Serum Cryptococcal Antigen in Patients with AIDS

Marta Feldmesser, Carol Harris, Samuel Reichberg, Shahida Khan, and Arturo Casadevall

Cryptococcus neoformans is the most common life-threatening fungal pathogen in patients with AIDS. Most AIDS patients with C. neoformans infection present with meningitis, but infection of almost every organ has been described [1]. The latex agglutination (LA) test for serum cryptococcal antigen is widely used for detecting cryptococcal polysaccharide in patients with AIDS, as an initial screening test for patients with fever of unclear etiology or neurological symptoms and for asymptomatic patients. The diagnosis of cryptococcosis is often inferred from the detection of cryptococcal polysaccharide in sterile body fluids.

Because the incidence of cryptococcal meningitis varies widely with geographic location, different conclusions have been drawn regarding the value of screening [2, 3]. Nonetheless, screening has become common practice in the care of patients with AIDS in many locations. The identification of occasional patients with antigenemia whose cultures are not positive has raised many questions regarding the significance and appropriate management of isolated cryptococcal antigenemia.

What is serum antigen testing? What is the meaning of a positive test? Antibody-coated latex particle agglutination detects capsular polysaccharide in body fluids. Immunologic detection of cryptococcal capsular antigen is a sensitive and specific procedure. False-positive results are few with use of commercially available LA tests. Tanner et al., using 90 serum specimens from patients with concurrent culture results, compared four LA tests and found that the sensitivity ranged from 83% to 97% and the specificity from 93% to 100%; false-positive rates ranged from zero to 5%. All but one of the false-positives was associated with titers of \( \geq 1:8 \) [4].

False-positive LA reactions have resulted from infection with Trichosporon beigelii [5] and with the DF-2 bacillus [6], presumably because these pathogens produce antigens that are cross-reactive with C. neoformans polysaccharide. These conditions are unlikely to be confused clinically with cryptococcal infection in AIDS patients. It is possible that additional pathogens produce antigens that are cross-reactive with C. neoformans polysaccharide. For example, types II and XIV antipneumococcal sera can react with C. neoformans serotype A polysaccharide [7]. Although pneumococcal infection has not been reported to cause false-positivity for serum cryptococcal antigen, it may someday become so. If, for example, a patient with pneumococcal bacteremia was newly positive for serum antigen, one could repeat the test after resolution of the acute process. Again, the clinical conditions should be easily distinguishable. Cross-reactivity with rheumatoid factor is eliminated by the treatment of samples with proteinase and by boiling. False-positive test results can also result from contamination from syneresis fluid (i.e., fluid on wire loops), but this can be prevented by laboratory-personnel awareness [8].

Polysaccharide detection can also be accomplished by enzyme-linked immunosorbent assay (ELISA) antigen capture. This test is based on capture of the antigen in the patient sample with use of the same anticytrococcal rabbit polyclonal immunoglobulin used in the latex procedure, followed by detection with a monoclonal anticytrococcal globulin conjugated with horseradish peroxidase. While most LA tests detect intact cryptococcal polysaccharide, the only ELIA licensed for commercial use measures glucuronoxylomannan, the major component of the polysaccharide. Testing by EIA does not require pretreatment of serum samples with proteinase and provides spectrophotometrically determined objective results [9]. The EIA has sensitivity (93%) and specificity (96%) comparable to those of LA methods for serum testing [4].

What is the clinical course of cryptococcal antigenemia in patients with AIDS? We recently attempted to answer this question by reviewing serology records from a 1-year period. We identified 10 patients who met the Centers for Disease Control and Prevention’s 1993 case definition for AIDS and whose serum LA cryptococcal antigen tests were positive; because of their antigenemia, they had undergone diagnostic evaluation for the presence of active infection, and the CSF cryptococcal cultures and antigen tests were negative. All had a change in clinical status at the time the positive test result was obtained.

Serum LA antigen test results were verified for all patients and were confirmed for four by retesting in a reference laboratory and by EIA. A positive titer was defined as \( \geq 1:4 \). In
addition to lumbar puncture, further diagnostic testing included fungal blood cultures, hepatic enzyme level determinations, fungal urine cultures, and chest radiography.

The mean CD4 cell count in our patients was 97/mm³ (median, 53; range, 5–379). All patients had serum cryptococcal antigen titers of ≥1:8. Six patients had negative blood cultures, were treated with fluconazole, and did not have cryptococcal meningitis within the follow-up period, which ranged from 3 to 22 months. A seventh was not initially treated, acquired cryptococcal liver disease followed by meningitis, and died from disseminated disease.

The remaining three were found to have disseminated disease during the evaluation conducted because of the positive serum antigen test. Two of these three patients were found to be fungemic. One was treated intermittently with fluconazole but had meningitis 5 months later, from which she died. The other, who did not have an identifiable focus of infection, was treated with amphotericin B and then fluconazole. She subsequently was negative for serum antigen and died 9 months later of an unrelated process. In the third patient, yeast forms consistent with C. neoformans were noted on pleural biopsy. We found that our ability to define the natural history of cryptococcal antigenemia was limited by the high percentage of patients who were treated at the time their positive serum antigen test result was obtained.

A similar study at the University of Pennsylvania identified 13 HIV-infected patients with serum cryptococcal antigen titers of ≥1:8 but no other evidence of cryptococcal infection [10]. Ten patients received systemic antifungal therapy, and disseminated disease developed in none. Two of the three patients who did not receive antifungal therapy had cryptococcal meningitis 2 and 4 months after the initial positive serum antigen test. The authors concluded that patients with antigenemia may benefit from antifungal therapy to prevent or delay the development of cryptococcal meningitis, but they also recognized the limit of the conclusions they could draw and the desirability of a larger study [10].

Does antigenemia imply systemic infection? An unequivocal answer to this question is not possible because of the paucity of clinical data. However, evidence from animal studies strongly suggests that the presence of polysaccharide in the blood implies infection. Cryptococcal disease is presumed to be acquired via the pulmonary tree [11]. It is not clear whether cryptococcal meningitis follows acute dissemination or represents reactivation of latent infection [12].

Evidence from animal studies strongly suggests that serum antigenemia is a manifestation of extrapulmonary disease. First, serum antigen levels are low or negative in rats given experimental pulmonary infection that does not disseminate [13]. Second, serum antigenemia develops in mice with experimental pulmonary infection that does disseminate [14]. Third, intratracheal administration of C. neoformans antigen does not result in measurable serum levels, a finding suggesting that absorption from pulmonary surfaces is minimal [15]. These models support the supposition that the presence of serum antigen reflects the presence of active extrapulmonary infection.

Beyond its role as a marker for disseminated infection, does cryptococcal polysaccharide directly affect the host? This question has not been definitively answered for human disease. However, cryptococcal polysaccharide has been shown to enhance HIV infection in vitro, raising the possibility of deleterious synergistic interactions between these organisms in HIV-infected patients [16].

Administration of polysaccharide predisposes mice to early death when they are subsequently challenged with C. neoformans [17]. A variety of literature reports have described detrimental effects of capsular polysaccharide on host immunity, including (1) induction of an immune paralysis-like effect in mice that is characterized by antibody unresponsiveness [18]; (2) production of brain edema in the rat [19]; (3) inhibition of leukocyte migration [20, 21]; and (4) induction of L-selectin shedding from human neutrophils [22].

The difficulty in treating cryptococcal meningitis has led to interest in identifying patients for whom primary prophylaxis may be appropriate and those with subclinical disease. Although current guidelines of the U.S. Public Health Service and the Infectious Diseases Society of America do not recommend routine serum cryptococcal antigen testing or routine prophylaxis [23], the ease of administration of fluconazole and its relatively low toxicity have created interest in its prophylactic administration to patients with CD4 cell counts of <250/mm³ [24] or <250/mm³ [25]. A recent randomized prospective trial (ACTG 981) revealed a reduction in the incidence of cryptococcal infection among patients who received primary prophylaxis with fluconazole and whose mean CD4 cell counts were 122–141/mm³, but there was no difference in survival rates and >10,000 doses were given to prevent each case [26].

The prophylactic use of fluconazole is not without risk, as the incidence of thrush and esophagitis due to fluconazole-resistant Candida species has increased with the growing use of azoles [27]. C. neoformans relapse-associated isolates in cases of meningitis do not appear to be resistant to fluconazole [28], but there have been anecdotal reports of increased cryptococcal resistance [29, 30]. Furthermore, animal studies have shown that the outcome of experimental infection is worse in treated animals when the MICs of this agent against isolates are high [31].

In addition, culture-negative cryptococcal disease is associated with fluconazole use by patients with AIDS who receive this agent for indications other than cryptococcal disease [32]. The expanded use of fluconazole for prophylaxis may increase the incidence of cryptococcal antigenemia that is not associated with positive cultures.

Hence, it appears that the outcome of cryptococcal antigenemia has not been well studied, but patients are frequently treated. What is known about the course of cryptococcal dis-
ease? Before the development of amphotericin B, untreated cryptococcal infection was almost uniformly fatal, although rare cases of chronic meningitis and one case of spontaneous cure were reported [33]. Untreated disseminated cryptococcal infection is life-threatening in patients with AIDS [34]. When a serum antigen test is positive, negative cultures do not exclude the presence of disseminated disease.

It is unethical to conduct a prospective trial to determine the natural history of any condition that is associated with significantly high morbidity and mortality and for which sensitive, specific noninvasive screening tests exist and nontoxic therapy is available. Considering that cryptococcal infections are life-threatening and that oral fluconazole therapy has low toxicity, as well as the experience with our patient group, we cannot justify a prospective study to determine the natural course of untreated cryptococcal antigenemia. More meaningful retrospective analysis, including study of cost efficacy, would require pooling data from a large number of centers.

In the meantime, testing patients with AIDS for serum cryptococcal antigen at the time of a change in clinical status (such as the development of fever, malaise, headache, or respiratory or neurological symptoms) and treating them after a positive test result may be more cost-effective than general screening and may reduce the potential for emergence of azole-resistant fungi that could follow widespread primary prophylactic use of fluconazole [35].

The clinical manifestations of cryptococcal infection are protean, and cryptococcosis is usually included in the differential diagnosis of clinical symptoms of patients with HIV infection. We propose that patients with AIDS be tested for serum cryptococcal antigen at the time of a change in clinical status, and if the test is positive (defined as a titer of \( \geq 1:4 \)), it should be repeated. If it is again positive, the patient should undergo lumbar puncture. If CSF studies are negative, further evaluation should include blood culture, urine culture, chest radiography, and determination of liver enzyme levels.

In our view, persistence of antigenemia implies cryptic systemic infection, even if culture studies are nondiagnostic. We advocate treatment of patients with persistent titers of \( \geq 1:4 \), recognizing that a small number of patients with false-positive results may be treated. Alternatively, one may consider retesting to determine the serum titer until it reaches 1:8 and then initiating treatment.

There are no clinical data for firm recommendations regarding the length of therapy. A conservative approach to persistent antigenemia in the setting of negative culture data may be to treat with fluconazole until the serum titer is negative and to then test for serum antigen at regular intervals. We believe that the presence of serum cryptococcal antigen necessitates careful evaluation for a site of infection and that treatment of isolated serum antigenemia be strongly considered, as empirical therapy for presumptive disseminated disease may prevent development of overt infection.