CLINICAL OUTCOMES OF CRYPTOCOCCAL MENINGITIS AMONG HIV-INFECTED PATIENTS IN THE ERA OF ANTIRETROVIRAL THERAPY

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Abstract. Cryptococcal meningitis (CM) is a common opportunistic infection in HIV-infected patients and the clinical outcome can be severe. This study aimed to determine the survival rate and prognostic factors among HIV-infected patients with CM in the era of antiretroviral therapy (ART). Understanding of these facts may help clinicians to manage CM patients efficiently and patients with poor prognostic factors could be closely monitored. We conducted a retrospective cohort study among new cases of HIV-associated CM who were treated at Ramathibodi Hospital, Mahidol University, Thailand, during 2002-2013. Of 195 patients, 119 (61%) were male; the median (interquartile range, IQR) age was 33 (29-39) years. The median (IQR) CD4 cell count was 20 (9-44) cells/mm^3. The median survival time was >12 years and the 75% survival time was 5 years. Using the Cox proportional hazard model, the factors associated with mortality were impaired consciousness [hazard ratio (HR)=2.38; 95% confidence interval (CI): 1.03-5.50], low initial cerebrospinal fluid (CSF) protein (≤60 mg/dl) (HR=2.88; 95%CI: 1.13-7.35), low initial CSF glucose (≤30 mg/dl) (HR=2.36; 95%CI: 1.01-5.51), high opening pressure during induction therapy (>25 cmH$_2$O) (HR=2.90, 95%CI: 1.21-6.94), no ART (HR=14.8; 95%CI: 5.39-40.7) and relapse of CM (HR=4.31; 95%CI: 1.42-13.1). The HIV-associated CM survival rate in the ART era is higher than it was during the pre-ART era.

Keywords: cryptococcal meningitis, HIV, AIDS, outcome, antiretroviral therapy, Thailand

INTRODUCTION

Opportunistic infections (OIs) are a major concern among acquired immu-nodeficiency syndrome (AIDS) patients. Cryptococcal meningitis (CM) is a major OI and is usually severe. It is estimated worldwide 957,900 cases of CM occur each year, resulting in 624,700 deaths within 3 months after the diagnosis (Park *et al*, 2009). Introduction of combined antiretroviral therapy (ART) has substantially reduced CM mortality and relapse rates (Jongwutiwes *et al*, 2007). A French cohort

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study found the mortality rate of HIV-infected patients with CM decreased significantly after ART was introduced (15.3/100 person-years) compared with the pre-ART era (63.8/100 person-years) (Lortholary *et al*, 2006). Although the mortality rate of CM has decreased following the introduction of ART, it is still high, particularly in resource-limited settings (Manosuthi *et al*, 2008). To reduce CM deaths, studies of outcomes and prognostic factors are warranted. However, these studies are few in resource-limited countries. We conducted a retrospective cohort study of adult HIV-infected patients with CM at a university hospital in Thailand, to determine the survival rate and identify the prognostic factors in the ART era.

MATERIALS AND METHODS

This study was a retrospective cohort study. The study population was adult HIV-infected patients with CM newly diagnosed between January 2002 and December 2013, treated at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand. Inclusion criteria were HIV-infected patients aged >15 years with newly diagnosed CM during the study period. CM was diagnosed with a positive culture for *Cryptococcus neoformans*, a positive India ink smear for encapsulated yeast, or a positive latex agglutination test for cryptococcal antigen on the cerebrospinal fluid (CSF) with typical symptoms. Exclusion criteria were patients who had other known central nervous system (CNS) co-infections or other significant concurrent CNS diseases which might influence the outcome of CM.

Patients were followed until the end of December 2013. Cases were removed from the study at the date of the last visit if they were lost to follow-up or if they were referred to another hospital. A relapsed case was defined as a patient who developed a new episode of CM after the original clinical manifestations of CM had disappeared and the CSF culture had become negative and were confirmed by a positive CSF culture for *C. neoformans* (Lortholary *et al*, 2006). Immune reconstitution inflammatory syndrome (IRIS) cases were defined as those who developed new symptoms of clinical meningitis after the original clinical manifestations had resolved and the patient had been on ART and had a negative CSF culture for *C. neoformans* (Shelburne *et al*, 2005). All alternative etiologies of symptoms other than IRIS must have been ruled out.

Proportions, medians and interquartile ranges (IQR) were calculated. To compare demographic and clinical characteristics, we used chi-square tests or Fisher's exact tests for categorical variables and Mann-Whitney *U* tests for continuous variables. Kaplan-Meier survival analysis and Cox's proportional hazards model were used to evaluate survival and factors associated with outcomes. Hazard ratios and 95% confidence intervals (CI) were calculated and used to measure the strength of associations between outcome variables and their predictors. A two-tailed alpha level of 0.05 was used to determine statistical significance. Statistical analyses were performed using SPSS software, version 20 (IBM, Armonk, NY).

This study was approved by the Ethics Committee, Faculty of Tropical Medicine, Mahidol University, and the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University. The data were evaluated anonymously.
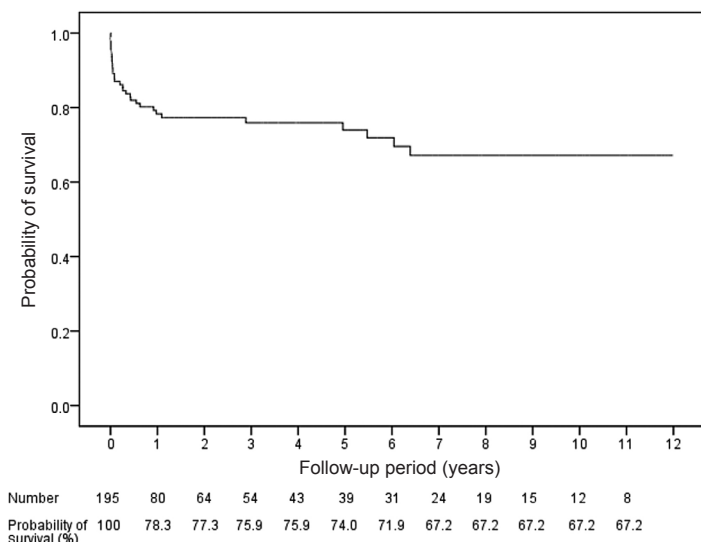
Follow-up period (years)	0	1	2	3	4	5	6	7	8	9	10	11	12
Number	195	80	64	54	43	39	31	24	19	15	12	8	
Probability of survival (%)	100	78.3	77.3	75.9	75.9	74.0	71.9	67.2	67.2	67.2	67.2	67.2	

Fig 1–Kaplan-Meier curve of study subjects.

RESULTS

Baseline characteristics

The study population was comprised of 195 patients. Of these, 119 patients (61%) were males. The median (IQR) age was 33 (29-39) years old and the median (IQR) CD4 cell count was 20 (9-44) cells/mm^3. Presenting symptoms and signs included fever (75.1%), headache (82.6%), impaired consciousness (25.3%) and stiffness of neck (66.1%). The median (IQR) duration from symptom onset until the first visit was 14 (5-21) days. All patients had a lumbar puncture performed. The median (IQR) opening pressure was 25 (18-37) cmH_2O. Median (IQR) CSF white blood cell (WBC) count, CSF protein, and CSF glucose were 7 (0-45) cells/mm^3, 60 (43-93.5) mg/dl, and 43 (31.5-52.5) mg/dl, respectively. Sixty-eight point four percent of patients had a positive India ink smear for encapsulated yeast. The CSF culture for *C. neoformans* was positive in 93.8% and the CSF cryptococcal antigen was positive in 97.7%. A blood culture for *C. neoformans* was positive in 69.6%.

Treatment of CM and HIV/AIDS

Of the 195 studied patients, 92.8% were treated with antifungal drugs; the others died or were referred to other hospitals prior to initiation of antifungal therapy. Eighty-six point seven percent of patients received amphotericin B (AmB, 0.7 mg/kg/day) for induction therapy [70.8% had AmB alone and 15.9 % had AmB with fluconazole (FLU)]; 6.2% received FLU only. During antifungal therapy and regular lumbar puncture to release intracranial pressure, the median (IQR) opening pressure was 28 (19-40) cmH_2O. Of the 195 studied patients, 53.3% received ART: 9.7% had started ART prior to the presentation of CM [median (IQR) time from ART initiation to onset of CM was 27 (11-60) days]; and 43.6% were initiated ART after diagnosis of CM [median (IQR) time from start of antifungal therapy to ART initiation was 68 (46-119) days]. The others died or were referred to other hospitals prior to ART initiation.

Outcomes

The median (IQR) follow-up period was 141 (10-1,307) days. Fig 1 shows the Kaplan-Meier curve of survival. The median survival time was more than 12 years and the time when 75% of patients survived (75% survival time) was 5 years. The probabilities of survival (95% CI) at 2 and 10 weeks, 6, 12, 24, 36, 48 and 60 months were 91.8% (87.7-95.9), 87% (81.7-92.3), 82% (75.7-88.3), 78.3% (71.2-85.4), 77.3% (70.2-84.4), 75.9% (68.5-83.3), 75.9%

Table 1
Factors associated with mortality in study subjects.

Characteristics	HR (95% CI)	p-value
Impaired consciousness	2.38 (1.03-5.50)	0.043
CSF WBC count (≤ 5 cells/mm^3)	2.25 (0.99-5.08)	0.052
CSF protein level (≤ 60 mg/dl)	2.88 (1.13-7.35)	0.027
CSF glucose level (≤ 30 mg/dl)	2.36 (1.01-5.51)	0.047
CSF opening pressure (> 25 cmH$_2$O)[a]	2.90 (1.21-6.94)	0.017
Not initiating ART	14.8 (5.39-40.7)	<0.001
Relapse of CM	4.31 (1.42-13.1)	0.010

[a]During induction therapy of CM. ART, antiretroviral therapy; CI, confidence interval; CM, cryptococcal meningitis; CSF, cerebrospinal fluid; HR, hazard ratio; WBC, white blood cell.

(68.5-83.3) and 74% (65.8-82.2). Of the 195 studied patients, 37 (19%) died, of whom 24 (12.3%) had a CM-related death.

Paradoxical IRIS and cryptococcal relapse

Of the 104 patients who received ART, 20 (19.2%) had 23 episodes of IRIS. The median (IQR) time from starting antifungal therapy to ART initiation among the IRIS patients was 77 (58-120) days and the median (IQR) time from ART initiation to onset of IRIS was 148 (48-288) days. Of the 20 IRIS patients who had an IRIS episode, during a median (IQR) follow-up period of 2.6 (1-6.2) years, 3 (15%) died due to IRIS.

Of the 195 studied patients, 15 (7.7%) had a relapse of CM comprising a total of 19 episodes. The median (IQR) time from start of antifungal therapy to relapse was 137 (92-254) days. Of the 15 relapse patients, during a median (IQR) follow-up period of 410 (119-1965) days, 6 (40%) died due to cryptococcal relapse.

Factors associated with mortality among studied patients

Of the 195 studied patients, factors significantly associated with mortality using the Cox proportional hazard model were: impaired consciousness [hazard ratio (HR)=2.38; 95% CI: 1.03-5.50], low initial CSF protein (≤ 60 mg/dl) (HR=2.88; 95% CI: 1.13-7.35), low initial CSF glucose (≤ 30 mg/dl) (HR: 2.36; 95% CI: 1.01-5.51), high opening pressure during induction therapy (>25 cmH$_2$O) (HR=2.90; 95% CI: 1.21-6.94), no ART initiation (HR=14.8; 95% CI: 5.39-40.7) and relapse of CM (HR=4.31; 95% CI: 1.42-13.1) (Table 1). Fig 2 shows the Kaplan-Meier curve for survival probability stratified by significant prognostic factors.

DISCUSSION

In our study, the median survival rate among studied subjects with HIV and CM was greater than 12 years and the 75% survival was 5 years. This survival rate is better than the results from other studies in the pre-ART era. In one study from Thailand, the reported 12-month survival rate for those with HIV and CM was 15% (Pitisuttithum *et al*, 2001). Other reports from resource-limited settings in the ART era found survival rate among HIV-infected patients with CM of 46-65% at 6 months and 59% at 12 months (Kambugu *et al*, 2008; Day *et al*, 2013; Jarvis *et al*, 2014).
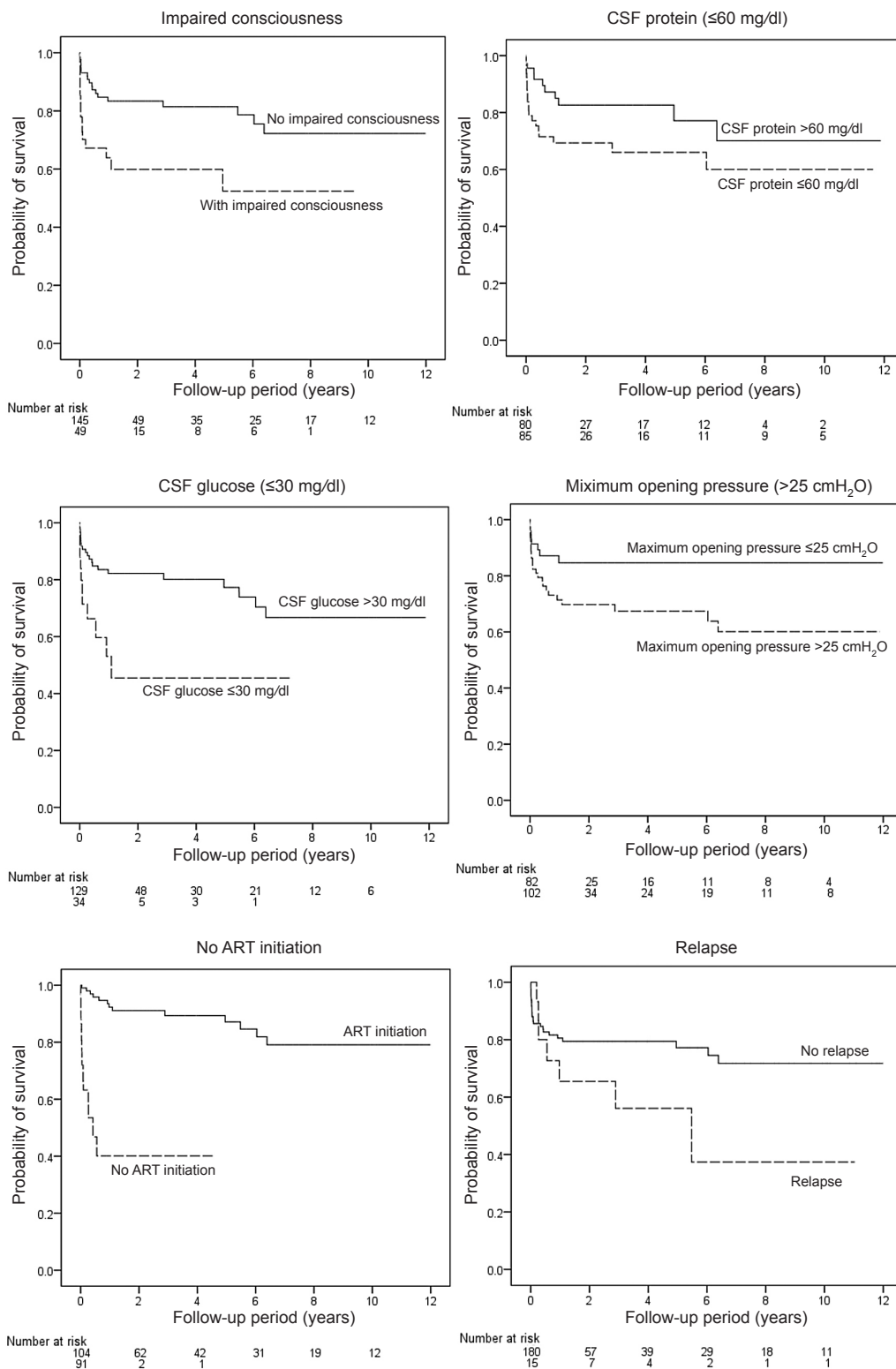
Fig 2–Kaplan-Meier curve stratified by associated factors.

Reasons for the improved survival rates in the ART era may be differences in the healthcare infrastructure and improved resources and experience in managing the patients with CM.

Although improved survival has been demonstrated by a previous study (Lortholary *et al*, 2006), the disease is still dangerous and lethal, even in the ART era. Understanding prognostic factors may help clinicians better manage these patients. We found 6 factors to be significantly associated with death in our study subjects: impaired consciousness, a low CSF protein level (≤ 60 mg/dl), a low CSF glucose level (≤ 30 mg/dl), a high opening pressure during treatment (> 25 cmH$_2$O), no ART initiation, and relapse of CM.

Impaired consciousness had also been found to be associated with death in these patients from other studies (Saag *et al*, 1992; Pitisuttithum *et al*, 2001; Kambugu *et al*, 2008; Majumder *et al*, 2011; Vidal *et al*, 2012; Jarvis *et al*, 2014). The association between death in an HIV-infected patient with CM and a low CSF protein has been reported in a previous study (Kambugu *et al*, 2008). An elevated CSF protein in patients with meningitis suggests the presence of inflammatory proteins that eliminate organisms, such as cytokines and immunoglobulins. A high CSF IFN-γ level in an HIV-infected patient with CM is associated with rapid clearance of *C. neoformans* from the CSF; CSF IFN-γ levels were significantly higher among survivors of CM than among those who died (Siddiqui *et al*, 2005). A low CSF protein may reflect a poor immune response in CSF of HIV-infected patients.

A low CSF glucose has been found to be associated with death among HIV-infected patients with CM in a previous study (Darras-Joly *et al*, 1996). In that study, a CSF glucose level < 2 mmol/l (36 mg/dl) was significantly associated with mortality within three months after CM diagnosis. Low CSF glucose may reflect high burden of *C. neoformans*. In bacterial meningitis, bacterial consumption of glucose is one of the mechanisms for reduced CSF glucose levels (Venkatesh *et al*, 2000). A high pre-treatment CSF fungal burden has also been found to be associated with mortality in HIV patients with CM (Jarvis *et al*, 2014). However, *C. neoformans* quantitative cultures are difficult to perform. A CSF glucose level might be a useful predictor of fungal burden and probability of mortality.

A high CSF opening pressure during induction therapy (> 25 cmH$_2$O) was significantly associated with mortality in our cohort. This finding was also reported in a previous study (Graybill *et al*, 2000). A study from Brazil found an elevated opening pressure (≥ 50 cmH$_2$O) at days 7 to 14 of induction therapy was significantly associated with in-hospital mortality (Vidal *et al*, 2012). The Infectious Disease Society of America (IDSA) guidelines recommend patients with an opening pressure > 25 cmH$_2$O should have CSF drainage (Perfect *et al*, 2010). If an opening pressure > 25 cmH$_2$O persists, the patients should have daily lumber punctures. Failure to follow this recommendation is significantly associated with neurological complications (Shoham *et al*, 2005). Our results suggest the opening pressure should be monitored during induction therapy. CSF drainage should be performed regularly to maintain a CSF opening pressure < 25 cmH$_2$O.

Not initiating ART was significantly associated with mortality in our cohort and had been described as a risk factor for death due to CM in several previous studies (Lortholary *et al*, 2006; Chottana-

pund *et al*, 2007; Jongwutiwes *et al*, 2007; Mathiesen *et al*, 2012). ART has led to a significant decline in deaths due to OIs including CM. In our study, some of the patients die before they were started on ART.

An association between relapse of CM and death has been reported previously (Pitisuttithum *et al*, 2001). The mortality in relapsing CM has been reported to range from 25% to 41.7% (McCarthy *et al*, 2006; Espie *et al*, 2010). IRIS was not significantly associated with death in our study. One in 13 patients with IRIS died in one prospective Thai-American study (Sungkanuparph *et al*, 2009) but IRIS was not significantly associated with death. However, 16 of 46 IRIS patients died in a prospective Ugandan study and IRIS was significantly associated with death (Boulware *et al*, 2010). Differences in infrastructure, medical care, resources, trained personnel, experience in managing HIV-infected CM patients with IRIS and severity of IRIS may account for these differences in association with IRIS.

The median (IQR) time from induction therapy to ART initiation was 68 (46-119) days in our study. This may explain the reason why time to initiate ART was not significantly associated with mortality. Most patients had a delay in starting ART of >5 weeks after initiation of induction therapy. Early ART has been reported to be significantly associated with CM mortality (Makadzange *et al*, 2010; Boulware *et al*, 2014). Deferring ART for 5 weeks after initiating CM treatment has been associated with significantly better survival than initiating ART 1 to 2 weeks after initiating CM treatment (Boulware *et al*, 2014).

In conclusion, we found introduction of ART improved survival in HIV-infected patients with CM but initiation of ART should be delayed for at least 5 weeks. The mortality rate due to CM in HIV-infected patients is still high. Further studies are needed to improve morbidity and mortality among HIV-infected patients with CM. Factors associated with mortality were impaired consciousness, a low initial CSF protein level, a low initial CSF glucose level, a high CSF opening pressure during induction therapy, no initiation of ART and relapse of CM. HIV-infected patients with CM who have these factors should be monitored closely.

ACKNOWLEDGEMENTS

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