 2006, Thailand instituted national guidelines recommending that patients with a CD4 cell count <100 cells/mL receive fluconazole prophylaxis. Despite these guidelines, the frequency of cryptococcal antigenemia in our study, at least among patients hospitalized with ARI, trended upward over time (although not statistically significantly). The temporal increases in the prevalence of antigenemia are important and should be monitored. Although cryptococcal antigenemia can persist for long periods after CM [14, 15], the development of cryptococcal antigenemia in patients without a history of CM and who reported taking fluconazole is concerning and deserves further study.

Our analysis has several limitations. First, patient medical records were available for only a subset of patients, who might not be representative of the overall patient population. In addition, few follow-up data were available, which inhibited our ability to monitor the development of CM and survival among CrAg+ patients without evidence of meningitis. Second, our study was performed in rural areas of Thailand; because most HIV-infected patients in Thailand live in Bangkok, where exposures (such as to *Cryptococcus* species) of HIV-infected patients might differ from exposures in rural areas, the results might not be generalizable to the entire population. Third, *P. jirovecii*, a well-known fungal agent of pneumonia in HIV-infected patients in Southeast Asia [34, 35], was not included in the diagnostic testing for the pneumonia etiology study. Although few of the patients with medical records were found to have discharge diagnoses of *P. jirovecii* pneumonia, we cannot rule out the presence of this agent in these or other patients as a cause of their respiratory symptoms. Finally, we did not attempt to detect *Cryptococcus* species from lung tissue or respiratory specimens from patients enrolled in this study and, thus, cannot demonstrate conclusively that it was the cause of ARI in these patients. However, antigenemia indicates active infection, and many patients did not have other etiologic agents identified, despite testing for multiple common pathogens. Even without definitive evidence of a causal link with ARI, the high prevalence of cryptococcal antigenemia in hospitalized HIV-infected patients with ARI in Thailand supports testing this population, regardless of signs and symptoms of meningitis.

In summary, cryptococcal infection was the second-leading infection detected in HIV-infected patients presenting with ARI in Thailand. A high proportion of patients with cryptococcal infection had no alternate etiology of ARI identified. Among HIV-infected patients hospitalized with ARI in Thailand, cryptococcal infection should be among the differential diagnoses, even in the absence of meningitis. Healthcare providers in Thailand should test such patients for *Cryptococcus*, because treatment with antifungal agents at this point may provide a window of opportunity to prevent serious health outcomes.

## Notes

**Author contributions.** J. H. analyzed the data for this study and drafted the article. M. L., N. P., and N. M. performed the laboratory testing and provided critical revision to the manuscript. S. H. carried out chart reviews and provided critical manuscript revision. S. N., P. P., S. C., F. R., M. C., L. P., S. A. M., and H. C. B. were involved in the study conception and design, data acquisition, and critical revision of the article. L. C. provided initial data analysis and critical manuscript revision. B. P. provided assistance with study design and contributed significantly to critical manuscript review. All authors have seen and approved the final version of the manuscript.

**Acknowledgments.** We thank the staff of participating hospitals in Thailand for their assistance with this study.

**Disclaimer.** The findings and conclusions in these reports are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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