A prospective observational study was conducted over a 10-month period to determine the clinical and laboratory manifestations of cryptococcal meningitis in Zimbabwe, a country where antifungal agents are not widely available. Eighty-nine patients with cryptococcal meningitis (median age, 34 years; range, 11–63 years; 56 males) were identified from 406 patients for whom a clinical diagnosis of meningitis had been made. All patients tested were positive for antibody to human immunodeficiency virus. Cryptococcal meningitis was the first AIDS-defining illness in 88% of patients. Typical presentations were headache, mental impairment, and meningism (median duration, 14 days; range, 1–180 days). The median CD4⁺ cell count was 70/μL (range, 0–651/μL). The cumulative median survival from the time of diagnosis was 14 days (range, 0–233 days); 22% of patients survived for >30 days. Independent indicators of a good prognosis were not identified. This study provides a unique basis for the development of novel management strategies for patients with cryptococcal meningitis who reside in resource-poor countries.

Infection due to Cryptococcus neoformans, a ubiquitous encapsulated fungus [1–3], is the third most frequent opportunistic infection of the CNS in patients with AIDS [4]. Cryptococcosis has been extensively described in patients from Europe and North America [5–11] and has been recognized as a prominent AIDS-associated opportunistic infection in sub-Saharan Africa for >10 years [12–18]. Studies conducted largely in the United States suggest that between 6% and 10% of patients with AIDS will develop cryptococcal meningitis [7, 19]. In 40% of these patients, cryptococcosis is the first AIDS-defining condition [6–10, 19, 20]. Zimbabwe has a comprehensive list of essential drugs that covers treatment of a wide spectrum of infectious and noninfectious conditions; this list constitutes the national formulary for government hospitals [21]. The drug list does not include antifungal agents for the treatment of cryptococcosis. The cost of the initial induction phase of therapy for cryptococcal meningitis amounts to several times the average monthly salary in Zimbabwe, and thus, as in many resource-poor sub-Saharan African countries [17], cost-benefit considerations have prevented the provision of these expensive drugs as a priority of health care expenditures. However, the section of society affected by cryptococcal meningitis is socioeconomically very important and is often responsible for the support and care of others [22]. This circumstance has resulted in increased demand for effective treatment and, in the absence of antifungal agents, has made it extremely difficult for the attending physician to manage patients with cryptococcal meningitis. Comprehensive prospective studies from this region on which to base new approaches to therapy are scarce.

The aim of this prospective study was therefore to define the clinical features, laboratory findings, immunologic status, and outcome for patients with cryptococcal meningitis who present to the teaching hospitals in Harare, Zimbabwe. We have attempted to identify a group of patients with good prognoses who are suitable for targeting the development of new treatments or novel therapeutic strategies appropriate for the region.

Methods

Study Population

Harare and Parirenyatwa Central Hospitals are tertiary referral teaching hospitals that also provide district hospital services to the local community in Harare (population, ~1.25 million).
Consecutive patients with cryptococcal meningitis presenting to three of 10 adult general internal medical teams were identified as part of a large meningitis study conducted between 1 January and 31 October 1995. The admission policy for these teams has a lower age limit of 8 years and no upper age limit. Meningitis was considered if the patient had meningism (signs of neck stiffness with photophobia, vomiting, or headache); fever with fits, change in mental state, or headache; unexplained change in mental state with either fits or headache; or a severe unremitting headache. All patients received treatment with benzylpenicillin and chloramphenicol before confirmation of the diagnosis; this therapy was modified as appropriate when the culture results became available. Informed consent was obtained from the patients or, when necessary, their relatives. The study was approved by the University of Zimbabwe Research Board and Medical Research Council of Zimbabwe.

Clinical and Laboratory Evaluation

Patient demography, clinical presentation, evidence of AIDS-defining illnesses, and features of HIV infection [23, 24] were recorded on a standardized form. This information was obtained from the patient if possible, their relatives, or their hand-held medical records and by physical examination. Mental status was assessed by using the Glasgow coma score (GCS) [25]. A lumbar puncture was performed and blood for cultures was obtained immediately at the time of admission. CSF was routinely plated onto sheep blood, MacConkey, and chocolate agars. All cryptococcal isolates were subcultured onto Sabouraud dextrose medium, and their identity was confirmed with use of API 20 C kits (bioMérieux, Marcy l’Etoile, France). The biovar was determined by culture on glycine-L-canavanine bromothymol blue agar [26]. Cryptococcal polysaccharide antigen in CSF was detected by latex agglutination testing (Omega Diagnostics, Alloa, United Kingdom). Anonymous serological testing for HIV was performed by using a dipstick antibody screening assay (Immuno Chemical Laboratory, Bangkok, Thailand); this assay was previously shown to be sensitive and specific under local conditions [27]. Absolute CD4+ lymphocyte counts were quantified on whole blood smears by immunostaining with a mouse monoclonal antibody to CD4 (Dako, Glostrup, Denmark) that was detected by using the alkaline phosphatase/antibody to alkaline phosphatase technique [28].

Evaluation of Outcome

In Zimbabwe, once a diagnosis of cryptococcal meningitis is made at a government hospital, patients are routinely discharged to the care of their families with supportive advice and analgesic treatment. Antifungal agents are not on the Essential Drugs List for Zimbabwe [21], and although these agents are available in the private sector, they are prohibitively expensive for this patient population. To assess outcome, patients were followed up at home at regular intervals until death. Post-mortem examinations were not performed.

Statistical Analysis

The cumulative median survival (95% CI) was calculated from the date of presentation by using the Kaplan-Meier method. The influence of admission clinical and laboratory features [5–7, 9–11, 29] on outcome was assessed at 3 days and 14 days. Univariate analysis included determination of odds ratios (95% CI) and the \( \chi^2 \) or Fisher’s exact test as appropriate. Stepwise logistic regression analysis was employed to identify independent factors.

Results

Population Characteristics

During the study period, 6,144 patients were admitted to the hospital under the care of the participating general medical teams. Meningitis was suspected on clinical grounds in a total of 406 patients (median age, 33 years; range, 8–78 years; 234 males). The diagnosis of meningitis was confirmed for 200 patients (median age, 33 years; range, 8–65 years; 124 males). Eighty-nine of these patients had cryptococcal meningitis: 56 males (median age, 34 years; range, 20–63 years) and 33 females (median age, 33 years; range, 11–51 years). Seventy-nine (100%) of 79 patients tested were positive for antibody to HIV.

Clinical Features

The presenting clinical features of the patients are detailed in table 1. Cryptococcosis was the first known AIDS-defining illnesses in 78 (88%) of 89 patients. A diagnosis of active pulmonary tuberculosis (typical radiographic changes and/or a positive sputum smear) had been made for six patients before admission. Treatment with a standard regimen of rifampin, isoniazid, pyrazinamide, and ethambutol had been commenced. Two patients had Kaposi’s sarcoma, and three patients had bacteremia due to non-typhi Salmonella. At least one other feature of HIV infection was identified in 60 (75%) of 80 patients examined.

The median time from the onset of symptoms to diagnosis was 14 days (range, 1–180 days). Patients typically presented with headache, mental impairment, and features of meningoencephalitis in the absence of focal neurological signs. Twenty-six (35%) of 74 patients had received antimicrobial chemotherapy before admission. Extraneuronal manifestations of cryptococcal disease were not identified in this cohort.

Concurrent respiratory disease was identified in 30 (34%) of 89 patients, 25 of whom had an acute cough and 11 of whom...
had an abnormality on a chest radiograph (lobar shadowing, 7; patchy infiltrates, 2; and interstitial shadowing, 2). Sputum collection or bronchoalveolar lavage was not performed. It is therefore unclear whether these clinical and radiological features constituted pulmonary cryptococcosis or intercurrent bacterial pneumonia.

### Laboratory Findings

Anemia in both men (hemoglobin level, <13 g/dL) and women (hemoglobin level, <12 g/dL) was a common finding in this population (68% and 87%, respectively). However, only three patients had a hemoglobin level of <8 g/dL. Twenty-three percent of the patients had hyponatremia (serum sodium level, <128 mmol/L) at the time of admission. The cellular and biochemical CSF abnormalities observed were modest: median WBC count, 5/mm³ (range, 0–1,250/mm³; predominately mononuclear cells); median glucose level, 2.1 mmol/L (range, 0.1–5.1 mmol/L); and median protein level, 1.1 g/L (range, 0.1–10.6 g/L). Seven patients (8%) had no detectable CSF abnormalities (WBC cell count, <5/mm³; protein level, <0.45 g/L; glucose level, >2.2 mmol/L). India ink-stained CSF preparations were positive for 76 (85%) of 89 patients. CSF culture was positive for C. neoformans for 77 (87%) of 89, and testing for CSF cryptococcal antigen was positive for 79 (92%) of 86. In six cases (7%) in which there was CSF evidence of nonpyogenic meningitis, testing for cryptococcal antigen was positive, but India ink staining and culture of CSF were negative.

Cultures of blood from eight patients (9%) yielded C. neoformans. Two cryptococcal isolates were identified as C. neoformans var. gattii (recovered from CSF but not from blood); the remaining strains were identified as C. neoformans var. neoformans. Concurrent bacteremia was detected in 11 patients (12%); bacteremia was due to Staphylococcus aureus in 7 patients, Salmonella arizonae in 3, and a group A β-hemolytic streptococcus in 1. Coagulase-negative staphylococci were cultured from blood specimens from 14 patients; these isolates were not thought to be clinically significant. Ring forms of Plasmodium falciparum were detected in two patients. CD4⁺ lymphocyte counts were determined for 70 unselected patients with cryptococcal meningitis. The median cell count for this group was 70/μL (range, 0–651/μL). Only a few patients had a CD4⁺ cell count of ≥200/μL (eight [11.4%] of 70); 19 (27%) of 70 had a CD4⁺ cell count of 100–199/μL, and 43 (61.4%) of 70 had a CD4⁺ cell count of <100/μL.

### Outcome

Patients were hospitalized for a median of 4 days (range, 0–37 days). Fifty patients were discharged alive, 11 of whom were lost to follow-up (median follow-up, 4 days; range, 0–14 days). The cumulative median survival was 14 days (95% CI, 9.9–18.1; range, 0–233 days), and 17 (22%) of 78 survived for >30 days. None of the patients were known to have received antiretroviral or antifungal therapy. Analysis of clinical and laboratory features predictive of outcome revealed that severely impaired mental status—i.e., GCS of <13 (10 of 67 patients; OR, 6.9; 95% CI, 1.5–33; P = 0.023)—and hyponatremia (16 of 71 patients; OR, 7.9; 95% CI, 1.6–38; P = .012) were associated with rapid deterioration following admission (survival, <3 days).

Both patients with coexistent P. falciparum parasitemia died on the day of admission. Neither bacteremia at the time of presentation nor a diagnosis of Kapoisi’s sarcoma was associated with rapid deterioration following admission. No association between severe mental impairment or hyponatremia and a more indolent illness (survival >14 days) was observed. However, a CSF glucose level of <2.2 mmol/L (40 of 72 patients; OR, 2.8; 95% CI, 1.1–7.6; P = .037) was associated with survival of >14 days. No independent association was found between admission clinical and laboratory features and
Table 2. Cryptococcal meningitis in Zimbabwe: comparison with cryptococcosis before the introduction of amphotericin B and with cryptococcal disease in HIV-infected patients receiving treatment in the United States and Europe.

<table>
<thead>
<tr>
<th>Origin of report(s), [reference] (no. of patients)</th>
<th>Mean age (y)</th>
<th>Percent of males</th>
<th>Percentage with cryptococcosis as first AIDS-defining illness</th>
<th>Percentage with CSF WBC count of &lt;20/mm³</th>
<th>Mean CD4 cell count (μL)</th>
<th>Mean survival (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harare, Zimbabwe, [PR] (89)</td>
<td>35</td>
<td>63</td>
<td>88</td>
<td>63</td>
<td>70</td>
<td>14</td>
</tr>
<tr>
<td>Before the introduction of amphotericin B,* [31–33] (172)</td>
<td>34.5</td>
<td>71</td>
<td>NA</td>
<td>6</td>
<td>NA</td>
<td>60</td>
</tr>
</tbody>
</table>

NOTE. NA = not applicable; PR = present report.
* Data not available for all cases.
† A range is given for the studies reviewed.
‡ Not all patients received suppressive antifungal therapy following induction therapy.

Discussion

Stoddard and Cutler [30] were the first to comprehensively describe the clinical characteristics and laboratory diagnosis of systemic infection with C. neoformans. In a monograph published in 1916, they differentiated the disease from other fungal infections and named the organism Torula histolytica. Before the epidemic of HIV infection, cryptococcal meningitis was a rare but well-recognized disease worldwide [4, 13, 19, 31–35]. Reports of cryptococcal disease in HIV-infected individuals from sub-Saharan Africa appeared in the early 1980s [12, 13]. At Harare Central Hospital, there has been a 12-fold rise in the number of diagnoses of cryptococcal meningitis in patients in the period between 1990 and 1995 (Central Hospital Statistics). This large prospective study demonstrates that C. neoformans, almost exclusively var. neoformans [1, 2, 14, 36], now accounts for 45% of all laboratory-proven cases of meningitis in adults.

In common with reports spanning the last 80 years [5–11, 17, 18, 31–33], most patients with cryptococcal disease in our study were men in their fourth decade of life (table 2). All subjects tested were HIV-positive, and 88% presented with what was frequently their first AIDS-defining illness. As in previous studies conducted both before and during the era of HIV infection, these individuals were generally symptomatic for at least 2 weeks before admission with nonspecific features of subacute meningitis or meningoencephalitis and headache. It is interesting that, as was found before the introduction of amphotericin B [31–33] and in marked contrast to studies of HIV-infected patients from the United States and Europe [6, 7, 9–11], overt meningism was frequently observed in our population. It seems likely that this difference reflects severity of disease at presentation rather than immunologic differences, but this speculation remains to be confirmed.

Before the introduction of amphotericin B in the middle 1950s, an underlying immunodeficiency was absent in most patients with cryptococcal meningitis [31–33, 37]. We and other researchers have demonstrated a profound immunodeficiency in HIV-infected individuals with cryptococcal meningitis who frequently have CD4⁺ cell counts of <100/μL [4, 5, 10, 29]. The modest biochemical and cytological changes seen in CSF specimens from our patients have been described in HIV-infected patients elsewhere [6, 9–11, 17]. These changes could easily be overlooked in the context of the frequently observed abnormalities associated with invasion of HIV into the CNS alone [38]. Indeed, it is important to point out that seven (8%) of our patients for whom microbiological evidence of infection had been found had no detectable CSF abnormalities.

Current treatment regimens for cryptococcal meningitis are associated with a high rate of acute mortality during initial therapy (10%–25%), a 12-month survival rate among all patients of 30%–60% [4, 7, 11, 20], and a high relapse rate without maintenance treatment [6, 7, 9]. A number of investigators have attempted to define the clinical and laboratory characteristics associated with a poor prognosis to optimize antifungal treatment [5–7, 9–11, 29]. Analysis of these factors in our population revealed a high prevalence of patients younger than 35 years of age, impaired mental status, hyponatremia, low CSF leukocyte counts, and a low CSF glucose level. With the exception of one patient, all individuals who completed follow-up had at least one poor prognostic indicator at the time of admission (median number of factors, 2; range, 0–4). It is therefore remarkable that although the cumulative median survival for our study group was 14 days (range, 0–233 days), 22% survived for >30 days without treatment. It appears that while some patient’s conditions deteriorate rapidly after admis-
sion, as reported in the early studies of cryptococcosis [31–33], many seem to have a more indolent course even without treatment.

It is uncertain which clinical and laboratory features separate the two patient groups. We have failed to identify prognostic factors that are discriminatory in our cohort. Although univariate analysis revealed that severely impaired mental status and hyponatremia were associated with survival of <3 days and that a low CSF glucose level was associated with survival of >14 days, no factor was identified as an independent determinant of survival at these times. The relationship between severely impaired mental status and hyponatremia and rapid deterioration was not unexpected. The association between a more indolent clinical course and a low CSF glucose level was contrary to a previous retrospective report of a cohort receiving treatment [10]. In the absence of specific therapy, this relationship may reflect a more effective meningeal inflammatory response rather than a higher fungal load.

In conclusion, as a result of HIV infection. C. neoformans var. neoformans has become the commonest cause of meningitis in adults in Zimbabwe. As the numbers of patients presenting with cryptococcal meningitis grow in resource-poor countries such as Zimbabwe, the need for appropriate and sustainable treatment approaches to the disease increases. This study provides a basis for the development of new strategies for the management of cryptococcal meningitis. Whether these strategies include targeting patient groups with good prognoses, intermittent suppressive therapy, or the use of novel agents remains to be determined by carefully designed clinical trials.

Acknowledgments

The authors are grateful to Mrs. E. Mushangi and Mrs. P. Manjoro for their assistance in the follow-up of these patients; James Mazorodze and Thenjiwe Chikumba for their technical assistance; Jane Wodsworth for her statistical expertise; Dr. T. J. Allain and Professor R. Gillon for their helpful comments; and the hospital staff and the patients and their relatives who participated in this study.

References

28. Mason DY. Immunocytochemical labelling of monoclonal antibodies by the APAAP immunokaline phosphatase technique. In: Bullock GR,