Baseline Correlation and Comparative Kinetics of Cerebrospinal Fluid Colony-Forming Unit Counts and Antigen Titers in Cryptococcal Meningitis

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Cerebrospinal fluid (CSF) cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers serve as alternative measures of organism load in cryptococcal meningitis. For these measures, we correlated baseline values and rates of decline during the first 2 weeks of therapy in 68 human immunodeficiency virus–seropositive patients with cryptococcal meningitis. At baseline, there was a strong correlation between CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers. During the first 2 weeks of therapy, CSF cryptococcal colony-forming unit counts decreased by >5 logs, and CSF cryptococcal antigen titers decreased by 1.5 dilutions. In individual patients, there was no correlation between the rate of decline in CSF cryptococcal colony-forming unit counts and that in CSF cryptococcal antigen titers.

Cryptococcal meningitis is a common life-threatening infection in patients with advanced HIV disease; it accounted for 13%–44% of all deaths in 2 cohorts of HIV-infected patients in Africa [1, 2]. In a recent study in Thailand, we demonstrated the value of serial quantitative cerebrospinal fluid (CSF) cultures in assessment of the early fungicidal activities of new drug regimens [3]. In addition, the baseline quantitative culture result (colony-forming unit count) was found to be a powerful prognostic marker. Cryptococcal capsular polysaccharide is shed by the organism into tissues and body fluids; latex agglutination and ELISA are sensitive and specific diagnostic tests for its detection in CSF. The CSF antigen titer, like the colony-forming unit count, may be a marker of organism load and has been shown to be of some value in predicting relapse of infection [4]. However, the precise relationship between CSF cryptococcal antigen titers and CSF cryptococcal colony-forming unit counts has not been studied. Therefore, here, we correlate baseline CSF cryptococcal colony-forming unit counts with CSF cryptococcal antigen titers and compare their rates of decline during the first 2 weeks of therapy.

Patients, materials, and methods. The study was approved by the ethical and scientific review subcommittee of the Thai Ministry of Public Health and by the research ethics committee of St. George’s Hospital, London, and was performed at Sappasitprasong Hospital in Ubon Ratchathani in northeastern Thailand, as described elsewhere [3]. Between May and December 2002, we enrolled 64 patients experiencing a first episode of cryptococcal meningitis that had been diagnosed by use of CSF India ink and a cryptococcal antigen test. All patients were treated with amphotericin B (0.7–0.8 mg/kg/day) alone or in combination with flucytosine (100 mg/kg/day), fluconazole (400 mg/day), or both. After 2 weeks, therapy was switched to fluconazole (400 mg/day for 8 weeks and 200 mg/day thereafter). We performed follow-up lumbar punctures on days 3, 7, and 14. An additional 8 patients, who were treated at Los Angeles County Hospital as part of a clinical trial approved by the ethics committee of the University of Southern California, underwent baseline determination of CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers. These data were included in the baseline correlation analysis of CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers.

With a mean delay of <2 h after lumbar puncture, CSF was serially diluted 10-fold, and 100 μL of each dilution was spotted onto each half of a Sabouraud dextrose agar plate.
the colony-forming units from the plate with the lowest dilution that was at least 40 cfu. The rate of decline in log cryptococcal colony-forming units per milliliter of CSF per day was derived from the slope of the linear regression of log cryptococcal colony-forming units against time for each patient, as described elsewhere [3]. Quantitative cultures for patients treated in Los Angeles were performed using serial 10-fold dilutions with 1.0-mL aliquots placed in Sabouraud dextrose agar plates.

Cryptococcal capsular polysaccharide antigen titers in samples from patients from Thailand and Los Angeles were determined by use of a standard commercial assay (Immuno-Mycologics), which included pronase treatment for CSF samples. We measured cryptococcal antigen titers in CSF obtained at baseline with a mean delay of <1 h after lumbar puncture. After follow-up lumbar punctures, CSF was frozen at −80°C, with a mean delay of <3 h, and cryptococcal antigen titers were determined during a 2-day period. There were no differences in cryptococcal antigen titers between samples assayed directly at the time of diagnosis and those frozen first and analyzed later. Therefore, not all baseline samples were reanalyzed. The rate of decline in cryptococcal antigen titers was calculated from the slope of the linear regression of cryptococcal antigen titers against time for each patient. For comparison of the rates of decline in cryptococcal colony-forming units and cryptococcal antigen titers, only samples from patients for whom rates could be calculated for both markers were used.

The association between CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers was tested by use of Spearman’s rank correlation. Pearson’s correlation coefficient was used to test the association between the rate of decline in CSF cryptococcal colony-forming unit counts and that in CSF cryptococcal antigen titers. The Mann-Whitney U test was used to test the association between cryptococcal antigen titers and death at 2 and 10 weeks.

**Results.** The characteristics of the Thai patients have been reported elsewhere [3]. In brief, 64 patients were enrolled. One patient, who was HIV seronegative, was excluded. For 3 patients, baseline CSF quantitative cultures could not be assessed, because the cultures were contaminated. In addition to baseline CSF cryptococcal colony-forming unit counts, baseline CSF cryptococcal antigen titers were available for the remaining 60 patients. CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers at day 14 were available for 47 patients. Fifty-two patients had at least 1 follow-up CSF cryptococcal colony-forming unit count and cryptococcal antigen titer available, allowing the rate of decline in CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers to be calculated. Paired baseline CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers were available for 8 patients from Los Angeles.

At baseline, there was a highly significant positive correlation between CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers (Spearman’s $r = 0.63$ [95% confidence interval (CI), 0.46–0.76]; $P < .0001$) (figure 1A). There was still a significant correlation at days 7 and 14, although, at day 14, the strength of the association was re-
duced (Spearman’s $r = 0.46$ [95% CI, 0.20–0.66]; $P = .001$). As previously reported, the baseline CSF cryptococcal colony-forming unit count is a powerful prognostic marker for death at 2 and 10 weeks in both univariate ($P = .0007$ and $P = .004$, Mann-Whitney U test) and multivariate analyses [3]. The baseline cryptococcal antigen titer was also associated with death at 2 and 10 weeks, but not as strongly ($P = .01$ and $P = .02$, Mann-Whitney U test).

During the first 2 weeks of antifungal therapy, CSF cryptococcal colony-forming unit counts decreased rapidly, from a median of 595,000 cfu/mL of CSF (interquartile range [IQR], 68,875–2,020,750 cfu/mL of CSF) at baseline to a median of 595,000 cfu/mL (IQR, 64–1:32 at 10 weeks [14]; and, in a retrospective study by Eng et al. [15], by 4 months of therapy, the CSF cryptococcal antigen titer decreased to $≤ 1:8$ in 6 of 7 patients with AIDS. However, during the first 2 weeks of therapy, cryptococcal antigen titers are unlikely to be helpful in monitoring the response to therapy in individual patients or as a surrogate marker for response in clinical trials. Although there were clear differences in the rates of decline in CSF cryptococcal colony-forming unit counts between antifungal treatment groups [3], there were no significant differences in the rates of decline in cryptococcal antigen titers between treatment groups (data not shown). Thus, although there was a strong correlation between CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers at baseline, the rates of decline in these 2 markers during the first 2 weeks of therapy differed, reflecting the related but distinct processes of fungal killing and clearance of cryptococcal capsular polysaccharide from the CSF.

**Discussion.** We found a strong correlation between CSF cryptococcal antigen titers and CSF cryptococcal colony-forming unit counts at baseline. We found an association between CSF cryptococcal antigen titers and mortality at 2 and 10 weeks but an even stronger association between CSF cryptococcal colony-forming unit counts and mortality at 2 and 10 weeks [3]. A previous study found a strong correlation between baseline CSF cryptococcal antigen titers and CSF culture status (positive vs. negative) at 2 weeks. The CSF culture status at 2 weeks was, in turn, strongly associated with clinical outcome at 10 weeks [5]. Mortality in both non–HIV-associated and HIV-associated cryptococcal meningitis has been associated with high baseline CSF cryptococcal antigen titers in some studies [6–8], although not in all [9, 10]. The cryptococcal antigen titer does reflect the viable organism load, as was directly assessed by quantitative cultures, and may also be influenced by duration of infection, given the slow clearance of cryptococcal capsular polysaccharide [11] and the other genetic and physiological factors affecting the rate of capsule synthesis and shedding [12].

CSF cryptococcal antigen titers and CSF cryptococcal colony-forming unit counts became less closely linked after initiation of treatment. During the first 2 weeks of therapy, CSF cryptococcal colony-forming unit counts decreased rapidly, whereas CSF cryptococcal antigen titers decreased less rapidly. Furthermore, in individual patients, the rate of decline in CSF cryptococcal colony-forming unit counts and that in CSF cryptococcal antigen titers were not correlated. Slow and variable clearance of cryptococcal capsular polysaccharide may contribute to the lack of correlation between the rate of decline in CSF cryptococcal colony-forming unit counts and that in CSF cryptococcal antigen titers. In patients who die of HIV-associated cryptococcal meningitis, cryptococcal capsular polysaccharide is widespread in the brain, particularly in the midbrain and basal ganglia [13], and, in animal models, cryptococcal capsular polysaccharide appears to be sequestered in the cells of the mononuclear phagocyte system and is eliminated only slowly from tissues [11]. During longer treatment periods, CSF cryptococcal antigen titers do decrease further. In the last Mycoses Study Group trial, CSF cryptococcal antigen titers decreased from a median of 1:1024 at baseline to a median of 1:64–1:32 at 10 weeks [14]; and, in a retrospective study by Eng et al. [15], by 4 months of therapy, the CSF cryptococcal antigen titer decreased to $≤ 1:8$ in 6 of 7 patients with AIDS. However, during the first 2 weeks of therapy, cryptococcal antigen titers are unlikely to be helpful in monitoring the response to therapy in individual patients or as a surrogate marker for response in clinical trials. Although there were clear differences in the rates of decline in CSF cryptococcal colony-forming unit counts between antifungal treatment groups [3], there were no significant differences in the rates of decline in cryptococcal antigen titers between treatment groups (data not shown). Thus, although there was a strong correlation between CSF cryptococcal colony-forming unit counts and CSF cryptococcal antigen titers at baseline, the rates of decline in these 2 markers during the first 2 weeks of therapy differed, reflecting the related but distinct processes of fungal killing and clearance of cryptococcal capsular polysaccharide from the CSF.

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