Etiology and Clinical Management of Adult Meningitis in Indonesia

Ahmad Rizal Ganiem

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Publication of this thesis was financially supported by:
Departement of Medicine, Radboud University Medical Centre, Nijmegen.
KNCV Tuberculosis Foundation.

Design cover by Herlan Hazil A.
Layout by satia nugra-ha.
Printed and bound by Gita Print, Bandung, Indonesia.

ISBN 978-60295204-5-3

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Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen,
op gezag van de Rector Magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op Maandag 8 April 2013
om 13.30 uur precies

door

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geboren op 26 mei 1966
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Doctoral thesis

To obtain the degree of doctor
from Radboud University, Nijmegen
on the authority of the Rector Magnificus prof. mr. S.C.J.J. Kortmann,
according to the decision of the Council of Deans
to be defended in public on Monday April 8, 2013
at 13:30 hours.

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Contents

Chapter 1

Introduction and thesis outline

MENINGITIS, A GLOBAL CHALLENGE

Meningitis can be defined as inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The majority of meningitis patients present with at least two of four signs/symptoms of fever, headache, neck stiffness, and altered mental status [1]. Other signs reflecting complications like cerebral nerve palsies or motor deficits may also be present, and this may result in death or sequalae in those who survive [2,3]. Meningitis is mostly caused by infection with bacteria or fungi. Patient characteristics, signs and symptoms and routine cerebrospinal fluid (CSF) examination can help make a diagnosis and guide treatment. However, these parameters are not 100% specific, and often no diagnosis can be made, as shown by a report from South Africa which revealed that almost half of meningitis cases had no definite diagnosis despite extensive microbacteriological testing [4].

Meningitis poses a big clinical challenge to physicians. Diagnosis is often difficult, and outcome is often poor. Despite the fact that many thousands of patients die each year from meningitis, this severe disease is not yet a public health priority.

Etiology and diagnosis

A common type of meningitis is acute bacterial (septic) meningitis that is mainly caused by *Streptococcus pneumonia, Hemophilus influenza,* and *Neisseria meningitidis*. It has a worldwide distribution with its causative pathogens varying by geographic distribution, age, and underlying medical and/or surgical condition [5]. Its estimated incidence is 2.6 – 6 per 100,000 adults per year in developed countries and up to 10 times higher in less developed countries [6]. Tuberculous (TB) meningitis usually has a somewhat more gradual onset ('subacute meningitis'), developing over a period of days to weeks. It is estimated that 1-5% of TB cases may develop central nervous system TB [3,7,8]. Without treatment, death occurs in virtually all patients with TB meningitis, while delay in treatment results in a considerable risk of death or irreversible neurological damage. Bacteriological confirmation is the key to diagnosis, but using conventional methods its positivity rate is not satisfactory and molecular diagnosis is also not very sensitive [3,9–11].

HIV infection complicates diagnosis and management of meningitis. HIV infection increases the risk of developing active TB by more than 20 times, and the risk of getting meningitis increases even more [12–15]. Further, meningitis in HIV-positive cases has a broader differential diagnosis [4,16]. Cryptococcal meningitis has been closely related to HIV infection, and along with TB meningitis comprises more than 80% of meningitis in HIV positive patients [4]. Other HIV-related CNS infection like toxoplasmosis and neurosyphilis also need to be considered.

Treatment and outcome

Prompt treatment of meningitis is crucial, but diagnostic uncertainty may delay proper treatment. The current treatment regimen for TB meningitis is suboptimal as shown by the fact that up to 50% of patients with TB meningitis die or remain neurologically disabled. So far no randomized clinical trials have compared different antibiotic regimens for TB meningitis. Corticosteroids, used as adjuvant treatment for TB meningitis, have shown a survival benefit, although its long-term effects are somewhat disappointing [17,18]. Cryptococcal meningitis also has a high mortality, accounting for a large proportion of 'early deaths' in patients with advanced HIV infection, especially in sub-Saharan Africa. Obviously, for all types of meningitis late presentation is a major contributor to the high mortality [19].

MENINGITIS IN INDONESIA

Indonesia, like other low-resource countries, still faces many cases of meningitis with significant mortality and morbidity. Indonesia has the fourth largest TB caseload worldwide, with an estimated prevalence of 680,000 cases of TB in year 2011 [20]. In Hasan Sadikin hospital, when we started this study in 2006, 60-80 adult patients were admitted with clinical meningitis annually. The microorganisms causing meningitis were mostly unknown, as bacteriological confirmation of disease was rarely achieved and no survey had been conducted on this topic. Many patients were suspected of suffering from TB meningitis, mostly in the advanced stage, and more than 50% died during hospitalization.

In year 2006 when we started the study, Indonesia was experiencing a rapid

growth of HIV. The HIV prevalence in the general population was low (0.2%), but estimated prevalence rates in certain risk groups, especially injecting drug users (IDUs) were 50% or more [21]. Uptake of HIV testing was low, and many patients were not aware of their HIV status until hospitalization for advanced HIV infection or AIDS. This condition probably fuelled the development of TB and cryptococcal meningitis. However, when we started our study, there were no data regarding the HIV prevalence among meningitis patients, or regarding the etiology of meningitis in this group, neither in our hospital nor anywhere else in Indonesia. Meningitis patients were rarely tested for HIV infection, and no survey had been conducted. Still, clinicians had the impression that HIV was increasing the numbers of patients presenting with meningitis in Indonesian hospitals. Of note, HIV incidence rate among adults 15-49 years old in Indonesia is increasing more than 25% in 2011 as compared to year 2001 [22].

In summary, adult meningitis is common in Indonesia, but bacteriological confirmation is extremely rare, and epidemiological data regarding the cause, presentation and outcome were lacking prior to my PhD. Also, the impact of HIV on cause and outcome of meningitis in Indonesia was unknown. During my PhD I have therefore tried to answer some of these questions related to meningitis in Indonesia.

THESIS OUTLINE

This thesis consists of studies related to the clinical presentation, diagnosis, optimal treatment and prevention of adult meningitis in Indonesia, both in HIV-infected and non-infected individuals. The studies were conducted in years 2006 – 2012, and involved both HIV-infected and non-infected individuals presenting at Hasan Sadikin hospital in Bandung, the referral hospital for West Java province (population: 43 million people), Indonesia.

During the last ten years there has been a gradual increase in the number of meningitis patients presenting to our hospital. However, before 2006 very few cases were bacteriologically confirmed, and systematic data regarding their severity and outcome were lacking. Therefore, as a first step towards

improvement of patient management, in **Chapter 2** we examined the cause, clinical presentation and outcome of a cohort of adult patients presenting with meningitis.

This initial study showed that the majority of our patients had a diagnosis of probable TB meningitis. Bacteriological confirmation of TB meningitis is difficult. Microscopic examination of cerebrospinal fluid (CSF) is generally insensitive (2–20 %), while culture is also not very sensitive and too slow to guide treatment. Nuclear acid amplification (NAA) tests may provide rapid confirmation of TB meningitis, but a systematic review and metaanalysis of the accuracy of NAA tests for diagnosis of TB meningitis showed that commercial NAA tests have a high specificity but a low sensitivity when compared with culture. There is some reason to believe that in-house PCR may be more sensitive, and in **Chapter 3** we asked ourselves the question if an in-house PCR could help improve the diagnosis of TB meningitis.

Despite a thorough bacteriological workup, we were unable to make a definite diagnosis in many meningitis patients, especially among those who were HIV-infected. In **Chapter 4** we investigated the possibility that cerebral toxoplasmosis accounted for some cases of meningitis of unknown origin. Toxoplasmosis, a common opportunistic infection in advanced HIV infection, usually presents as space occupying lesions in the brain, but since imaging of the brain is rarely performed in meningitis patients in our setting, cerebral toxoplasmosis may be missed. We hypothesized that cerebral toxoplasmosis may falsely be diagnosed as TB meningitis.

TB meningitis has a very high mortality and morbidity. Its treatment follows that of pulmonary TB. Some TB drugs, especially rifampicin, show poor penetration into the brain and cerebrospinal fluid. Rifampicin is pivotal in the treatment of TB meningitis, but the current dose of rifampicin is at the lower end of the dose-response curve. Higher dose of rifampicin has been studied in pulmonary TB by our group and others [23,24]. Intravenous rifampicin treatment, that probably leads to higher drug exposure, might be preferable in meningitis patients, many of whom are severely ill or unconscious. However, so far, no study has examined

a higher dose rifampicin, or the use of intravenous rifampicin in TB meningitis. Another option to intensify treatment and improve outcome of TB meningitis might be to add a fluoroquinolone. Among the fluoroquinolones, moxifloxacin has the highest activity against *M. tuberculosis* in vitro and in murine models, and has been proven to penetrate well into CSF of patients with TB meningitis. In **Chapter 5** we asked ourselves the question whether a higher dose of rifampicin and adding moxifloxacin may lead to a better drug exposure with accepted tolerability, and whether this intensified treatment results in better clinical outcome.

Early recognition can prevent the development or severe outcome of meningitis. Several studies in countries with a high burden of cryptococcal meningitis have found that the presence of cryptococcal antigen in the blood of HIV-infected patients is a risk factor for the development of cryptococcal meningitis and subsequent death. In **Chapter 6** we asked ourselves the question whether this also holds true for Indonesia, and whether this may contribute to the high mortality among HIV patients in our setting.

Delay in accessing medical help has been related to high mortality in TB meningitis, as clearly shown in a Vietnamese study, where mortality increased in accordance to TB meningitis grade; i.e. 16.7%, 31.1% and 58.8% in grade 1, 2, and 3, respectively [17]. Most patients in our setting present with grade 2 and 3 TB meningitis, reflecting delay in presentation. In **Chapter 7** we asked ourselves the question why patients present at such late stage to the hospital, using in-depth interviewing with a group of meningitis patients.

The main findings of these studies are summarized and discussed in **Chapter 8**, and an outline of further research is proposed. It is hoped than this body of work will contribute to better management of meningitis, particularly TB meningitis, in Indonesia and elsewhere.

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Chapter 2

The effect of HIV infection on adult meningitis in Indonesia: a prospective cohort study

AIDS 2009, 23:2309–2316

ABSTRACT

Objective

Indonesia has a concentrated but rapidly growing HIV epidemic. We examined the effect of HIV on causative organisms, clinical features and prognosis of adult meningitis.

Design

A prospective cohort study.

Methods

All adult patients at a referral hospital who underwent cerebrospinal fluid examination for suspected meningitis were examined for HIV and included in a prospective cohort study. Microbiological testing was done for common bacterial pathogens, mycobacteria and fungi. Patients were followed for at least 6 months, and logistic regression models were used to identify risk factors for mortality.

Results

Among 185 patients who mostly presented with subacute meningitis, 60% were male and the median age was 30 years. HIV infection was present in 25% of the patients; almost two-thirds were newly confirmed, and all presented with severe immunosuppression (median CD4 cell count 13/ µL, range 2–98). One-third of HIV-infected patients had cryptococcal meningitis whereas two-thirds suffered from tuberculosis.

After 1 month, 41% of patients had died. HIV infection was strongly associated with 1-month mortality (adjusted odds ratio 12.15; 95% confidence interval 3.04 – 15.72) and death during extended follow-up (hazard ratio 2.48; 95% confidence interval 1.97–5.74).

Conclusion

Although HIV is still uncommon in the general population in Indonesia, its prevalence among adult meningitis cases already seems high. *Mycobacterium tuberculosis* and *Cryptococcus neoformans* are the main causes of meningitis in this setting, and mortality is very high, especially in HIV-infected patients. Our data suggest that adult meningitis cases in Indonesia should be screened routinely for HIV infection. Further studies are needed to address the high mortality.

INTRODUCTION

Indonesia is witnessing one of the most rapidly growing HIV epidemics in Asia. Except for the region Papua, Indonesia has a concentrated epidemic largely driven by injecting drug use. The estimated prevalence of HIV among the general population is only 0.2%, but among risk groups, especially injecting drug users (IDUs), rates have been reported up to 50% [1]. Uptake of HIV testing is low, and many patients are only tested when they have advanced HIV infection or AIDS. Among patients diagnosed with HIV in 2006 in the top referral hospital for West Java, the median CD4 cell count was 32/µL (R. Wisaksana, personal communication). HIV patients with severe immunosuppression have a high risk of certain regular and opportunistic infections. Although definite diagnosis of such infections may be difficult in this setting, anecdotal data from Indonesian hospitals suggest that HIV infection is a major determinant of presentation and outcome of certain diseases, such as pulmonary infections, chronic diarrhea and dermatological problems.

Patients with advanced HIV infection often have severe neurological manifestations, especially meningitis. HIV infection is associated with a higher risk of cryptococcal, tuberculous and pneumococcal meningitis [2–4], and with a higher mortality of meningitis [5]. There are neither any data regarding the epidemiology of common adult meningitis in Indonesia, nor about a possible effect of HIV on its cause, presentation and outcome. We conducted a prospective study in a hospital setting in Indonesia to determine the HIV prevalence, causative agents, clinical and laboratory features and prognosis of adult patients with meningitis.

METHODS

Patient population and study design

Patients described in this study were admitted to Hasan Sadikin Hospital Bandung, Indonesia. This hospital serves the local community and acts as the top referral hospital for West Java Province (population about 40 million). Each year, about 2000 neurology patients are admitted to the hospital, around 10% with central

nervous system (CNS) infections. Clinical data were recorded prospectively from all adult patients (≥ 18 years old) admitted with suspected meningitis to the ward between November 2006 and November 2008. The study protocol was approved by the Hospital Ethical Committee.

Study procedures

After providing informed consent, each patient underwent standard history taking, physical and neurological examination and lumbar puncture. The Glasgow Coma Scale (GCS) was used; an altered consciousness was defined as GCS < 14 and coma as GCS < 9 [6]. In the absence of computed tomography (CT) scan, considerations to perform lumbar puncture were based on clinical signs and symptoms.

Serological testing for HIV was done with informed consent during hospital admission for patients unaware of their HIV status and done anonymously for those who had died before consent was obtained. Follow-up was done until hospital discharge or death. At time of discharge, the outcome was graded according to the Glasgow Outcome Scale (GOS) [6]. All patients were reevaluated monthly until treatment was completed. Home visits and phone calls were made for those who were lost to follow up. The cause of death was determined by clinical signs only; autopsy was not performed.

Laboratory examinations

Cerebrospinal fluid (CSF) measurements consisted of standard macroscopic and biochemical analysis. Microscopy was done for cryptococci, acid-fast bacilli and bacterial pathogens using India Ink, Ziehl Nielsen and Gram staining, respectively. Culture was done for *Mycobacterium tuberculosis* (solid Ogawa and liquid MB-BacT; Biomerieux, Durham, North Carolina, USA), bacterial pathogens (blood agar, chocolate agar and brain–heart infusion) and fungi (Sabouraud). Cryptococcal antigen (CALAS; Meridian Diagnostics, Cincinnati, Ohio, USA) testing was done on CSF samples. All investigations were done at Department of Clinical Pathology, Hasan Sadikin Hospital. In a subset of patients, PCR was done on CSF for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus suis* [7] (courtesy Menno de Jong, Oxford Medical

Research Unit, Ho Chi Minh City, Vietnam). Species identification of *Cryptococci* was done at the Faculty of Medicine, University of Indonesia. Routine blood examination consisted of white blood cell count and differentiation, random blood glucose and sodium level. Mild and severe hyponatremia was defined as a serum sodium concentration less than 136 mEq/L and 125 mEq/L or less, respectively. Serum alanine transferase was measured as a baseline value and repeated in the case of suspected drug-induced hepatitis.

All blood samples were screened for syphilis using Venereal Disease Research Laboratory (VDRL) and for anti-hepatitis C virus (HCV) antibodies. Measurement of CD4 cell count for HIV patients only became available during the study. Chest radiograph abnormalities were recorded and classified as miliary, infiltrative, cavitary or other.

Diagnostic criteria

Diagnosis of meningitis was made using clinical or CSF criteria or both. Clinical criteria of meningitis included headache, fever and neck stiffness, with or without altered consciousness. CSF criteria were cell count more than 10 cell/mL, protein concentration more than 45 mg/dl or the CSF : blood glucose ratio less than 0.5; either alone or in combination [8].

Definite tuberculous meningitis (TBM) was diagnosed if CSF microscopy or culture or both were positive for *M. tuberculosis* [4]. Probable TBM was defined as meningitis with typical CSF findings in conjunction with suspected active pulmonary tuberculosis (TB) (using chest radiography), clinical sign of other extra pulmonary TB or bacteriologically confirmed TB outside the CNS.

Definite bacterial meningitis was defined as meningitis with detection of bacteria in CSF by microscopy or culture or characteristic CSF findings (a predominance of polymorphonuclear cells and a CSF : blood glucose ratio <0.4) in combination with a positive blood culture [9,10]. Probable bacterial meningitis was diagnosed in patients with clinical meningitis and characteristic CSF findings, without confirmatory bacteriological result, but with clinical improvement with antibiotic treatment.

Cryptococcal meningitis was diagnosed if India Ink examination or cryptococcal antigen testing or both of CSF were positive [11].

The neurological status of patients with TBM was classified according to the British Medical Research Council (BMRC) definition as follows: grade I, normal consciousness, no neurological signs; grade II, confusion or neurological signs (meningeal or focal) and grade III, stupor or coma [4].

Drugs and dosage

TBM was treated with fixed dose combination of rifampicin (450 mg), isoniazid (300mg), ethambutol (750mg) and pyrazinamide (1500mg). For unconscious patients, drugs were given via nasogastric tube. Dexamethasone was given following recent recommendations [12]. Bacterial meningitis was treated with ceftriaxone intravenous (i.v.) 2–4 g/day for 10–14 days with administration of i.v. dexamethasone 20mg/day for 4 days [8,10].

Cryptococcal meningitis was treated with oral fluconazole (800mg/day) or intravenous amphotericine-B (0.7–1mg/kg bodyweight) if available [13]. HIV treatment was done in accordance with national guidelines, with nevirapine, lamivudine and zidovudine; patients on TB treatment received efavirenz instead of nevirapine.

Data analysis and statistics

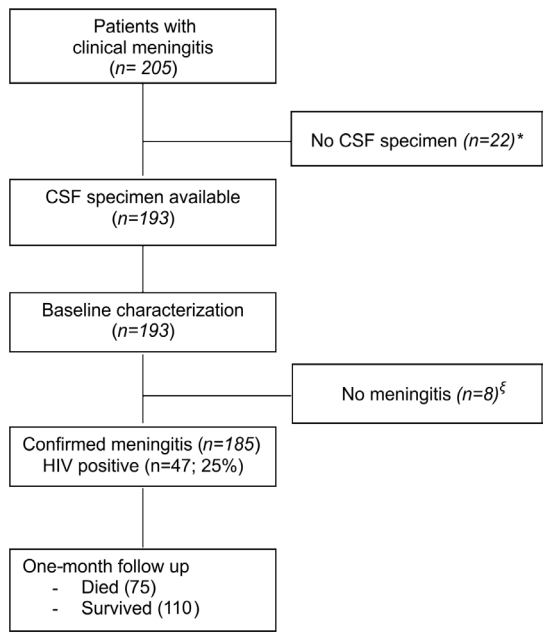
Data were expressed as indicated using proportion (%), median and interquartile range (IQR). Parametric and nonparametric tests were used to identify differences between groups in continuous outcomes, and χ^2 tests were used to compare categorical outcomes. Factors associated with 1-month mortality were identified using logistic regression. Factors significantly associated in univariate analysis and factors significantly different between HIV positive and negative patients were included in multivariate regression analysis. Progression to death was investigated by Kaplan–Meier estimates, and logistic regression was used to identify prognostic factors of death. Statistical analysis was done by SPSS version 13. Survival curve was drawn using GraphPad Prism version 4.0.

RESULTS

Patient characteristics

During the study period, 215 patients presented with suspected meningitis. CSF could not be obtained in 22 of the patients, and eight patients had other diagnoses, resulting in 185 patients in this study (**Figure 1**). Sixty patients were referred from other hospitals: 28 from hospitals in Bandung and the remaining from other cities.

Figure 1. The case of 215 patients presenting with suspected meningitis.



Symbol * denotes declined lumbar puncture (16), contraindication to do lumbar puncture (5), and dry tap (1).
ξ denotes toxoplasmosis (3), vascular lesion (1), miliary TB (2), space-occupying lesions (2)

A total of 51 patients (27.6%) had a previous diagnosis of TB (pulmonary or extra pulmonary or both). Previous antibiotic treatment for bacterial infections (other than TB) was reported for 19 patients. Patients were mostly young males

Table 1. Demography, history, and physical examination (n=185)

Characteristics	HIV-positive (n=47)	HIV-negative (n=138)	P-value	OR (95%CI)
General				
Age in year - median (IQR)	30 (27-33)	29 (23-37)	.57	
Male sex *	36 (76.6)	75 (54.3)	.01	2.59 (1.21-5.55)
History of TB *	19 / 46 (41.3)	32 / 129 (24.8)	.04	2.13 (1.05-4.34)
Duration of illness in days – median (IQR) *	30 (14-60)	14 (7-30)	.01	
Signs and symptoms on presentation				
Headache **	37 / 46 (80.4)	122 / 130 (94.2)	<.01	0.27 (0.10-0.75)
Neck stiffness	43 / 47 (91.5)	126 / 130 (96.9)	.14	0.35 (0.08-1.46)
Body temperature $\geq$ 38°C	17 / 41 (41.5)	48 / 122 (39.8)	.81	1.09 (0.53-2.24)
Altered consciousness (GCS < 14)	20 / 43 (46.5)	57 / 126 (45.2)	.89	1.05 (0.53-2.11)
Coma (GCS <9)	1 / 43 (2.3)	10 / 125 (8.0)	.19	0.27 (0.03-2.20)
Hemiparesis	12 / 47 (25.5)	28 / 121 (23.1)	.74	1.14 (0.52-2.48)
Paraparesis *	0 / 47 (0)	13 / 123 (10.6)	.02	1.43 (1.29-1.58)
Cranial nerve palsy	12 / 47 (25.5)	39 / 127 (30.2)	.51	0.77 (0.36-1.65)

Data are presented as no. of patients / no. evaluated (%) unless stated otherwise. CI, confidence interval; CSF, cerebrospinal fluid; IQR, interquartile range; GCS, Glasgow Coma Scale; OR, odds ratio.

*p < .05

**p < .01

presenting after a median duration of 2 weeks of complaints, with headache, neck stiffness, altered consciousness and focal neurological signs (**Table 1**). Forty-seven patients (25%) were HIV-positive, 30 of which were newly diagnosed. CD4 cell counts, available for 13 HIV patients, were very low (median 13/μL; range 2–98). General and clinical characteristics were not associated with HIV infection, except for male sex, history of TB infection, duration of illness, headache and paraparesis (**Table 1**). The classic triad of headache, fever and neck stiffness was found in 61%. Cranial nerve palsies were present in 29%, hemiparesis in 23% of patients and paraparesis in 8% of the patients. The most common cranial nerves

affected were abduscens and oculomotor nerves. Eighteen patients (9.7%) had had seizures at some points, mostly generalized.

Laboratory findings

CSF findings confirmed meningeal inflammation in most of the patients. However, only 62% showed all the three diagnostic criteria of meningeal inflammation, and 14% had only one criterion or no abnormalities. Hyponatremia was common (68%) and was severe in 52% and mild in 16% of patients. Laboratory examinations showed lower CSF and peripheral leukocyte counts and higher plasma sodium among HIV-infected patients (**Table 2**). Reflecting the nature

Table 2. CSF, blood and radiological examination findings (n=185)

Characteristics	HIV-positive (n=47)	HIV-negative (n=138)	p-value
Indexes of CSF inflammation			
Leukocytes / mL – median (IQR) **	18 (2-143)	95 (17-232)	0.003
% lymphocytes – median (IQR)	60 (22-90)	65 (38-87)	0.75
% neutrophils – median (IQR)	22 (0-49)	27 (10-59)	0.16
Protein in mg/dL – median (IQR)	120 (64-200)	140 (58-363)	0.27
Glucose in mg/dL – median (IQR)	36 (14-45)	26 (11-45)	0.24
CSF:blood glucose ratio – median (IQR)	0.37 (0.13-0.43)	0.23 (0.11-0.43)	0.12
Blood examination			
Leukocytes - $x10^3$/ mL – median (IQR) **	5.50 (4.30-9.15)	11.50 (8.50-14.43)	<0.01
Plasma Na^+ (mEq/L) – median (IQR) *	132 (125-138)	128 (121-134)	0.05
Radiological Examination			
Abnormal Chest X-ray – no. (%)**	23/45 (51.1%)	91/125 (72.8%)	<0.01
Infiltration – no. (%)	15	48	
Miliary TB – no. (%)	7	33	
Other – no. (%)	1	10	

No blood examination was done in three HIV-negative patients, no chest X-ray in one HIV-positive and five HIV-negative patients. CSF, cerebrospinal fluid; IQR, interquartile range.

*$p < .05$

**$p < .01$

of the HIV epidemic in Indonesia, which is mostly driven by IDU, 71.8% of HIV-infected and 4.7% of HIV-uninfected patients had antibodies against HCV.

Radiology

Chest radiograph abnormalities, mostly showing infiltrative and miliary lesions suggesting TB, were found in 67% of patients (**Table 2**). Chest radiograph abnormalities were more common among HIV-negative patients ($p<0.01$). Head CT scans were only available for 17 patients and were abnormal in 11. CT-scan abnormalities included cerebral edema, hydrocephalus and meningeal enhancement; no mass lesions were found.

Etiology

A definite cause of meningitis could be established in 102 patients (55%) (**Table 3**). *Mycobacterium tuberculosis* was detected in 86 patients, mostly confirmed by culture. *Cryptococcus* species were identified in 15 patients (8%); 14 by means of India ink and confirmed by cryptococcal antigen test and one by cryptococcal antigen test only. Pneumococcal meningitis was diagnosed in one patient, confirmed by bacterial culture. Among 83 patients (45%) without microbiological confirmation, 67 had a probable diagnosis of TBM based on duration of symptoms (median 21 days), moderate CSF leukocytosis mostly consisting of mononuclear cells (median 69%) and a high prevalence (85%) of

Table 3. Final diagnosis according to HIV-status

Diagnosis	HIV-positive (n=47)	HIV-negative (n=138)	Total population (n=185)
Tuberculous meningitis (TBM)	31 (66.0%)	122 (88.4%)	153 (82.7%)
Definite TBM	*10*	*76*	*86*
Probable TBM	*21*	*46*	*67*
Cryptococcal meningitis	14 (29.8%)	1 (0.7%)	15 (8.1%)
Definite bacterial meningitis	-	1 (0.7%)	1 (0.5%)
Probable bacterial meningitis	-	10 (7.2%)	10 (5.4%)
Unknown cause	2 (4.3%)	4 (3.6%)	6 (3.2%)

Data are presented as number of patients (percentage)

chest radiograph abnormalities typical for TB; 10 had a probable diagnosis of bacterial meningitis based on typical CSF presentation (median CSF cell count 1168/µL with 90% neutrophils) and good response to antimicrobial therapy.

No cause of meningitis could be ascertained in six patients. Syphilis serology was negative in all patients. Thirty-nine patients (25%) with TBM were admitted to the hospital with BMRC grade I, 89 (58%) with grade II and 25 (16%) with grade III. The severity (grading) of TBM was not significantly associated with bacteriological confirmation or HIV status (t-test; p=0.33 and 0.50, respectively).

Treatment

TB treatment was given to 155 patients with an initial diagnosis of TBM: as continuation of ongoing TB treatment for 18 patients and newly started for 137. Ceftriaxone was added in 12 patients; nine with pneumonia and three with suspected bacterial meningitis. The patient with definite bacterial meningitis and 10 patients with suspected bacterial meningitis were treated with ceftriaxone for 7–14 days. Ten patients with cryptococcal meningitis were treated with amphotericine-B (n=4) or oral fluconazole (n=6), whereas two patients died before initiation of treatment, and treatment was unavailable for three patients. Dexamethasone was administered to 88 of 155 patients (57%) with suspected TBM, but none exactly followed the prolonged course as recently suggested [12] for logistical reasons and toxicity (gastrointestinal bleeding). The median initial dose of dexamethasone was 20 mg (range 10–40 mg), and duration of treatment varied (range 1–14 days). Eight out of 155 patients (5%) on TB treatment developed drug-induced hepatitis, four with grade 3. In these patients, rifampicin, isoniazid and pyrazinamide were substituted by ciprofloxacin and streptomycin. Four HIV-patients were on antiretroviral therapy (ART) prior to admission, and three newly diagnosed patients started ART after at least 2 weeks of meningitis treatment. Ventriculo-peritoneal shunting was performed in three patients with hydrocephalus.

Outcome

Mortality was high and strongly associated with HIV infection. Seventy-six patients (41%) died within the first month of follow-up, mostly in the first week.

Table 4. Factors associated with one-month mortality

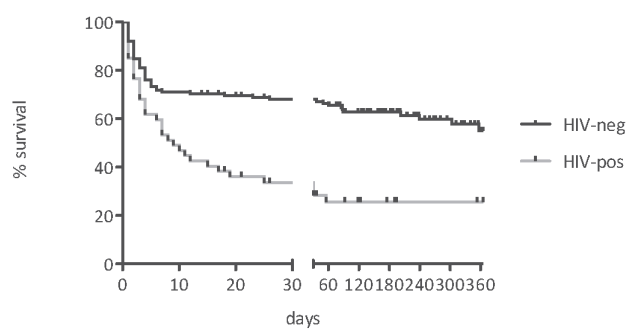
Characteristics	Dead (n=76)	Alive (n=109)	p-value	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio	
					Model without HIV	Model with HIV
HIV – positive	31 / 76 (40.8)	16 / 109 (14.7)	<0.001	4.00 (1.99-8.07)	-	12.15 (3.04-15.72)
Altered consciousness (GCS < 14)	44 / 66 (67)	35/105 (33)	<0.001	4.00 (2.08-7.69)	2.39 (0.94-6.09)	2.14 (0.77-5.95)
Fever on presentation	35/65 (48.2)	31/100 (29.1)	0.003	2.60 (1.36-4.96)	2.26 (0.94-5.43)	1.79 (0.68-4.72)
Focal neurological sign	42/73 (57.5)	49/108 (45.4)	0.108	1.63 (0.90-2.97)	1.47 (0.60-3.59)	1.66 (0.63-4.43)
CSF leukocyte count > 70/mm^3	37/75 (49.2)	56/109 (49.4)	0.785	0.92 (0.51-1.66)	1.13 (0.45-2.87)	1.25 (0.46-3.39)
Peripheral leukocyte > 10,000/ mm^3	41/70 (57.1)	49/103 (47.6)	0.155	1.56 (0.84-2.88)	1.23 (0.51-3.00)	3.84 (1.12-13.18)
Abnormal Chest X-ray	46/69 (66.7)	70/103 (68.0)	0.859	0.94 (0.49-1.81)	1.95 (0.69-5.48)	2.86 (0.90-9.08)
Plasma sodium < 136 mEq/L	35/59 (59.3)	73/99 (73.7)	0.06	0.52 (0.26-1.03)	0.43 (0.16-1.13)	0.49 (0.17-1.41)

Data are presented as no. of patients / no. evaluated (%) unless stated otherwise. CI, confidence interval; CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale.

The suspected cause of death was available in 68 patients and was neurologic in 42 (62%) and systemic in 26 patients (38%). Of the patients who had undergone ventriculoperitoneal shunting, one survived and two died after 3 and 6 weeks, respectively. Among HIV-positive patients, seven out of 10 with TBM, and eight out of 14 with cryptococcal meningitis died. In univariate analysis, HIV infection was strongly associated with 1-month mortality [odds ratio (OR) 4.00; 95% confidence interval (CI) 1.99–8.07]. The effect of HIV was similarly strong when the comparison was limited to patients with TBM (OR 4.31; 95% CI 1.85–10.00). Other factors associated with 1-month mortality were altered consciousness and fever on admission. In multivariate analysis, only HIV status remained significantly associated (**Table 4**). No significant correlation was found between CSF biochemical parameters and death.

Sixty-three out of 111 patients (57%) left the hospital with a maximal GOS score and 48 (43%) with neurologic sequelae. All surviving patients were followed (median follow-up 56 days; IQR 4–214). During this follow-up time, 17 additional patients died after a median of 60 days (range 5–438). Using t-test, the score on the GOS upon hospital discharge was associated with this late mortality (p=0.002). Using Kaplan–Meier estimates, the death rates at 6 months were 25.6% (95% CI 17.3–30.9%) for HIV-negative and 62.8% (95% CI 57.9–73.1%) for HIV-positive patients (**Figure 2**).

Figure 2. Longterm survival of HIV-positive and HIV-negative patients



DISCUSSION

In this cohort of Indonesian adults presenting with subacute meningitis, HIV infection was unexpectedly common and strongly associated with mortality. More than one-quarter of patients presented with HIV infection, which was mostly newly diagnosed. TB was the most common cause, and among HIV-positive patients, almost one-third suffered from cryptococcal meningitis. Mortality, both early and late, was more than twice as high among HIV-positive patients as compared with HIV-negative patients.

The most important finding in our study is the high HIV seroprevalence. HIV among the general population in Indonesia is still low at 0.2%, but 25% of this cohort of patients with meningitis were HIV-positive. Although HIV prevalence rates in some other Southeast Asian countries are declining, Indonesia is witnessing an accelerating growth [1]. It seems to be a matter of time before Indonesia, which has the fourth biggest population in the world, will have a more generalized HIV epidemic. Our data support the need for effective prevention and early detection of HIV in Indonesia. The low CD4 cell counts, confirming late presentation, further stresses the importance for earlier detection and timely HIV treatment.

TB was the main causative agent of meningitis. The prevalence of TBM (83%) in this series is much higher compared with studies from low-income countries. In a large cohort of adult meningitis in Vietnam, TBM was diagnosed in 40% of patients [14]. Smaller case-series in Asia and Africa found prevalence of TBM ranging from 13 to 46% [15–18]. The high prevalence of TBM in our setting was not due to the definitions we used. Bacterial confirmation of TBM in our cohort (56%) was higher than in previous studies from low-income countries whose diagnostic yields were 5–20% [4,19], though one small study from South Africa revealed a positive result in 60% of patients [20]. We believe that good flow of samples and use of large CSF samples and liquid culture contributed to our result. In our cohort, confirmation of TBM was lower in HIV-positive than in HIV-negative patients. This is different from other reports from Argentine and Vietnam, which reported a higher positivity rate among HIV positive patients [4,21]. The use of molecular

testing might further increase the number of confirmed cases [22]. The second most common pathogen was *Cryptococcus neoformans*, which accounted for almost one-third of cases among HIV patients. This finding reemphasizes the notion of cryptococcal meningitis as an important opportunistic infection among HIV patients [2]. The global burden of cryptococcal meningitis has been estimated at one million infections in 2006, with half a million deaths [23]. To our knowledge, this is the first publication of proven cryptococcal meningitis from Indonesia. Interestingly, we only confirmed one case of acute bacterial meningitis. This finding is in contrast with various reports from other Asian countries [7,14,24]. In the Vietnam cohort, 30% of its participants had bacterial meningitis, mainly caused by *Streptococcus suis* [14]. Bacterial meningitis due to *S. suis* is linked to contact with pigs or uncooked pig products [7,25], which is rare in Indonesia. Despite the difference with other studies, we believe that our findings do reflect the causative pattern of meningitis in patients who visit this referral hospital. We performed an extensive microbiological workup to all specimens, and CSF patterns and other supporting evidence (history, chest radiograph abnormalities) were consistent with TBM in the majority of patients. It could be that patients with acute bacterial meningitis may never have reached our hospital, either because they had died earlier or because they had received antibiotic treatment elsewhere. Although no viral diagnostic assays were performed, it seems unlikely that patients in this cohort suffered from viral meningoencephalitis. First, cryptococcal TB and bacterial meningitis altogether accounted for almost all cases. Second, the subacute presentation, frequent history of previous TB treatment and chest X-rays suggesting TB, the common presence of meningismus and cranial nerve palsies and the CSF patterns all point against herpes or enteroviruses as a cause of disease in this cohort.

Within 1 month, 41% of patients had died and another 9% died during further follow-up. The high mortality was strongly associated with HIV. Literature reports suggest that for cryptococcal meningitis, a low GCS score, a high fungal burden, a high CSF opening pressure and a poor inflammatory response (white blood cells <20) seem predictive of a poor prognosis [2]. For TBM, a low GCS score, advanced stage of disease on admission, and raised intracranial pressure seem predictive of a poor outcome [15,19,20]. In addition, given the high proportion of patients with a previous history of TB treatment, it is likely that at least some

patients in our cohort had multidrug resistant (MDR) TB, which strongly increased mortality in a study in Vietnam [26]. Unfortunately, drug resistance testing is not routinely available in our setting, and government regulations largely exclude the possibility of sending samples abroad. Possibly, neurosurgical intervention for hydrocephalus might have improved survival, but in our cohort, this was severely hampered by the low availability of CT scan. There are also opportunities to improve pharmacological treatment. The most effective drugs to treat cryptococcal meningitis [13] are not consistently available (amphotericine B) or not available at all (flucytosine) in Indonesia. Fluconazole prophylaxis and preemptive fluconazole therapy for those who screen positive for cryptococcal antigen may prevent meningitis and death [27,28]. With regard to TB, intravenous antituberculous drugs are unavailable, and one may question the bioavailability of oral drugs, especially when administered through a nasogastric tube in comatose patients. In addition, corticosteroid treatment for TBM was often not given in accordance with evidence from a large clinical trial [12]. Of note, so far there is not enough evidence to support the use of corticosteroids for TBM in HIV-positive patients [29]. In addition, it is yet unknown whether immediate initiation of ART can improve outcome. Obviously, earlier detection and treatment of HIV will prevent cryptococcal and TBM and possibly improve its outcome.

Our study suffers from certain limitations. CT scanning was rarely available, and decisions to perform lumbar puncture were based on clinical signs only. In addition, there may have been selection of patients as we included patients in a single (large) hospital only. However, despite these limitations, we believe we are able to make some important conclusions. HIV is unexpectedly high and associated with increased mortality, cryptococcus is common, and TB is the main cause of adult meningitis in this setting. Our study shows that all adult meningitis patients in this setting should be tested for HIV and that there is a great need for effective prevention and early HIV testing in light of the growing HIV epidemic in Indonesia. The high mortality among our patients warrants further study to explore the benefit of earlier diagnosis, possible neurosurgical interventions and better drug treatment.

ACKNOWLEDGEMENTS

This study was financially supported by the Royal Academy of Arts and Sciences (KNAW), the Netherlands, Foundation for Advancement of Tropical Research (NWO-WOTRO), and by IMPACT, a 5-year HIV program supported by the European Commission (www.impactbandung.org). RvC has a fellowship from the Netherlands Organization for Health Research and Development (ZonMw), DvB is supported by a personal grant of the Netherlands Organization for Health Research and Development (ZonMw): NWOVeni grant 2006 (916.76.023).

We thank the patients, dr. Asep Zainuddin for setting up the laboratory work, dr. Regina Loprang, dr. Mia Yasmina and dr. Laila Mahmudah (field physicians), Lies Ratnasari and Witri Indrasari (laboratory technicians), Kristianto (social worker) and the residents of the Department of Neurology, Hasan Sadikin Hospital, Bandung for their help in conducting this study. We also thank Dr. Menno de Jong (Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam), Prof. Dr. Retno Wahyu (University of Indonesia, Jakarta, Indonesia) and dr. Dede Gunawan for their support. Prof. Cissy B. Sudjana Prawira, Director of Hasan Sadikin General Hospital, is thanked for encouraging and accommodating research in her institution.

There was no conflict of interests.

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Chapter 3

Comparison of real time IS*6110*-PCR, microscopy, and culture for diagnosis of tuberculous meningitis in a cohort of adult patients in Indonesia

PLoS ONE, 2012;7(12):e52001

ABSTRACT

Background

Bacteriological confirmation of tuberculous (TB) meningitis is difficult. Culture is slow and microscopy has insufficient sensitivity. We evaluated real time PCR targeting insertion sequence IS*6110* among 230 consecutive adult patients with subacute meningitis in a referral hospital in Indonesia.

Methods

Cerebrospinal fluid (CSF) samples were examined using microscopy, solid and liquid culture, and real time IS*6110*-PCR with a fluorescence-labeled probe using DNA extracted from CSF Forty. CSF samples of non-infectious neurology patients were used as negative controls. IS*6110*-PCR results were linked with clinical and CSF characteristics.

Results

Most patients presented with subacute meningitis, after a median of 14 days of symptoms (range 7-30). After exclusion of cryptococcal and bacterial meningitis, 207 patients were classified as definite or probable TB meningitis; 17.9% with HIV infection. In this group IS*6110*-PCR gave the highest positivity rate (68%, 95% CI 62-74%) compared with microscopy of ZN-stained slides (11%, 95% CI 7-15%), and mycobacterial culture using solid (36%, 95% CI 29-42%) and liquid (44%, 95% CI 37-51%) media. IS*6110*-PCR was positive in 92% of patients with culture-positive and 42% of patients with culture-negative probable TB meningitis. Among culture-negative patients, a positive PCR was associated with a history of TB treatment, a longer duration of illness, a higher CSF cell count and protein, and a lower CSF glucose. IS*6110*-PCR was negative in all CSF samples from non-meningitis control patients.

Conclusions

Real time IS*6110*-PCR is a quick, sensitive, and specific test for diagnosing of TB meningitis in this setting. Its performance in other (less-developed) settings needs further study.

INTRODUCTION

Tuberculous (TB) meningitis is the most severe form of tuberculosis and causes substantial morbidity and mortality in adults and children [1]. Early recognition and treatment of the disease is believed to be able to reduce the burden of this disease, but this is hampered by the fact that it is often difficult to find bacteriological proof for TB meningitis. [2,3]. Microscopy of cerebrospinal fluid (CSF), although inexpensive and rapid, has a poor sensitivity, ranging from 1.9% to 20% in different series, with the exception of one study (58%) that used large volumes of CSF [3,4,5]. CSF culture, also lacks sensitivity for diagnosing TB meningitis [2,3,4,6]. Furthermore, the slow growth of *Mycobacterium tuberculosis,* that usually takes up to 4 to 6 weeks limits the role of culture in decisions regarding initiation of TB treatment [7]. Therefore, a rapid and accurate diagnostic test would greatly benefit timely and adequate management of patients with possible TB meningitis.

Nucleic acid amplification (NAA) tests seem an attractive diagnostic tool for TB meningitis because of their speed and expected high sensitivity. However, a systematic review and metaanalysis of the accuracy of NAA tests for diagnosis of TB meningitis showed that commercial NAA test had a high specificity (98%; 95% CI 97-99%) but a low sensitivity when compared with culture (56%; 95% CI 46-66%) among 14 studies combined [8]. The recently developed GeneXpert system, combining DNA extraction with a real time PCR that simultaneously detects both *M. tuberculosis* and rifampin resistance, has a lower sensitivity compared to culture, but to our knowledge only one study has reported use in suspected meningitis [9]. In that study none of 19 CSF samples were positive with GeneXpert. This method was not available in Indonesia when the study was performed.

In-house PCR for diagnosis of TB meningitis may be more sensitive, possibly due to the use of nested PCR, DNA extraction methods, or use of different molecular targets. However, the precise role of in-house PCR for TB meningitis remains uncertain. Many in-house assays have been evaluated without adequate standardization and using small groups of patients [10,11,12,13]. The present

study therefore evaluated in-house real time PCR targeting IS*6110* in CSF samples from a well-characterized cohort of 230 adult patients with suspected meningitis, making comparisons with CSF *M. tuberculosis* culture and microscopy.

METHODS

Ethics statement

Anonymized CSF samples were used from an already-existing hospital collection, collected as part of a project 'Optimization of diagnosis of meningitis', approved by the Ethical Committee of Hasan Sadikin Hospital / Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia (No. 85/FKUP-RSHS/KEPK/Kep/EC/2006). The current study made use of an already existing sample collection, no separate patient consent was asked for this study. HIV testing is done routinely after verbal informed consent for all patients with suspected meningitis in Hasan Sadikin hospital. Consent is obtained from closest relatives (husband/wife or parents) for those patients who are unstable or unconscious at time of presentation. HIV testing was done anonymously afterwards for those who had died before consent could be obtained. This study was approved by the ethical review board of Padjadjaran University / Hasan Sadikin Hospital, Bandung, Indonesia.

Setting and patients

Patients were recruited at Hasan Sadikin Hospital, top referral hospital for West Java, Indonesia, where approximately 100 adult patients present with subacute meningitis each year. A clinical diagnosis of meningitis in this setting is based on clinical findings, CSF criteria or both. Clinical criteria of meningitis included headache, fever and neck stiffness, with or without altered consciousness. CSF criteria were cell count >10 cell/mm^3, protein concentration > 45 mg/dL, or the CSF: blood glucose ratio < 0.5; either alone or in combination. For this study, definite TB meningitis was defined as CSF microscopy or culture positive for *M. tuberculosis*. Probable TB meningitis was defined as meningitis with typical CSF findings in conjunction with either: suggestive chest X-ray abnormalities; suggestive TB lymphadenitis; bacteriologically confirmed TB outside the CNS. Definite bacterial meningitis was diagnosed if bacteria were detected in the CSF,

while probable bacterial meningitis was diagnosed in patients with characteristic CSF findings without a confirmatory bacteriological result. Cryptococcal meningitis was diagnosed if either India Ink examination or cryptococcal antigen testing of CSF were positive [14]. Final diagnoses was reviewed by a panel of experts. Patients with suspected or proven bacterial meningitis are prescribed ceftriaxone with corticosteroids, those with cryptococcal meningitis amphotericin B, and those with presumed TB standard TB treatment with adjunctive corticosteroids according to national and international guidelines [15].

Clinical evaluation

All consecutive patients presenting with clinical meningitis between 2006 and 2008 were included for this study. Signs and symptoms were recorded using standardized forms, and CSF was obtained for routine examination and microbiological testing as described below. Further routine examinations included chest X-ray examination and sputum examination if indicated. Neuroradiology is not routinely done in this setting since it is not covered by the government health insurance for the poor. For this study the HIV-status was determined anonymously for those patients who had died before consent was obtained.

Routine CSF examination

Following a lumbar puncture, 5-10 mL CSF was obtained, transported to the laboratory within one hour, and divided into two tubes: one (0.5 – 1 mL) for CSF cells, protein and glucose, and one (8 – 10 mL) for microbiological testing. The second tube was then concentrated by centrifugation at 3000 x g for 10 minutes. The CSF sediment was used to prepare smears for direct examination of acid-fast bacilli after Ziehl-Neelsen (ZN) staining, bacteria (Gram) and Cryptococci (India ink). For Mycobacterial culture, the sediment was inoculated onto two slants of Ogawa egg medium and on MB/BacT alert system (Biomerieux, Durham, North Carolina, USA) according to the manufacturer's instruction. Ogawa slants were incubated at 37°C and observed twice weekly for 3 months. For culture of bacterial pathogens some sediment was inoculated on blood agar and Trypton Soy Broth (TSB), while Sabouraud plates were inoculated for growth of fungi.

Distilled water was processed in parallel with each CSF sample as a negative control. Cryptococcal antigen (CALAS; Meridian Diagnostics, Cincinnati, Ohio, USA) testing was also done on all CSF samples. At the time of our study, the GeneXpert system was not yet operational in Indonesia.

Real time PCR for *M. tuberculosis*

DNA was extracted from 200 µl of CSF sediment by using QIAmp DNA mini kit (Qiagen, USA). CSF samples were spiked with a known concentration of PhHV as an inhibition control. Each batch of extraction included CSF negative controls to rule out any contamination, with a minimum of two negative controls per-18 CSF samples. Primers and probes used for real time IS*6110*-PCR amplify fragment of IS*6110*, a repeated insertion sequence specific for *M. tuberculosis complex* [16]. The total PCR volume was 20 µl (7 µl extracted DNA in 13 µl PCR-mix). The PCR-mix consisted of Platinum Quantitative PCR Supermix-UDG (Invitrogen), 300 nM of each primer of IS*6110*, 150 nM of the FAM-labeled *M. tuberculosis* probe, 200 nM of each primer of PhHV inhibition control, and 150 nM of the Cy5-labeled PhHV inhibition control probe. PCR amplification was carried out during 2 min at 50°C for UDG activation, 10 min at 95°C and 40×15 s at 90°C, 1 min 60°C. Each run of the assay included two negative controls to rule out any contamination along the preparation of PCR-mix and sample addition, respectively. The positive control included the DNA of H37Rv with the Ct value 29 ± 1. PCR was performed in Chromo4™system real time PCR detector (Biorad laboratories, Inc.).

Adequate care was taken to prevent carry over amplicon contamination by performing the PCR in three separate rooms (clean room, extraction room, and amplification room), by using barrier tips, and also by using PCR-mix that contained Uracyl DNA-Glycosylase (UDG). CSF samples from additional 40 non-infectious neurology patients were used as negative controls. For all samples, PCR was done blinded to results of CSF microscopy and culture and clinical data.

Data analysis and statistics

After exclusion of patients with bacterial and cryptococcal meningitis, the positivity rate of microscopy for acid-fast bacilli, *M. tuberculosis* culture using solid and liquid media, and IS*6110*-PCR was expressed as percentage (%) for the remaining group. The sensitivity of IS*6110*-PCR using culture as gold standard was

expressed as percentage (95% confidence interval). Comparisons were made of CSF and clinical characteristics of patients after stratification by *M. tuberculosis* culture- and PCR-results. Continuous variables are expressed as mean (SD) if normally distributed and median (interquartile range, IQR) if not normally distributed, and categorical variables as percentage. Differences between groups were compared using Chi-square test for proportions and Mann-Whitney U test for continuous variables, with p-values <0.05 considered statistically significant. Statistical analysis was done by SPSS version 16.

RESULTS

A total of 230 consecutive patients with suspected meningitis were included. Patients were mostly male (60%), with a median age of 30 years (range 24-36 years), presenting after a median of 14 days (range 7-30 days) of symptoms, mostly with headache (67%) and altered consciousness (31.3%) as chief complaints. On examination, nuchal rigidity (77.2%), lowered consciousness (52.6%), and focal neurological signs were common. HIV infection was present in 22.2%; one patient was not tested.

Based on CSF culture and microscopy, subjects were classified into four groups (**Figure 1**). TB meningitis was diagnosed in 207 patients (90%), cryptococcal meningitis in 13 (5.6%), bacterial meningitis in 7 (3%), and meningitis was excluded in 3 patients (0.1%). Among 207 patients with suspected TB meningitis, a definite diagnosis could be established in 105 patients (50.7%) based on culture and microscopy (n=102) and microscopy alone (n=3). We could classify 102 patients (49.3%) as probable TB meningitis. 17.9% of patients with TB meningitis were HIV-infected.

IS*6110*-PCR assay was positive in 143 patients, 140 patients with TB meningitis, two patients with confirmed cryptococcal meningitis, and one patient with suspected bacterial meningitis. For the patient with suspected bacterial meningitis, TB was also confirmed by direct spoligotyping of the CSF sample for *M. tuberculosis,* as described elsewhere [17]. None of the 40 patients in the control group had a positive IS*6110*-PCR result.

Figure 1. Diagnosis among 230 meningitis suspects based on clinical characteristics and CSF microscopy and culture.

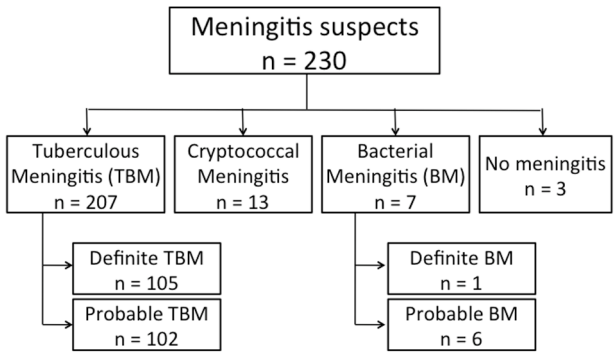
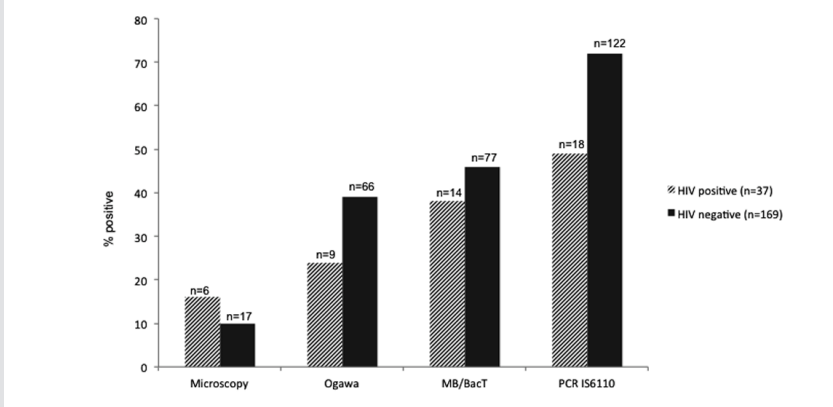


Figure 2 shows the comparison of diagnostic modalities for TB meningitis. The diagnostic yield of IS*6110*-PCR was much higher (68%, 95% CI 62-74%) compared to solid culture (36%, 95% CI 29-42%; p<0.001) and microscopy (11%, 95% CI 7-15%; p<0.001), and higher than liquid culture (44%, 95% CI 37-51%) although this was not statistically significant (p=0.262). As shown in **Figure 2**, this was true for HIV-infected and -non-infected patients although the positivity yield of IS*6110*-PCR was lower among HIV-infected patients (49%, 95% CI 33-65% vs 73%, 95% CI 66-80%; p=0.004). Larger CSF samples were more often PCR-positive; 50% of samples < 4 mL (n=22) were positive, compared with 71% of samples > 4 mL (n=181, p=0.05).

M. tuberculosis culture (either solid or liquid) was positive in 102 patients, and IS*6110*-PCR in 140 patients (**Table 1**). Using culture as the gold standard, IS*6110*-PCR had a sensitivity of 92% (95%CI 87-97%), compared with 21% (95% CI 13-29%) for microscopy. Among 46 culture-negative PCR-positive patients, 43 were clinically diagnosed as probable TB meningitis. For all of those patients there was supporting evidence for a diagnosis of TB: 17 patients had confirmed TB outside the CNS; 13 were cured after TB treatment; and 13 had been taking TB

Figure 2. Positivity rate of different diagnostic tests for TB among 207 meningitis patients.



Patients with cryptococcal (n=13), bacterial (n=7), and no meningitis (n=3) were excluded, HIV-status was unknown for one patient. ZN staining was used for microscopy, Ogawa for solid culture, and MB/BacT system for liquid culture. Differences between IS*6110*-PCR and solid culture (p<0.001) and microscopy (p<0.001) were statistically significant.

Table 1. Comparison of *M. tuberculosis* culture and IS*6110*-PCR results among 207 TB meningitis patients

		Culture		Total
		Positive	Negative	
IS*6110*-PCR	Positive	94 (45%)	46 (22%)	140 (67%)
	Negative	8 (4%)	59 (29%)	67 (33%)
	Total	102 (49%)	105 (51%)	207

drugs for some days before lumbar puncture, while it is known that CSF cultures quickly become negative after start of TB treatment.

Among patients with a negative *M. tuberculosis* culture, positive IS*6110*-PCR was associated with a lower CSF glucose, and a higher CSF cell count and protein, a longer duration of illness, and more frequent history of previous TB treatment (**Table 2**). Clinical features of this group of patient were in accordance with that of TB meningitis. On presentation 44% had decreased level of consciousness, 28% had fever, 86% had neck stiffness, 32% had motor deficit, and 22% had cranial nerve palsies. 14% of these patients were HIV-positive. Among 21 HIV-infected patients with negative *M. tuberculosis* culture, similar differences were found, although none were significant.

Table 2. CSF and clinical characteristics of 166 HIV-negative patients with suspected TB-meningitis according to culture and PCR-result

	Culture positive (n=88)	Culture negative (n=78)		
		PCR positive (n=39)	PCR negative (n=39)	p-value
Duration of illness (days) – median (IQR)	14 (7-30)	14 (7-21)	8 (6-14)	.104
History of TB – no. (%)	13/86 (15.1)	12/38 (31.6)	5/36 (13.9)	.068
CSF				
Cells/mL – median (IQR)	136 (47-262)	66.5 (7-121)	11 (0.8-334.8)	.302
PMN % – median (IQR)	37 (20-62)	13 (0.5-52)	10 (0-44)	.750
MN % – median (IQR)	63 (38-80)	72 (41-95.5)	72 (30-95)	.423
Protein (mg/dL) – median (IQR)	160 (63-370)	125 (40-342.5)	55 (40-412.5)	.620
Glucose (mg/dL) – median (IQR)	16 (9.8-32.0)	36.5 (18.8-48.8)	48 (37-60.8)	.026
CSF:blood glucose ratio – median (IQR)	0.13 (0.09-0.24)	0.34 (0.17-0.46)	0.46 (0.34-0.56)	.017

Three culture negative / PCR positive patients were not included because microscopy was positive.
PMN=polymorphonuclear cell, MN=mononuclear cell, IQR=interquartile range

DISCUSSION

In this study real time IS*6110*-targeted PCR had a high positivity rate (68%) among patients with suspected TB meningitis, higher than *M. tuberculosis* microscopy (11%), and *M. tuberculosis* culture using solid (36%) and liquid (44%) media, both

among HIV-infected and non-infected subjects. CSF characteristics and clinical data of culture-negative/PCR-positive patients were in line with a diagnosis of TB meningitis, and specificity of the IS*6110*-PCR was 100% in a control group of patients with alternative diagnoses.

Untreated TB meningitis is almost uniformly lethal and delay in treatment is associated with increased mortality and morbidity. Unfortunately, CSF microscopy has poor sensitivity, while *M. tuberculosis* culture is slow and therefore has a limited role in decisions about treatment of possible TB meningitis. *M. tuberculosis* PCR has the potential to be the ideal tool for rapid diagnosis of TB meningitis. Unfortunately, commercial PCR tests from Roche, Abbott, and Genprobe before 2003 only have a moderate sensitivity for diagnosis of TB meningitis, as summarized in a systematic review [8]. Some studies have reported higher sensitivity for in-house PCR assays, but their role in diagnosis of TB meningitis remains uncertain. In the present study we evaluated in-house real time IS*6110*-PCR in CSF samples from a well characterised cohort of 230 meningitis patients, larger than previous series [18,19]. The high prevalence of TB meningitis in our cohort was confirmed bacteriologically in the majority of patients, more than in most published case series [18,20].

In-house IS*6110*-PCR had a higher positivity rate (68%) in our cohort compared to many other case series evaluating in-house PCR, some using IS*6110* as a target [6,7,21,22,23]. This may have been due to the use of large volume CSF samples (5-10 mL), as was nicely shown in an earlier study [5], and IS*6110*, which has multiple copies present in the genome of *M. tuberculosis* complex, as the PCR target [16]. We used PhHV as an inhibition control in each sample to rule out the possibility of PCR inhibition with a false negative PCR result. The positivity rate of IS*6110*-PCR in our study was lower among HIV-infected patients, in line with a study from India [24], and possibly due to the fact that cerebral toxoplasmosis may mimic TB meningitis in the absence of neruradiology, as we have recently shown [25], underlining the need for more extensive microbiological testing in HIV-infected patients. Compared with culture, IS*6110*-PCR had a sensitivity of 92%, in line with some earlier previous studies [3,21,26] but superior compared to some other studies which reported sensitivity rates of 32-75% [7,12,22,23,27,28].

Two patients with cryptococcal meningitis, and one with bacterial meningitis had a positive IS*6110*-PCR result. Mixed infections of mycobacterial with cryptococcal meningitis [25,29] and bacterial meningitis have been reported previously [24,30]. The presence of *M. tuberculosis* DNA in our patient with bacterial meningitis was confirmed by *M. tuberculosis* spoligotyping.

The detection of IS*6110* PCR in culture-negative patients (n=46) was often (40%) supported by TB outside the CNS or a good response to TB treatment. In addition, some patients had been taking TB drugs, which is known to sterilize CSF cultures within days [31]. The higher positivity of IS*6110*-PCR compared to culture (although not statistically significant compared with liquid culture) in these patients may have been due to amplification of DNA from non-viable organisms. Further support for the specificity of IS*6110*-PCR was derived from a closer examination of the group of culture-negative patients. Those with positive PCR results usually had a lower CSF glucose, raised protein level, and more pronounced pleiocytosis. A previous history of TB was more common in patients with a positive IS*6110*-PCR result (33% vs 14%), and duration of illness was typically longer (median 14 days vs 7 days). Obviously, the sensitivity of this assay will be lower in areas with a substantial prevalence of *M. tuberculosis* strains with no or only a few IS*6110* copies. However, this seems to be relatively rare, and multiple copies of IS*6110* were present in all strains from a previous study we conducted in Indonesia [32].

The positive IS*6110*-PCR results obtained from those patients could not be due to amplicon contamination as physical separation rooms were used for sample processing, PCR setup, amplification process, carefull handling procedure, the use of barrier tips, dUTP-uracil glycosylase system, and unambiguous negative results from our negative IS*6110*-PCR controls. Real time PCR allows direct observation of amplicon reaction without the need to open PCR tubes, thereby avoiding the possibility of amplicon contamination. Finally, none of the patients in the control group with non-infectious CNS disease had a positive IS*6110*-PCR result.

The strength of our study is the validation of in-house IS*6110*-PCR in a large group of TB meningitis patients, much larger than most previously published series. Other strenghts include the use of comparative *M. tuberculosis* culture

(solid and liquid) and microscopy, a work-up for other pathogens, and the fact that laboratory results were linked with clinical data. Unfortunately no direct comparison could be made with a commercial PCR. Despite this limitation, we can conclude that in-house PCR targetting IS*6110* is a very sensitive and specific method for diagnosis of TB meningitis, which may speed up diagnosis and timely treatment of this deadly disease. It should be noted that this assay, evaluated in a single highly-qualified laboratory, may not perform equally well in other settings.

ACKNOWLEDGEMENT

We would like to thank Dr. Bayu Wahyudi, Director of Hasan Sadikin hospital, and Prof. Tri Hanggono Achmad, Dean of the Faculty of Medicine, Universitas Padjadjaran, for encouraging and accommodating research at their institutions.

Part of these data has been presented as an oral presentation at the 42nd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (OP-930-28).

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Chapter 4

Cerebral toxoplasmosis mimicking subacute meningitis in HIV-infected patients: a cohort study from Indonesia

PLoS Neglected Tropical Disease, 2013; 7(1): e1994.

ABSTRACT

Background

HIV-associated subacute meningitis is mostly caused by tuberculosis or cryptococcosis, but often no etiology can be established. In the absence of CT or MRI of the brain, toxoplasmosis is generally not considered as part of the differential diagnosis.

Methodology / principal findings

We performed cerebrospinal fluid real time PCR and serological testing for *Toxoplasma gondii* in archived samples from a well-characterized cohort of 64 HIV-infected patients presenting with subacute meningitis in a referral hospital in Indonesia. Neuroradiology was only available for 6 patients.

At time of presentation, patients mostly had newly diagnosed and advanced HIV infection (median CD4 cell count 22/µl), with only 17.2% taking ART, and 9.4% PJP-prophylaxis. CSF PCR for *T. gondii* was positive in 21 patients (32.8%). Circulating toxoplasma IgG was present in 77.2% of patients tested, including all in whom the PCR of CSF was positive for *T. gondii.* Clinically, in the absence of neuroradiology, toxoplasmosis was difficult to distinguish from tuberculosis or cryptococcal meningitis, although CSF abnormalities were less pronounced. Mortality among patients with a positive CSF *T. gondii* PCR was 81%, 2.16-fold higher (95% confidence interval 1.04 – 4.47) compared to those with a negative PCR.

Conclusions / significance

Toxoplasmosis should be considered in HIV-infected patients with clinically suspected subacute meningitis in settings where neuroradiology is not available.

INTRODUCTION

In settings of Africa and Asia, the most common cause of subacute meningitis in patients with advanced HIV infection is either tuberculous or cryptococcal infection [1,2]. However, in many patients, the etiology of subacute meningitis cannot be established [1,3]. In line with a large retrospective cohort of adult meningitis patients in South Africa, where 52.8% had no definite diagnosis despite extensive microbiological testing [1], we could not identify the causative pathogen in 48.9% of HIV-infected meningitis patients in an Indonesian setting [4].

Toxoplasmosis is a common and serious central nervous system (CNS) infection in patients with advanced HIV infection [5-8], although its incidence has decreased with introduction of antiretroviral treatment (ART) [6,9]. Cerebral toxoplasmosis mostly presents as cerebral mass lesions with headache, confusion, fever, lethargy, seizures, cranial nerve palsies, psychomotor changes, hemiparesis and/or ataxia [10]. Some of these symptoms may also mimic meningitis, but cerebral toxoplasmosis is generally not considered as a differential diagnosis of subacute meningitis in HIV-infected patients. This is especially the case in low-resource settings where no CT or MRI can be performed. We have therefore examined if toxoplasmosis can be diagnosed in HIV-infected patients presenting with subacute meningitis of unknown origin in Indonesia, using cerebrospinal fluid (CSF) PCR for *T. gondii*.

METHODS

Ethics statement

Anonymized CSF and blood samples were used from an already-existing hospital collection, from a cohort of patients collected as part of a project 'Optimization of diagnosis of meningitis', approved by the Ethical Committee of Hasan Sadikin Hospital/Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia (No. 85/FKUP-RSHS/KEPK/Kep/EC/2006). As this study was done using already existing sample collection, no separate consent was asked for this study. HIV testing is done routinely with oral informed consent for all patients with suspected meningitis in Hasan Sadikin hospital, after 24% were found HIV-positive in

a previous cohort study of 185 patients in the same hospital [4]. Consent is obtained from closest relatives (husband/wife or parents) for those patients who are unstable or unconscious at time of presentation. With approval from the ethical committee HIV testing was done anonymously afterwards for those who had died before consent could be obtained.

Setting and patients

We included adult patients presenting with suspected meningitis at Hasan Sadikin Hospital, the top referral hospital for West Java Province, Indonesia, between December 2006 and October 2010. Clinical data including outcome was recorded in individual case report form. Definite TB meningitis was diagnosed if CSF culture or real time PCR were positive for *M. tuberculosis*, cryptococcal meningitis if either CSF India Ink examination or cryptococcal antigen testing were positive, and toxoplasmosis if CSF *T. gondii* PCR was positive. HIV testing is done routinely for patients presenting at this hospital, but cerebral CT-scanning is rarely done in this setting and is not covered by the government health insurance for the poor.

Laboratory examinations

CSF cell count and differentiation, protein and glucose were measured. CSF microscopy was done for cryptococci, acid-fast bacilli and bacterial pathogens. CSF was cultured for *Mycobacterium tuberculosis* (solid Ogawa and liquid MB-BacT, Biomerieux), bacterial pathogens (blood agar, chocolate agar, and brain-heart infusion) and fungi (Sabouraud). Cryptococcal antigen (CALAS, Meridian Diagnostics) testing was done on CSF samples following the manufacturer's instructions. Five to 7 mL CSF samples were used for molecular testing. After centrifugation of CSF samples at 3000 x g for 10 minutes, DNA was extracted from 200 µl of CSF sediment by using QIAmp DNA mini kit (Qiagen, USA). CSF *M. tuberculosis* real time PCR was done using *IS6110*, a repeated insertion sequence specific for *M. tuberculosis,* as a target [11]. Measurement of CD4-cell count for HIV-patients only became available during the time of the study and was measured only for those who survived for more than 4 days. Real time PCR for *T. gondii*, using the multicopy B1 gene of the *T. gondii* as the target as described elsewhere [12], was performed to archived CSF samples at Radboud University Nijmegen

Medical Centre. CSF specimens from 22 HIV-negative meningitis patients (16 with definite TB meningitis, 2 with bacterial meningitis, and 4 with no definite diagnosis), and nine patients with non-infectious CNS diseases, all recruited at Hasan Sadikin Hospital, were used as controls for *T. gondii* PCR. These samples were collected during the study period over a similar time scale compared to the case CSF samples. Toxoplasma immunoglobuline G (toxoplasma IgG) were measured by electro chemiluminescent assay (ECLIA, Elecsys, Roche) in archived serum samples of patients included in the study.

Data analysis and statistics

Characteristics of patients with definite tuberculosis, cryptococcosis and toxoplasmosis were compared using Chi-square test for proportions and Mann-Whitney U test for continuous variables. Progression to death using 2-month mortality data was examined by Kaplan–Meier estimates.

RESULTS

During the period, 401 patients presented with clinical meningitis, 76 were diagnosed with HIV infection, and 64 had archived CSF samples and were included in this study. Patients included in the study presented after a median 7 days, with meningismus (86.0%), headache (80.8%), lowered consciousness (33.3%), fever (28.8%), hemi- or tetraparesis (28.6%), cranial nerve palsies (12.5%), and seizures (10.9%). HIV was newly diagnosed in 53 patients (82.8%). All 11 patients previously diagnosed with HIV were taking ART, and 6 were using co-trimoxazole as *Pneumocystis jiroveci* (PJP) prophylaxis at time of presentation. The median CD4 cell count was 22/µl, and less than 200/µl in 22 out of 23 patients tested (96%).

CSF *T. gondii* PCR was positive in 21 of 64 HIV-infected patients (32.8%), with a median Ct-value of 36.0 (IQR: 34.2-39.3). None of the 22 HIV-negative control and 9 non-infectious CNS disease patients had a positive *T. gondii* PCR. Archived serum sample was not available in 14 patients. Toxoplasma IgG was positive in 78% of patients tested, including all patients with positive CSF *T. gondii* PCR. Toxoplasma IgG titers were higher among patients with a positive CSF *T. gondii* PCR (p=.017).

A definite diagnosis of TB meningitis was established in 21/64 patients (32.8%). Out of 21 patients with positive *T. gondii* PCR, five had combined tuberculosis and toxoplasmosis. Cryptococcosis was diagnosed in 15/64 patients (23.4%), including two who were also diagnosed with tuberculosis. In 14 patients (21.9%) no causative pathogen was isolated.

Neck stiffness, headache and fever, the classical signs of meningitis, were equally common in patients diagnosed with toxoplasmosis, cryptococcosis and tuberculosis, as were most other signs and symptoms, except hemiparesis (**Table 1**). None of the patients with toxoplasmosis had received ART or co-trimoxazole prophylaxis prior to admission with meningitis. CT scans were available for 6 patients, including 4 with a positive *T. gondii* PCR. Three showed signs of hydrocephalus, one a hypodense lesion that showed no enhancement using contrast, and two were normal. No mass lesions typical for cerebral toxoplasmosis were seen.

CSF cell count and protein were normal or mildly elevated in patients with toxoplasmosis, and hypoglycorrhachia was less common compared with tuberculosis or cryptococcosis (**Table 1**). CD4 counts, missing in two-thirds of patients due to early death or the unavailability of CD4 cell testing during the initial phase of the cohort study, were low in all but one patient. **Table 2** lists the CSF findings of individual patients, **Figure 1** is a graphic representation of the CSF cell count, protein and glucose ratio, showing the overlap in CSF findings between patients with toxoplasmosis, cryptococcal and tuberculosis CNS infection.

Patients with confirmed cryptococcosis received amphotericine B, followed by fluconazole; all others received empiric tuberculosis treatment combined with adjunctive corticosteroids [13]. No toxoplasmosis treatment was given, as *T. gondii* PCR was performed retrospectively and was not available at time of presentation. Eight patients were lost to follow up and were not included in Kaplan Meier analysis. Mortality among those with positive CSF *T. gondii* PCR was 2.16-fold (95% CI 1.04 – 4.47) higher compared to those who had a negative PCR result; median survival was 7 days for toxoplasmosis, 7 days for tuberculosis meningitis, 110 days for cryptococcosis, and 32 days for patients with an unknown cause of meningitis (**Figure 2**).

Table 1. Characteristics of patients according to microbiological diagnosis

	Tuberculosis (n=14)	Cryptococcosis (n=13)	Toxoplasmosis (n=16)
General			
Age in year - median (IQR)	30 (27-34)	30 (27-32)	31 (28-33)
Male sex - no. (%)	11 (78.6)	11 (84.6)	11 (68.8)
History of TB treatment	3 (21.4)	3 (23.1)	4 (25.0)
Duration of illness, days – median (IQR)	14 (6-28)	7 (2-14)	7 (4-12)
ART prior to admission – no. (%)	2 (14.3)	4 (30.8)	1 (6.3)
PJP-prophylaxis	0	3 (23.1)	0
Signs and symptoms on presentation – no. (%)			
Headache	10/12 (83.3)	10/11 (90.9)	10/12 (83.3)
Seizure	0/13 (0.0)	2/11 (18.2)	2/13 (15.4)
Neck stiffness	12/13 (92.3)	9/11 (81.8)	13/16 (81.3)
Body temperature ≥ 38°C	4/11 (36.4)	1/8 (12.5)	4/13 (30.8)
Altered consciousness (GCS < 14)	6/10 (60.0)	2/10 (20.0)	4/10 (40.0)
GCS – median (range)	13 (6-15)	15 (9-15)	14 (12-15)
Hemi- or tetraparesis	8 (57.1)	0/12 (0.0)	6 (37.5)
Cranial nerve palsy	1 (7.1)	2 (15.4)	3 (18.8)
CSF			
Leukocytes / mL – median (IQR) *	76 (6-272)	46 (24-188)	14 (2-26)
% lymphocytes – median (IQR)	49 (16-70)	70 (58-96)	70 (50-100)
Protein, g/dL – median (IQR)	140 (87-235)	151 (105-245)	130 (90-300)
CSF:blood glucose ratio – median (IQR) **	0.13 (0.06-0.43)	0.20 (0.09-0.41)	0.40 (0.33-0.53)
Blood examination			
CD4 cell count – range ***	2-98	4-45	9-30
Titer of toxo-IgG > 1:300	5/9 (55%)	3/10 (30%)	11/11 (100%)
Radiological Examination			
Abnormal Chest X-ray	8/11 (66.7)	4/9 (44.4%)	5/10 (50.0%)

Patients with combined TB-toxoplasmosis (n=5), combined TB-Cryptococcus (n=2), and no definite diagnosis (n=14) were excluded from the table. Data are presented as no. of patients / no. evaluated (%) unless stated otherwise.

GCS = Glasgow Coma Scale. *significantly different: TB vs. toxoplasmosis (.01); cryptococcosis vs. toxoplasmosis (.02). **significantly different: TB vs. toxoplasmosis (p=.03), cryptococcosis vs. toxoplasmosis (.02). ***CD4 cell counts were only available for 16 patients with definite diagnoses.

Table 2. Line list of all patients

No	Age	Sex	CSF examination*			Causative pathogen			Diagnosis
			Cell number	Protein	Glucose ratio	Toxo[φ]	TB[ε]	Crypto[ω]	
1	31	M	4	26	0.33	pos	neg	neg	toxoplasmosis
2	30	M	17	100	0.35	pos	neg	neg	toxoplasmosis
3	33	M	18	160	—	pos	neg	neg	toxoplasmosis
4	18	F	11	150	—	pos	neg	neg	toxoplasmosis
5	29	F	2	90	0.32	pos	neg	neg	toxoplasmosis
6	24	F	1	300	0.54	pos	neg	neg	toxoplasmosis
7	28	M	13	630	0.4	pos	neg	neg	toxoplasmosis
8	19	F	22	310	0.33	pos	neg	neg	toxoplasmosis
9	30	M	49	130	0.83	pos	neg	neg	toxoplasmosis
10	36	M	1	60	0.58	pos	neg	neg	toxoplasmosis
11	33	M	30	434	0.32	pos	neg	neg	toxoplasmosis
12	35	F	14	95	0.52	pos	neg	neg	toxoplasmosis
13	32	M	26	131	0.5	pos	neg	neg	toxoplasmosis
14	30	M	180	100	0.43	pos	neg	neg	toxoplasmosis
15	34	M	0	50	0.38	pos	neg	neg	toxoplasmosis
16	32	M	—	—	—	pos	neg	neg	toxoplasmosis
17	25	F	143	100	0.13	neg	pos	neg	TB
18	31	M	696	39	0.07	neg	pos	neg	TB
19	30	M	7	260	0.06	neg	pos	neg	TB
20	21	F	105	700	0.05	neg	pos	neg	TB
21	52	M	193	190	0.55	neg	pos	neg	TB
22	29	M	1120	—	0.03	neg	pos	neg	TB
23	32	M	104	160	0.04	neg	pos	neg	TB
24	30	M	48	40	0.24	neg	pos	neg	TB
25	28	M	507	6980	0.1	neg	pos	neg	TB
26	23	F	0	73	0.4	neg	pos	neg	TB
27	31	M	26	140	—	neg	pos	neg	TB
28	40	M	4	112	0.47	neg	pos	neg	TB
29	56	M	0	100	0.4	neg	pos	neg	TB
30	29	M	10	210	—	neg	pos	neg	TB
31	31	M	109	120	0.64	pos	pos	neg	TB-toxo
32	39	M	919	630	0.04	pos	pos	neg	TB-toxo

Table 2. (continued)

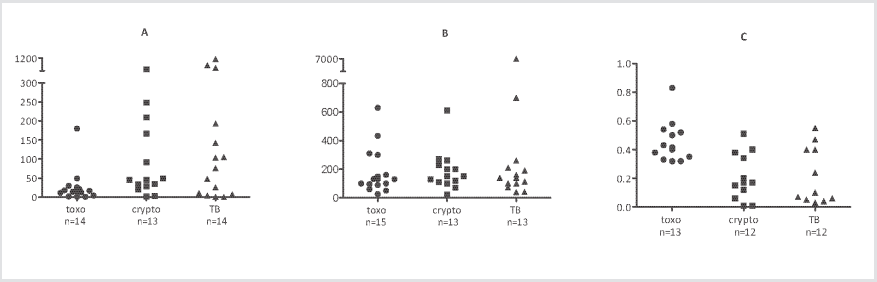
33	22	M	13	10	0.25	pos	pos	neg	TB-toxo
34	31	M	0	200	0.43	pos	pos	neg	TB-toxo
35	23	F	23	64	0.46	pos	pos	neg	TB-toxo
36	27	M	34	200	0.43	neg	neg	pos	Cryptococcosis
37	30	M	2	22	0.01	neg	neg	pos	Cryptococcosis
38	27	M	419	110	0.06	neg	neg	pos	Cryptococcosis
39	30	M	167	120	0.2	neg	neg	pos	Cryptococcosis
40	38	M	20	230	0.12	neg	neg	pos	Cryptococcosis
41	32	M	209	610	0.01	neg	neg	pos	Cryptococcosis
42	25	F	28	70	0.4	neg	neg	pos	Cryptococcosis
43	32	M	49	100	0.15	neg	neg	pos	Cryptococcosis
44	36	M	46	270	0.51	neg	neg	pos	Cryptococcosis
45	29	M	3	200	0.17	neg	neg	pos	Cryptococcosis
46	22	F	35	151	0.38	neg	neg	pos	Cryptococcosis
47	32	M	248	130	—	neg	neg	pos	Cryptococcosis
48	32	M	92	260	0.34	neg	neg	pos	Cryptococcosis
49	27	M	0	—	0.16	neg	pos	pos	TB-crypto
50	34	M	0	120	0.27	neg	pos	pos	TB-crypto
51	30	F	0	44	0.39	neg	neg	neg	unknown
52	27	M	0	180	0.49	neg	neg	neg	Unknown
53	33	M	259	110	0.57	neg	neg	neg	Unknown
54	32	M	2	130	0.48	neg	neg	neg	Unknown
55	30	M	—	—	—	neg	neg	neg	unknown
56	28	M	143	9850	0.04	neg	neg	neg	unknown
57	30	M	146	40	0.19	neg	neg	neg	unknown
58	36	M	17	16	0.51	neg	neg	neg	unknown
59	24	M	0	30	0.6	neg	neg	neg	unknown
60	29	M	43	44	0.21	neg	neg	neg	unknown
61	39	M	18	na	0.24	neg	neg	neg	unknown
62	32	M	184	480	0.08	neg	—	neg	unknown
63	28	M	298	317	0.34	neg	neg	neg	unknown
64	34	M	166	133	—	neg	—	neg	unknown

M = male; F = female; neg = negative; pos = positive

*cell numbers are in cells/µl; protein in mg/dL; glucose ratio = CSF glucose : blood glucose

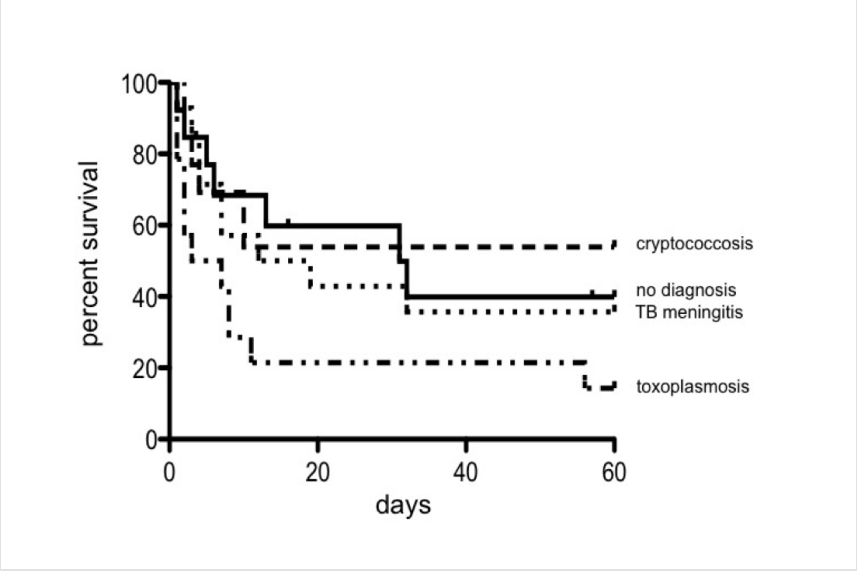
[φ] toxoplasmosis; [ε] TB meningitis; [υ]cryptococcosis

Figure 1. CSF characteristics according to causative pathogen



CSF cell count (A), protein concentration (B) and CSF:blood glucose ratio (C) for cases with confirmed toxoplasmosis (●), crypotoccococis (■), and tuberculosis (▲)
Toxo = toxoplasmosis; crypto = cryptococcosis; TB = TB meningitis

Figure 2. Kaplan Meier survival estimates.



Patients with available long term follow up data: Toxoplasmosis (n=14), Cryptococcosis (n=13), TB meningitis (n=14), no diagnosis (n=13)

DISCUSSION

In our cohort of HIV-infected patients presenting with clinical signs and symptoms of CNS infection, CSF *T. gondii* PCR was positive in 32.8% of patients, sometimes in conjunction with tuberculosis. In the absence of CT or MRI of the brain, toxoplasmosis could not be distinguished from tuberculosis or cryptococcosis. Mortality in this cohort of newly diagnosed and advanced HIV infection was extremely high and associated with a positive *T. gondii* PCR.

Cerebral toxoplasmosis typically causes space occupying lesion(s), leading to subacute or acutely developing confusion, with or without focal neurological deficits [14]. In the absence of CT or MRI of the brain, common findings like headache, fever, hemiparesis and decreased level of consciousness [10] may mimic those of meningitis [4, 15-17]. In previous series of cerebral toxoplasmosis [18,19], meningeal signs have been reported in 3 to 16 % of the patients, although in many reports neck stiffness is not mentioned [14]. Although rare, cases of spinal cord toxoplasmosis have also been reported [20]. No typical mass lesions were found in 6 patients with an available CT scan. This is not surprising, as this study depended on the availability of CSF samples, that would not have been obtained if typical mass lesions had been found.

We used *T. gondii* PCR for diagnosis of cerebral toxoplasmosis. In previous studies CSF *T. gondii* PCR had a sensitivity of 50-60% to confirm cerebral toxoplasmosis in HIV-infected patients [21, 22]. The sensitivity is possibly higher among patients with meningoencephalitis compared to those with space-occupying lesions only, but this has not been examined. Specificity of *T. gondii* PCR is high, between 97 and 100% [22-24]. The positivity rate of 32.8% in our study might therefore be an underestimate, especially in the category of patients in whom no other pathogen was isolated despite extensive microbiological testing.

In our cohort, toxoplasmosis could not be distinguished clinically from tuberculosis and cryptococcosis. From our previous series [4], CSF samples were available for the current study for 36 / 47 HIV-infected patients. Ten out of 17 patients who were diagnosed with 'probable TB meningitis' and 'unknown'

in the previous study were found to have a positive *T. gondii* PCR (and no bacteriological confirmation of tuberculosis) in the current study. Diagnosis of cerebral toxoplasmosis is usually based on clinical findings and CT or MRI of the brain. However, if cerebral imaging is lacking, toxoplasmosis may not be considered. Positive toxoplasma serology, which has a high sensitivity but very poor specificity, is helpful to exclude but not to confirm cerebral toxoplasmosis, although some reports suggest that high toxoplasma IgG titers are only found in patients with symptomatic toxoplasmosis [25]. Indeed, in our study, patients with a positive *T. gondii* PCR had a higher IgG titers compared to those who had a negative PCR. An autopsy study from India provides further support for the notion that cerebral toxoplasmosis is not always considered; among 233 HIV patients, toxoplasmosis accounted for 6.8% of deaths, but in none of these cases toxoplasmosis had been suspected clinically [26].

The incidence of cerebral toxoplasmosis varies between countries [14] and is related to the seroprevalence of toxoplasmosis in the general population [19, 25]. In the United States, toxoplasma seroprevalence varies from 3% to 30%, whereas in France 73%–90% of the population is infected [10]. Reported seroprevalence rates were varied from 13-31% in the general population, and 45-68% in HIV patients in studies from several developing countries [8, 27, 28]. In our study, 78% of patients had detectable toxoplasma IgG, but this does not reflect the seroprevalence in the general population or among unselected HIV-infected patients, as only meningitis patients were examined.

Mortality in this cohort of patients was very high, higher compared to reported rates in other series [8, 9, 29]. One explanation is that patients mostly presented with advanced and untreated HIV infection. In addition, no toxoplasmosis treatment was provided, as PCR was done retrospectively on archived samples. In our previous study, HIV infection was associated with a 2.5-fold increased mortality among patients presenting with meningitis [4]. Data from the current study suggests that this may is at least in part attributable to a high prevalence of (unrecognized and untreated) toxoplasmosis. Future studies should examine the benefit of timely diagnosis and/or empiric treatment of toxoplasmosis for patients in settings like ours. Empiric treatment for subacute meningitis in HIV-

infected patients should probably also include tuberculosis, which is difficult to exclude as culture is slow and microscopy and commercial PCR assays have insufficient sensitivity [30].

Our study suffers from several limitations. Most importantly, no CT or MRI of the brain could be performed. In addition, clinical data, CD4 cell counts and other laboratory parameters were missing in a number of patients. Despite these limitations the data strongly suggest that toxoplasmosis should be included in the differential diagnosis of HIV-infected with clinically suspected subacute meningitis, and that molecular testing or empiric treatment for toxoplasmosis should be considered in these patients, especially if no CT or MRI can be performed. Obviously, timely diagnosis and treatment of HIV will help prevent this severe opportunistic infection.

ACKNOWLEDGEMENTS

We thank the patients, dr. Regina Loprang, dr. Ela Hayati, and dr. Christian Budiman (field physicians), Lies Ratnasari and Witri Indrasari (laboratory technicians), Kristianto (social worker), and the residents of the Department of Neurology, Hasan Sadikin Hospital, Bandung for their help in conducting this study.

Part of these data have been presented at the 43rd World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease 2012 (*abstract number: 0P-212-17*).

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Chapter 5

Intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis: an open-label, randomized controlled phase 2 trial

Lancet Infectious Diseases, 2013;13(1):27-32

SUMMARY

Background

Intensified antibiotic treatment might improve the outcome of tuberculous meningitis. We assessed pharmacokinetics, safety, and survival benefit of several treatment regimens containing high-dose rifampicin and moxifloxacin in patients with tuberculous meningitis in a hospital setting.

Methods

In an open-label, phase 2 trial with a factorial design in one hospital in Indonesia, patients (aged >14 years) with tuberculous meningitis were randomly assigned to receive, according to a computer-generated schedule, first rifampicin standard dose (450 mg, about 10 mg/kg) orally or high dose (600 mg, about 13 mg/kg) intravenously, and second oral moxifloxacin 400 mg, moxifloxacin 800 mg, or ethambutol 750 mg once daily. All patients were given standard dose isoniazid, pyrazinamide, and adjunctive corticosteroids. After 14 days of treatment all patients continued with standard treatment for tuberculosis. Endpoints included pharmacokinetic analyses of the blood and cerebrospinal fluid, adverse events attributable to tuberculosis treatment, and survival. Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01158755.

Findings

60 patients were randomly assigned to receive rifampicin standard dose (12 no moxifloxacin, ten moxifloxacin 400 mg, and nine moxifloxacin 800 mg) and high dose (ten no moxifloxacin, nine moxifloxacin 400 mg, and ten moxifloxacin 800 mg). A 33% higher dose of rifampicin, intravenously, led to a three times higher geometric mean area under the time-concentration curve up to 6 h after dose (AUC_{0-6}; 78.7 mg.h/L [95% CI 71.0–87.3] *vs.* 26.0 mg.h/L [19.0–35.6]), maximum plasma concentrations (C_{max}; 22.1 mg/L [19.9–24.6] *vs.* 6.3 mg/L [4.9–8.3]), and concentrations in cerebrospinal fluid (0.60 mg/L [0.46–0.78] *vs.* 0.21 mg/L [0.16–0.27]). Doubling the dose of moxifloxacin resulted in a proportional increase in plasma AUC_{0-6} (31.5 mg.h/L [24.1–41.1] *vs.* 15.1 mg.h/L [12.8–17.7]), C_{max} (7.4 mg/L [5.6–9.6] *vs.* 3.9 mg/L [3.2–4.8]), and drug concentrations in the cerebrospinal fluid (2.43 mg/L [1.81–3.27] *vs.* 1.52 mg/L [1.28–1.82]). Intensified treatment did not result in increased toxicity. Six-month mortality was substantially lower in patients

given high-dose rifampicin intravenously (ten [35%] *vs.* 20 [65%]), which could not be explained by HIV status or severity of disease at the time of presentation (adjusted HR 0.42; 95% CI 0.20–0.91; p=0.03).

Interpretation

These data suggest that treatment containing a higher dose of rifampicin and standard-dose or high dose moxifloxacin during the first 2 weeks is safe in patients with tuberculous meningitis, and that high-dose intravenous rifampicin could be associated with a survival benefit in patients with severe disease.

INTRODUCTION

Meningitis is the most severe form of tuberculosis, resulting in death or neurological disability in 50% of patients [1,2]. The treatment in patients with tuberculous meningitis follows the model for short-course chemotherapy in patients with pulmonary tuberculosis, but the optimum drug regimen and duration have not been established.

Rifampicin is important in the treatment of tuberculous meningitis as shown by the high mortality in patients with rifampicin-resistant tuberculous meningitis [3,4].

However, the dose used is at the low end of the dose response curve [5,6], and the penetration of rifampicin into cerebrospinal fluid is low [7]. Higher doses of rifampicin for pulmonary tuberculosis have been assessed in several clinical trials reported before 1985 [8,9]. Until now, no data were available for the use of high-dose rifampicin in tuberculous meningitis, although one clinical trial is underway in Vietnam [10]. Apart from a higher dose of rifampicin, intravenous rather than oral administration might improve the drug penetration into the plasma and cerebrospinal fluid.

Penetration of other standard drugs for tuberculosis, isoniazid and pyrazinamide, into the cerebrospinal fluid is good and they are important for treatment of tuberculous meningitis. By contrast, neither ethambutol nor streptomycin, both commonly used drugs, show good penetration into the cerebrospinal fluid in the absence of inflammation. Fluoroquinolones might be good alternatives to these drugs. Of the fluoroquinolones, moxifloxacin has the highest activity against *Mycobacterium tuberculosis* in vitro and in mice [11,12]. The combination of rifampicin and moxifloxacin has been assessed in clinical trials with the aim of shortening the treatment in patients with tuberculosis [13–15]. Moxifloxacin has a good penetration in cerebrospinal fluid [16] and in patients with tuberculous meningitis [17]. Of note, data from in-vitro studies and those in animals suggest that the optimum dose of moxifloxacin for tuberculosis could be higher than the standard dose of 400 mg once daily [18,19] particularly because rifampicin lowers

the plasma concentrations of moxifloxacin by about 30% [20]. Therefore, in view of the high morbidity and mortality associated with tuberculous meningitis, we have assessed intensified treatment regimens containing high-dose rifampicin and moxifloxacin.

METHODS

Study design

This study was an open-label, randomized, phase 2, clinical trial with a factorial design. It was done at Hasan Sadikin Hospital, Bandung, Indonesia—the referral hospital for West Java province (population 43 million).

High-dose rifampicin and standard-dose or high-dose moxifloxacin were assessed as part of a four-drug regimen for tuberculous meningitis. Patients were given intensified regimens for the first 2 weeks of treatment and then all patients were given standard treatment. Most deaths occur during the first few weeks, and longer intravenous treatment would be difficult because most patients who survive the initial stage return home after 2 weeks of hospital admission.

The primary objectives were to assess the pharmacokinetics and safety or tolerability of the intensified treatment regimens. Secondary objectives were to compare neurological response and mortality in patients with tuberculous meningitis given intensified or standard treatment.

The study was approved by the ethical review board of Hasan Sadikin Hospital and Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia, and the ethical committee of Radboud University, Nijmegen, Netherlands. Compliance with the study protocol was assessed by internal monitoring and two external audits. Written informed consent for participation in the trial, and rapid HIV testing, was obtained from patients or close relatives of patients who were unconscious.

Patients

All patients older than 14 years admitted with clinically suspected meningitis between October 2010 and December 2011 had an initial screening that included standard cerebrospinal fluid and blood tests, and chest radiography.

In Indonesia, neuroradiology is rarely done in patients in this setting and is not covered by government health insurance for the poor.

Microbiological testing included microscopy for cryptococci, acid-fast bacilli, and bacterial pathogens in the cerebrospinal fluid; culture for *M tuberculosis*, bacterial pathogens, and fungi; cryptococcal antigen testing; and *M tuberculosis* drug-resistance testing with proportional methods.

CD4-cell testing and antiretroviral treatment were available for individuals who tested positive for HIV. Hepatitis C virus antibodies and hepatitis B virus surface antigen were also tested, and a 12-lead electrocardiogram (ECG) was done to check for possible ECG abnormalities, including prolonged QTc (>0.42 s for male and >0.45 s for female patients).

All patients with definite, probable, or possible tuberculous meningitis [21] were eligible for the study.

Exclusion criteria were failure to do a diagnostic lumbar puncture or evidence of bacterial or cryptococcal meningitis. Other exclusion criteria were treatment for tuberculosis for more than 7 days before admission, a history of tuberculous meningitis, pregnancy, lactation, a known contraindication to moxifloxacin, alanine aminotransferase activity more than five times the upper limit of normal, known hypersensitivity or intolerance to rifampicin or moxifloxacin, rapid clinical deterioration (e.g., signs of sepsis, decreasing consciousness or signs of cerebral edema, or herniation) during the screening process, and no informed consent. The neurological status of patients was classified according to a modification of the British Medical Research Council (BMRC) grading system as 1 (Glasgow Coma Scale [GCS] 15 with no focal neurological signs), 2 (GCS 11–14 or 15 with focal neurological signs), or 3 (GCS <10) [22].

Randomization and masking

An independent person, not involved in patient care, computer generated the randomization list. None of the physicians or investigators had access to this list. Investigators, notified by treating physicians, enrolled patients meeting the inclusion criteria into the study.

Patients were randomly assigned to one of six groups after stratification for HIV infection as the strongest risk factor for death in tuberculous meningitis [1]. In accordance with the factorial design, patients were first randomly assigned to receive a regimen with rifampicin at a standard dose (450 mg once daily, about 10 mg/kg, orally) or high dose (600 mg once daily, about 13 mg/kg, intravenously). The patients were then randomly assigned to receive oral ethambutol (750 mg), standard dose moxifloxacin (400 mg), or high-dose moxifloxacin (800 mg). All patients were given oral isoniazid (300 mg/day), pyrazinamide (1500 mg/day), and pyridoxine (50 mg/day). The fixed dosing of standard first-line drugs for tuberculosis (irrespective of weight) was in accordance with prevailing practice. The allocated treatment regimen was not masked from doctors, nurses, and patients because intravenous and oral routes of rifampicin were compared.

Treatment

After 14 days, all patients continued taking the standard regimen, consisting of oral isoniazid 300 mg/day, rifampicin 450 mg/day, pyrazinamide 1500 mg/day, and ethambutol 750 mg/day for 2 months, with subsequent rifampicin 450 mg/day and isoniazid 300 mg/day for 4 months in accordance with the Indonesian National Tuberculosis Program. All participants were given adjunctive dexamethasone intra venously for the first 6–8 weeks, starting with 0.4 mg/kg for grade 2 or 3, and 0.3 mg/kg for grade 1 tuberculous meningitis, with dose reduction as described previously [22]. Patients with both tuberculous meningitis and newly diagnosed HIV infection were started on antiretroviral treatment 4–8 weeks after the start of treatment [23].

For the intervention groups, intravenous rifampicin (Rifadin, Sanofi Aventis, Gouda, Netherlands) and moxifloxacin 400 mg tablets (Avelox, Bayer, Jakarta, Indonesia) were used. Government-approved standard treatment consisted of oral rifampicin, isoniazid, pyrazinamide, and ethambutol (Ethambutol, PT Kimia Farma, Bandung, Indonesia). These drug formulations had previously been assessed in this setting [8], and the Indonesian rifampicin formulation had shown bioavailability equal to the international reference [24]. All oral drugs were given once daily on an empty stomach.

For unconscious patients who could not swallow, drugs were dissolved in 30 mL plain water in a closed syringe and delivered through a nasogastric tube, which was then flushed. For a more reliable route of administration in the intensified regimen, 600 mg of rifampicin was diluted in 250 mL of normal saline infusion (0.9% sodium chloride) and given over 90 min. During the first 2 weeks of treatment, facility-based directly observed treatment was used by the physicians or nurses to administer the drugs. Intake of drug was checked with the hand-and-mouth procedure. After these 2 weeks, adherence was monitored by pill count every 2–4 weeks.

Pharmacokinetic assessment

Pharmacokinetic sampling was done in the first 3 days of drug administration. Serial blood sampling was done just before and at 1 h, 2 h, 4 h, 6 h, and 24 h after dosing as previously described [8]. Two samples of cerebrospinal fluid were taken on the same day as the blood samples and more than 24 h after the first sampling of cerebrospinal fluid, between 3 h and 6 h and starting from 6 h until 9 h after drug administration, respectively.

The total (protein-bound plus unbound) plasma and cerebrospinal concentrations of rifampicin and moxifloxacin were assessed at Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, using high performance liquid chromatography assays that were validated and that performed well in an international quality control program [8,20]. For rifampicin, accuracy for standard concentrations was between 99.8% and 100.4% dependent on the concentration. The intra-assay and inter-assay coefficients of variation were less than 4% for a concentration of 0.26–30 mg/L. The lower limit of quantification was 0.26 mg/L. For moxifloxacin, accuracy was greater than 95% at all standard concentrations. Intra-assay and inter-assay coefficients were from 1.4% to 5.4% and 0.2% to 3.9%, respectively. The lower limit of quantification was 0.03 mg/L.

Pharmacokinetic parameters for rifampicin and moxifloxacin in plasma were assessed non-compartmentally with WinnonLin Professional (version 5.0). The highest plasma concentrations were defined as C_{max}, with the corresponding times as T_{max}. The area under the time-concentration curve up to 6 h after dose

(AUC_{0-6}) and up to 24 h after dose in plasma were calculated with the log-linear trapezoidal rule from zero up to 6 h and 24 h, respectively. If the concentration at 24 h after dose (C_{24}) was less than the limit of quantification, it was calculated with the formula:

$$C_{24} = C_{last} \times e^{-\beta x(24 - T_{last})}$$

in which C_{last} is the last measurable concentration at T_{last} and β is the first-order elimination rate constant. β was obtained with least squares linear regression analysis on log C versus time, with the absolute slope of the regression line being β/2.303. If β could not be estimated (in view of the sampling only being done up to 6 h after dose), only AUC_{0-6} was assessed. C_{max} in the cerebrospinal fluid was estimated as the highest concentration measured in the intervals of 3–6 h and 6–9 h after dose.

Follow-up

Patients were followed up until 6 months after the start of treatment. In the first 2 weeks of treatment, possible drug-related adverse events were monitored daily, with twice weekly ECG, full blood count, and liver transaminases. More frequent or other investigations were done as clinically indicated. After discharge, patients were reviewed monthly. Because of the severity of tuberculous meningitis, with a six-month mortality of 47% in our previous series [1], adverse events were defined as those possibly or probably related to treatment (i.e., hepatotoxicity, cardiotoxicity, hypersensitivity, and hematological changes). All other adverse events (e.g., new neurological signs and respiratory failure) were not incorporated in the assessment of safety and toxicity. Classification of adverse events was based on the US National Institutes of Health Common Terminology Criteria for Adverse Events version 4.0 (see http://evs.nci.nih.gov/ftp1/CTCAE). The various grades of toxicity were managed in accordance with our predetermined toxicity management guidelines; treatment was stopped in patients who had grade 4 toxicity.

Survival was monitored during hospital admission, and afterwards through social workers for patients who did not attend scheduled appointments. The cause of

death was ascertained with clinical and laboratory assessments, and classified as neurological, non-neurological, or uncertain. No post mortem was done.

Statistical analysis

Because this study was exploratory, no sample size calculation was done, but subgroups of 20 (moxifloxacin) and 30 patients (rifampicin) were judged to be sufficient to assess safety and pharmacokinetics of intensified versus standard treatment for tuberculous meningitis.

Patients' characteristics, and pharmacokinetic and safety or tolerability data were presented descriptively for each group. Differences in pharmacokinetic parameters between study groups were not tested. Groups were pooled to present pharmacokinetic data for rifampicin dose (three groups combined), or moxifloxacin dose (two groups combined) if descriptive analysis suggested differences no larger than 25% in geometric mean AUC or C_{max} values between groups. Differences in pharmacokinetic parameters were tested with the independent samples *t* test on logarithmically transformed values of AUC and C_{max}. T_{max} values were compared with the Wilcoxon rank-sum test. The χ^2 test was used to compare proportions of patients with rifampicin peak plasma concentrations within the reference range of 8–24 mg/L [25].

Data for safety or tolerability and survival were analyzed in accordance with the intention-to-treat principle. χ^2 tests were also used for comparison of proportions of patients with adverse events. A Cox regression analysis was used to assess the effects of rifampicin dose, moxifloxacin use and dose, HIV status, rifampicin resistance, and GCS at the time of treatment initiation on survival in all patients, and in those with culture-confirmed tuberculous meningitis. All analyses were planned and no post-hoc analysis was done. All statistical analyses were done with SPSS (version 18.0.2) for Windows. p values of less than 0.05 were judged significant in all analyses.

This trial is registered with ClinicalTrials.gov, number NCT01158755.

Role of the funding source

The funders of the study had no role in the study design, data gathering, analysis, or interpretation of the results, and writing of the report. All authors had full access to the data. The corresponding author had the final responsibility for the decision to submit the report for publication.

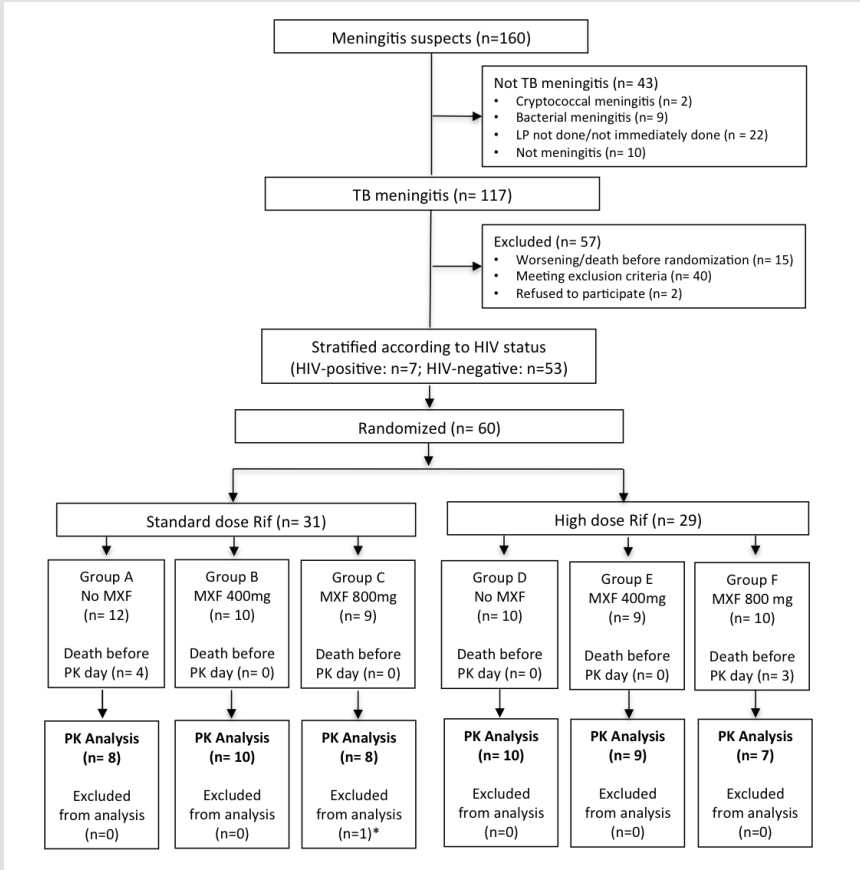
RESULTS

60 of 160 suspected cases of meningitis screened between October 2010 and December 2011, were randomly assigned to receive standard-dose or high-dose rifampicin (**Figure 1**). Informed consent was obtained from close relatives of 46 patients who were unconscious at presentation. Table 1 shows the baseline characteristics of patients randomly assigned to receive the two doses of rifampicin. 55% of patients were men (table 1); median duration of symptoms before presentation was 14 days (IQR 7–21). 12% of patients had an HIV infection (**Table 1**). The six groups were not equally balanced in terms of age and sex (**Table 1**). Symptoms at presentation included reduced consciousness (47 [78%]), headache (58 [97%]), seizures (four [7%]), weight loss (42 [70%]), and cough (34 [57%]). Six patients (10%) reported previous treatment for tuberculosis. On physical examination, lowered consciousness was noted in 47 patients (78%; median GCS 13, range 8–15). Neck stiffness (57 [95%]), cranial nerve palsies (23 [38%]), and motor deficit, most commonly hemiparesis (30 [50%]), were also noted. Most patients presented with BMRC grade 2 (49 [82%]) or grade 3 (seven [12%]) tuberculous meningitis. Chest radiography suggested pulmonary tuberculosis in 28 patients (47%), based on infiltrative (n=21), miliary (n=6), and cavitary (n=1) lesions.

Consistent with a diagnosis of tuberculous meningitis, cerebrospinal fluid showed pleocytosis with a predominance of lymphocytes (median 96 cells per µL [IQR 29–260]; 80% mononuclear cells [43–90]), a low ratio of cerebrospinal fluid to blood glucose (0.28 [0.11–0.41]), and raised protein concentration (1660 mg/L [1000–3130]).

Tuberculous meningitis was bacteriologically confirmed in 31 patients (52%),

Figure 1. Patient flow



Clinical follow-up was continued until 6 months of treatment. PK = pharmacokinetic assessment of blood and CSF; MXF = moxifloxacin.*This subject was excluded from rifampicin PK only, and not for moxifloxacin; leaving 52 patients available for rifampicin PK assessments, and 35 for moxifloxacin assessments.

based on a combination of culture (n=29) and microscopy (n=9). With the clinical scoring system [21], 20 remaining patients (33%) had probable tuberculous meningitis, whereas the other nine (15%) had possible tuberculous meningitis. Bacterial and cryptococcal meningitis were excluded in all patients.

Table 1. Patients' characteristics and drug doses

	All subjects (n=60)	Rifampicin 450 mg p.o. (n=31)			Rifampicin 600 mg i.v. (n=29)		
		No MXF (n=12)	MXF 400 mg (n=10)	MXF 800 mg (n=9)	No MXF (n=10)	MXF 400 mg (n=9)	MXF 800 mg (n=10)
Sex, male	33 (55%)	8 (67%)	6 (60%)	4 (44%)	4 (40%)	3 (33%)	8 (80%)
Age (years)	28 (16.64)	33.5 (19-47)	32.5 (19-50)	27.0 (18-57)	28.5 (16-49)	27.0 (18-60)	27.0 (19-64)
Body weight (kg)	48 (34-75)	50 (35-57)	49 (40-55)	48 (40-58)	46 (34-54)	47 (42-62)	49 (40-75)
Body mass index (kg/m2)	18.4 (15.1-26.0)	18.0 (16.0-23.3)	18.6 (15.6-22.9)	18.3 (15.4-23.3)	18.1 (15.1-21.6)	18.4 (16.3-24.7)	19.9 (16.5-26.0)
TB meningitis grade							
Grade 1	4 (7%)	0	0	1 (11%)	2 (20%)	1 (11%)	0
Grade 2	49 (82%)	12 (100%)	10 (100%)	5 (56%)	7 (70%)	7 (78%)	8 (80%)
Grade 3	7 (12%)	0	0	3 (33%)	1 (10%)	1 (11%)	2 (20%)
Infected with HIV	7 (12%)	2 (17%)	1 (10%)	1 (11%)	1 (10%)	1 (11%)	1 (10%)
GCS ≤ 14 on presentation	46 (78%)	11 (92%)	8 (80%)	8 (89%)	5 (50%)	5 (56%)	9 (90%)
Drug dose (mg/kg)							
Rifampicine	10.8 (7.8-17.6)	9.0 (7.9-12.9)	9.2 (8.2-11.3)	9.4 (7.8-11.3)	13.1 (11.2-17.6)	12.8 (10.0-14.3)	12.2 (8.0-15.0)
Isoniazide	6.3 (4.0-8.8)	6.0 (5.3-8.6)	6.2 (5.5-7.5)	6.3 (5.2-7.5)	6.5 (5.6-8.8)	6.4 (5.0-35.7)	6.1 (4.0-7.5)
Pyrazinamide	31.3 (20.0-44.1)	30.0 (26.3-42.9)	30.6 (27.3-37.5)	31.2 (25.9-37.5)	32.6 (27.8-44.1)	31.9 (25.0-35.7)	30.6 (20.0-37.5)
Ethambutol (n= 22)	15.6 (13.2-22.1)	15.0 (13.2-21.4)	—	—	16.3 (13.9-22.1)	—	—
Moxifloxacin (n=38)	10.3 (6.7-20.0)	—	8.2 (7.3-10.0)	16.7 (13.8-20.0)	—	8.5 (6.7-9.5)	16.3 (10.7-20.0)

Data are number (%) or median (range)

MXF = moxifloxacin, GCS = Glasgow Coma Scale

Seven patients died before the pharmacokinetic sampling was done (**Figure 1**). For 22 patients, sampling was done 1 day after the start of treatment. For logistical reasons (e.g., weekends and heavy patient loads), sampling was done on day 2 in 20 patients and on day 3 in 11.

In view of the short duration of sampling, up to 6 h after rifampicin dosing, assessment of plasma AUC0–24 was difficult in most patients. Geometric mean AUC_{0-6} and C_{max} for rifampicin in plasma were at least three times higher in patients given a high dose intravenously (**Table 2**). Five (19%) of 26 patients taking oral rifampicin at the standard dose (450 mg) had low C_{max} (<4 mg/L) compared with none of the 26 given a high dose intravenously. Standard dose oral rifampicin resulted in low concentrations in the cerebrospinal fluid (**Table 2**).

At least one sample of cerebrospinal fluid was available for 25 patients on standard dose rifampicin; in 16 of these patients (64%), rifampicin concentrations in cerebrospinal fluid were below the assay limit of quantification (0.26 mg/L). By contrast, rifampicin concentrations in the cerebrospinal fluid were below the limit of quantification in one (4%) of 25 patients on high dose intravenous rifampicin. Administration of the high dose increased rifampicin concentrations by almost three times in the cerebrospinal fluid (**Table 2**).

For moxifloxacin, plasma AUC_{0-24} could be reliably assessed in 24 of 35 patients; in the other 11 patients, plasma concentrations were still increasing at 6 h. Doubling the dose of moxifloxacin led to a roughly two times increase in plasma AUC_{0-24}, AUC_{0-6}, and C_{max}.

Moxifloxacin concentrations in the cerebrospinal fluid were high and above the limit of quantification in all patients from whom samples of cerebrospinal fluid were obtained. 800 mg moxifloxacin resulted in 1.6 times higher concentrations in the cerebrospinal fluid (**Table 3**).

Within the first 2 weeks of the study, 57% of patients had adverse events that were possibly or probably related to study drug (**Table 4**). Hepatotoxicity of all grades seemed to be equally distributed between rifampicin standard-dose and high-dose groups, and between the moxifloxacin standard-dose and high-

Table 2. Pharmacokinetic data of rifampicin (n= 52)

	Rifampicin dose		Ratio of intravenous to oral	p-value
	600 mg, i.v. (n=26)	450 mg, p.o. (n=26)		
Plasma				
AUC_{0-6} (mg.h/L)	78.7 (71.0 – 87.3)	26.0 (19.0 – 35.6)	3.0 (2.2 – 4.2)	<0.0001*
C_{max} (mg/L)	22.1 (19.9 – 24.6)	6.3 (4.9 – 8.3)	3.5 (2.6 – 4.8)	<0.0001*
C_{max} (≥ 8 mg/L) (n,%)	26 (100%)	13 (50%)	—	<0.0001†
T_{max} (h; median, range)	2 (1 – 2)	2 (1 – 6)	—	0.048 ‡
CSF				
C_{max} (mg/L) §	0.60 (0.46-0.78)	0.21 (0.16-0.27)	2.92 (2.03-4.20)	<0.0001*

Data are number (%) or geometric mean (95% CI), unless otherwise indicated. Rifampicin concentrations were measured for plasma AUC_{0-6} and CSF C_{max} in samples obtained during the first 3 days of treatment. Assessment of plasma AUC_{0-24} values was difficult in 41 patients because rifampicin concentrations at C_{24} were below the limit of quantification. Estimation of the C_{24} based on the last measurable concentration and the elimination rate constant was not possible because the elimination rate for rifampicin could not be assessed reliably with sampling at 2 h, 4 h, and 6 h after dose. For this reason, only AUC_{0-6} is shown. AUC_{0-6}=area under the time-concentration curve up to 6 h after dose. AUC_{0-24}=area under the time-concentration curve up to 24 h after dose. C_{max}=maximum plasma concentration. T_{max}=time to C_{max}. CSF=cerebrospinal fluid. C_{24}=rifampicin concentration at 24 h after dose.

* Independent-samples ***t*** test after log transformation. † $\chi 2$ test. ‡ Wilcoxon rank-sum test. § CSF samples were obtained from 25 patients on 600 mg intravenous and 25 patients on 450 mg oral rifampicin; in one and 16 patients, respectively, rifampicin, concentrations in CSF were below the limit of quantification of the assay (0.26 mg/L) and were set at half the limit of quantification (0.13 mg/L) to enable comparison of exposures in CSF.

dose groups. Grade 3 transaminase increases (n=7) were transient in all patients, despite continued antituberculous treatment. In accordance with our protocol, treatment was interrupted in patients with grade 4 hepatotoxicity. Hematological toxicity (thrombocytopenia, anemia, or leukopenia) did not occur. Mild QTc prolongation occurred in 20 patients (33%), including 15 (39%) of 38 patients given moxifloxacin (**Table 4**). Three patients, all in the rifampicin high-dose group,

Table 3. Pharmacokinetics data of moxifloxacin (n=35)

	800 mg (n=16)	400 mg (n=19)	Ratio of 800 mg to 400 mg	p-value
Plasma				
AUC_{0-24} (mg.h/L)*	60.4 (45.4 – 80.3)	28.6 (24.2 – 33.8)	2.1 (1.6 – 2.9)	<0.0001†
AUC_{0-6} (mg.h/L)	31.5 (24.1 – 41.1)	15.1 (12.8 – 17.7)	2.1 (1.5 – 2.8)	<0.0001†
C_{max} (mg/L)	7.4 (5.6 – 9.6)	3.9 (3.2 – 4.8)	1.9 (1.4 – 2.6)	<0.0001†
T_{max} (h; median, range)	2 (1 – 6)	2 (1 – 6)	—	0.301‡
CSF				
C_{max} (mg/L) §	2.43 (1.81 – 3.27)	1.52 (1.28 – 1.82)	1.60 (0.34 – 2.20)	0.006†

Data are number (%) or geometric mean (95% CI), unless otherwise indicated. Moxifloxacin concentrations were measured for plasma AUC_{0-24} and AUC_{0-6} and CSF C_{max} in samples obtained during the first 3 days of treatment.

AUC_{0-24}=area under the time-concentration curve up to 24 h after dose. AUC_{0-6}=area under the time-concentration curve up to 6 h after dose. C_{max}=maximum plasma concentration. T_{max}=time to C_{max}. CSF=cerebrospinal fluid.

* Could be assessed in 24 patients. † Independent samples ***t*** test after log transformation. ‡ Wilcoxon rank-sum test. § CSF samples were obtained in 15 patients on moxifloxacin 800 mg and 17 patients on 400 mg. All concentrations were above the limit of quantification of the assay.

had a hypersensitive reaction. Two reactions were mild and resolved within 3 days without stopping the drugs. One patient developed an anaphylactic shock on the first day of drug administration while receiving high-dose rifampicin and high-dose moxifloxacin, and died within 2 h after drug administration.

None of the patients were lost to follow-up. 30 patients (50%) died within 6 months, mostly in the first few weeks of treatment. For those who died within the 1-month follow-up (n=22), the main cause of death was respiratory failure (n=9), and then neurological deterioration (n=7), sepsis (n=2), and anaphylaxis (n=1). Cause of death was uncertain in the remaining three patients. Mortality was much lower in patients in the rifampicin high-dose group (**Figure 2a**), and when only patients with culture-confirmed tuberculous meningitis were

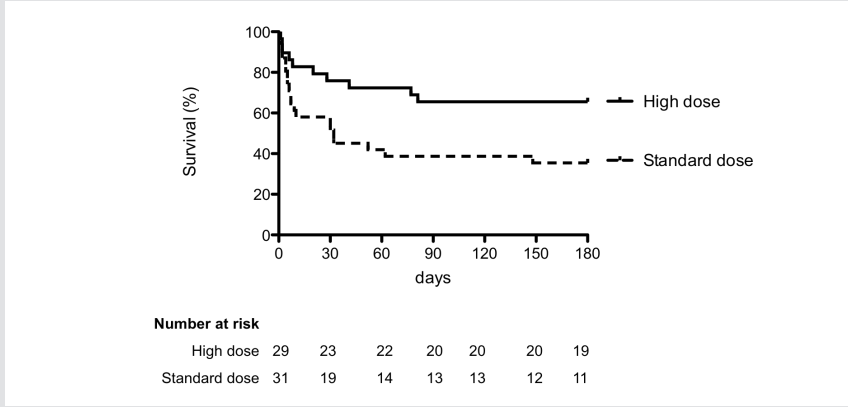
Table 4. Adverse events

	All subjects (n=60)	Oral rifampicin 450 mg (n=31)			Intravenous rifampicin 600 mg (n=29)		
		No MXF (n=12)	MXF 400 mg (n=10)	MXF 800 mg (n=9)	No MXF (n=10)	MXF 400 mg (n=9)	MXF 800 mg (n=10)
All Adverse events (grade)							
Mild (1 or 2)	22 (37%)	4 (33%)	6 (60%)	2 (22%)	1 (10%)	4 (44%)	5 (50%)
Severe (3 or 4)	12 (20%)	0	1 (10%)	4 (44%)	3 (30%)	2 (22%)	2 (20%)
Hepatotoxicity (grade)							
Mild (1 or 2)	23 (38%)	4 (33%)	6 (60%)	2 (22%)	1 (10%)	4 (44%)	6 (60%)
Severe (3)	7 (12%)	0	1 (10%)	3 (33%)	1 (10%)	1 (11%)	1 (10%)
Severe (4)	4 (7%)	0	0	1 (11%)	2 (20%)	1 (11%)	0
Hematological changes (grade)							
Mild (1 or 2)	0	0	0	0	0	0	0
Severe (3 or 4)	0	0	0	0	0	0	0
Cardiotoxity (grade)							
Mild (1 or 2)	20 (33%)	2 (17%)	2 (20%)	5 (56%)	3 (30%)	6 (67%)	2 (20%)
Severe (3 or 4)	0 (0%)	0	0	0	0	0	0
Hypersensitivity (grade)							
Mild (1 or 2)	2 (3%)	0	0	0	1 (10%)	0	1 (10%)
Severe (3 or 4)	1 (2%)	0	0	0	0	0	1 (10%)

Data are number (%)

Adverse events were graded in accordance with the U.S. National Institutes of Health Common Terminology Criteria for Adverse Events 4.0 (CTCAE, available online at http://evs.nci.nih.gov/ftp1/CTCAE); MXF = moxifloxacin

Figure 2a. Patient survival according to rifampicin treatment, all patients



Survival among all patients (n=60) treated with high dose i.v. (solid line) and standard dose p.o. (dashed line) rifampicin-containing treatment regimen during the first two weeks. Adjusted HR 0.42 (95% CI 0.20–0.87)

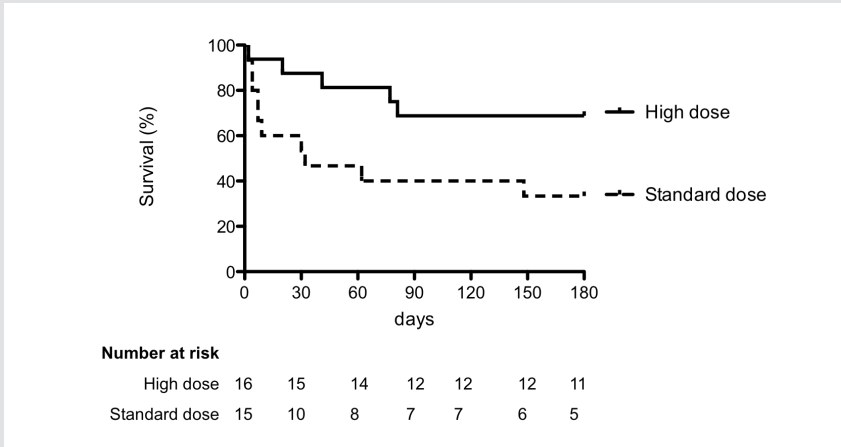
analyzed (**Figure 2b**). Table 5 shows the mortality for the various drug regimens. The effect of rifampicin was similar in both moxifloxacin groups and there was no interaction between the two drugs, confirmed with Cox regression analysis (p=0.54). However, the statistical power in this study was too small to exclude possible interaction between the two interventions. The cumulative 6-month mortality was 65% in patients in the rifampicin standard-dose group versus 34% in the high-dose group. In the multivariable analysis, corrected for moxifloxacin

Table 5. 6-month mortality by rifampicin regimen

	Oral rifampicin 450 mg	Intravenous rifampicin 600 mg	Total
Ethambutol 750 mg	7/12 (58%)	3/10 (30%)	10/22 (45%)
Moxifloxacin 400 mg	6/10 (60%)	2/9 (22%)	8/19 (42%)
Moxifloxacin 800 mg	7/9 (78%)	5/10 (50%)	12/19 (63%)
Total	20/31 (65%)	10/29 (34%)	30/60 (50%

Data are n/N (%). All patients received standard dose isoniazid, pyrazinamide, and adjunctive corticosteroids.

Figure 2b. Survival according to rifampicin treatment, bacteriologically proven TBM only



Survival among those with bacteriologically proven TBM (n=31) treated with high dose i.v. (solid line) and standard dose oral (dashed line) rifampicin-containing treatment regimens during the first two weeks. Adjusted HR 0.32 (0.11 – 0.92).

use, HIV status, and GCS at baseline, high-dose rifampicin remained a significant and strong predictor of survival (**Table 6**). In patients with culture-confirmed tuberculous meningitis, drug resistance did not affect outcome; resistance to streptomycin was present in two patients, but no resistance to rifampicin or isoniazid was noted (data not shown). Patients in the high-dose rifampicin group had more rapid resolution of coma (median 4 days [IQR 2–8] *vs.* 5 days [3–7]). Also, more patients in this group had a complete neurological recovery after 6 months of treatment (nine [31%] of 29 *vs.* four [13%] of 31).

DISCUSSION

Intensified treatment given for 2 weeks strongly increased drug exposure, did not increase drug-related adverse events, and improved the survival of patients. To our knowledge, this is the first report of a higher dose of intravenous rifampicin in patients with tuberculous meningitis (panel).

Table 6. 6-month mortality and Cox regression analysis

	Deaths	Univariable	Multivariable	p-value
Oral rifampicin 450 mg (n=31)	20 (65%)	1.00	1.00	0.03*
Intravenous rifampicin 600 mg (n=29)	10 (35%)	0.42 (0.20 – 0.91) †	0.42 (0.20 – 0.91) †	
No moxifloxacin (n=22)	10 (45%)	1.00	1.00	0.55 ‡
Moxifloxacin 400 mg (n=19)	8 (42%)	0.74 (0.29 – 1. 89) §	0.76 (0.30 – 1.94) §	
Moxifloxacin 800 mg (n=19)	12 (63%)	1.40 (0.60 – 3. 25) §	1.27 (0.53 – 3.02) §	
HIV positive (n=7)	4 (57%)	-	1.80 (0.59 – 5.53)	0.31
Glasgow Coma Scale at baseline	-	-	0.82 (0.68 – 0.99)	0.04

Data are number (%) or hazard ratio (95% CI), unless otherwise indicated.

*For differences between both rifampicin doses.

†Patients given 400 mg, 800 mg, or no moxifloxacin were pooled for comparison of rifampicin doses.

‡For differences between all moxifloxacin doses.

§ Patients given 450 mg or 600 mg rifampicin were pooled for comparison of different doses of moxifloxacin.

Previous research has shown that rifampicin, a key drug for treatment of tuberculous meningitis, does not penetrate well into the cerebrospinal fluid [7]. Rifampicin concentrations in cerebrospinal fluid have been reported in 18 studies and only in seven of these studies were mean or individual rifampicin concentrations of more than 1.0 mg/L in cerebrospinal fluid recorded at any time point [7]. Indeed, patients given standard-dose oral rifampicin in our trial had moderately low concentrations in plasma and very low concentrations in the cerebrospinal fluid. Of note, rifampicin concentrations in plasma and cerebrospinal fluid cannot be compared directly because plasma concentrations refer to total (i.e., protein-bound plus unbound) rifampicin, whereas only the unbound (active) fraction penetrates the cerebrospinal fluid because this fluid has a very low protein content compared with plasma. A 1.3 times higher dose of rifampicin given intravenously led to roughly three times higher plasma AUC_{0-6} and C_{max} values and also three times higher drug concentrations in the cerebrospinal fluid. In a previous study, we noted a nonlinear (1.65 times) average increase in systemic exposure to rifampicin when the daily oral dose was increased 1.3 times from 450 mg to 600 mg [8]. In another study, we assessed that rifampicin 450 mg intravenously resulted in a 40% higher drug

Panel: **Research in context**

Systematic review

We searched PubMed for articles published up to Sept 12, 2012, with the search terms "tuberculous meningitis", "TB meningitis", OR "high-dose rifampicin", in combination with ("randomized controlled trial"[Publication Type] OR ("randomized"[Title/Abstract] AND "controlled"[Title/Abstract] AND "trial"[Title/Abstract])), and "tuberculous meningitis" OR "TB meningitis" with "moxifloxacin".

69 studies were identified. No published data for patients with tuberculous meningitis given high-dose rifampicin were found, although the trial protocol of an ongoing assessment of high-dose oral rifampicin with levofloxacin was described in one study [10]. The pharmacokinetics of regimens containing four different fluoroquinolones were compared in 61 patients with tuberculous meningitis in a randomised study [26]. We identified a systematic review of high-dose rifampicin in patients with pulmonary tuberculosis [9]. Of 14 trials (4256 participants) included in this systematic review, 12 were done before 1980, and the only recent study judged to be of high quality was done by our group [8]. The pharmacokinetics of moxifloxacin in plasma and cerebrospinal fluid were described in four patients [17].

Interpretation

Our study is the first in which intensified antibiotic treatment including high-dose rifampicin was assessed in patients with tuberculous meningitis. The findings show that an increased dose of rifampicin, administered intravenously for the first 2 weeks, might improve the outcome of patients with tuberculous meningitis.

exposure than the same dose given orally [27]. Therefore, the higher exposure to rifampicin in our study suggests a combination of a higher dose, intravenous administration, and non-linear pharmacokinetics of this antibiotic. This increase in rifampicin exposure might be relevant because the antibiotic has exposure-dependent effects [5,6]. Average rifampicin concentrations were lower than in patients with pulmonary tuberculosis taking a similar oral dose of rifampicin in the same setting [8] possibly because of lower absorption when rifampicin is given through a nasogastric tube or when patients are severely ill.

Another possible approach for improving outcome of tuberculous meningitis might be the use of more effective antituberculous drugs like fluoroquinolones. After a pharmacokinetic study in 61 patients [26], a phase 3 trial is now underway to assess whether a combination of levofloxacin with oral rifampicin 15 mg/kg improves survival of patients with tuberculous meningitis [10]. We assessed another fluoroquinolone, moxifloxacin, which might have better pharmacokinetic properties [17], and stronger activity against *M tuberculosis* than did three other fluoroquinolones (ofloxacin, sparfloxacin, and ciprofloxacin) in animals [12]. Our study is the first in which the pharmacokinetics of moxifloxacin in the plasma and cerebrospinal fluid were assessed in a large series of patients with tuberculous meningitis. A higher dose of moxifloxacin (800 mg), which might be more potent, led to an almost proportional increase in drug concentrations in the plasma and cerebrospinal fluid.

Increasing the dose of rifampicin and moxifloxacin did not seem to greatly increase toxicity, which is in agreement with data for the use of a higher dose of rifampicin for tuberculosis [9] and other indications and with the few data available for high-dose moxifloxacin [17,28].

Although our study was not powered to detect a difference in mortality, high-dose intravenous rifampicin, when given for the first 2 weeks, led to a roughly 50% reduction in 6-month mortality. Moxifloxacin did not seem to be associated with a survival benefit. It should be noted that mortality in patients given standard-dose rifampicin was higher than in some other studies—e.g., 36.5% in the landmark corticosteroid trial from Vietnam [22].

One contributing factor to the fairly high mortality in our study might be that our study population consisted of mostly patients with very advanced disease. Only 7% of our patients presented with BMRC grade 1 tuberculous meningitis (table 1), compared with 32.2% in the Vietnam study. In patients given corticosteroids in the Vietnam study [22], the mortality was 16.7% in patients with grade 1 tuberculous meningitis, 31.1% with grade 2, and 54.8% with grade 3. In our study, the respective values for mortality were 25% (one of four), 50% (24 of 49), and 71% (five of seven).

Our study has several limitations. Because of the high early mortality, pharmacokinetic data were not available for all patients. Also no serial lumbar punctures were done, so no pharmacokinetic curves could be generated for cerebrospinal fluid. The apparent mortality benefit of high-dose intravenous rifampicin, although significant, should be interpreted with caution because of the size of the study. Since this study was open label we cannot exclude potential bias in people providing treatment and care for the patients. Despite these limitations, we feel that our results challenge the current treatment model for tuberculous meningitis. Definition of the optimum regimen, which might be given for more than 2 weeks and include an even higher dose of oral (rather than intravenous) rifampicin, would help implementation of intensified treatment for tuberculous meningitis in settings where it is most needed.

Conflicts of interest

We declare that we have no conflicts of interest.

ACKNOWLEDGMENTS

We thank Dr. Bayu Wahyudi, Hasan Sadikin General Hospital, for accommodating the research; Ela Hayati and Erik van den Hombergh for monitoring patients and running laboratory tests; and Wiel de Lange, University Medical Center Groningen, Netherlands, and Doortje Heemskerk, Oxford University Clinical Research Unit, Vietnam, for data safety monitoring.

RR was supported by the Royal Dutch Academy of Arts and Sciences and the ANDALAN-scheme from Universitas Padjadjaran, Bandung, Indonesia; ARG by fellowships from Radboud University Nijmegen; and RvC by a VIDI grant from the Netherlands Foundation for Scientific Research. The bioanalysis of tuberculosis drugs was sponsored by the Department of Pharmacy, Radboud University Nijmegen Medical Centre.

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Chapter 6

Asymptomatic cryptococcal antigenemia is strongly associated with mortality among HIV-infected patients in Indonesia

Submitted for publication

ABSTRACT

Background

Previous studies have shown that serum cryptococcal antigenemia may precede development of cryptococcal meningitis and accounts for early death among patients with advanced HIV infection. We examined the prevalence and significance of cryptococcal antigenemia in Indonesia, which is experiencing a rapidly growing HIV epidemic with a very high mortality.

Methods

We included ART-naïve HIV patients with CD4 cell count below 100 cells/μL and no signs of meningitis in a hospital setting. Baseline clinical data and follow up were retrieved from a prospective database, and cryptococcal antigen was measured in stored serum samples using a semiquantitative lateral flow assay. Cox regression analysis was used to identify factors related to mortality.

Results

Among 810 patients (median CD4 cell count 22), 58 (7.1%) had a positive cryptococcal antigen test with a median titer of 1:80 (range: 1:1 – 1:2560). Cryptococcal antigenemia at baseline was strongly associated with development of cryptococcal meningitis and early death and loss to follow up. After one year, both death (22.4% vs. 11.6%; p=0.016; adjusted HR 2.19; 95% CI 1.78 – 4.06) and the combined endpoint of death or loss to follow up (67.2% vs. 40.4%; p<0.001; adjusted HR 1.57; 95% CI 1.12 – 2.20) were significantly higher among patients with a positive cryptococcal antigen test, and this was not due to confounding.

Conclusions

Cryptococcal antigenemia is common and clinically relevant among patients with advanced HIV in this setting. Routine screening for cryptococcal antigen and preemptive antifungal treatment for those who are positive may help reducing early mortality.

Key words: cryptococcal meningitis; HIV infection; antigen testing; lateral flow assay; Indonesia

INTRODUCTION

Cryptococcal meningitis is a major cause of subacute meningitis and death in patients with advanced HIV infection, affecting an estimated one million people each year, especially in sub-Saharan Africa [1,2]. Cryptococcal meningitis is usually diagnosed by microscopic detection or culture of cryptococci from cerebrospinal fluid (CSF), or detection of cryptococcal antigen in CSF or serum [3–5]. Serum cryptococcal antigen has also been found in HIV patients without clinical meningitis. In a cohort in South Africa, cryptococcal antigen screening adequately identified patients at risk for cryptococcal meningitis and death [6]. Therefore, preemptive antifungal treatment for patients with asymptomatic serum cryptococcal antigenemia has been advocated [7,8]. Besides the South African study, several studies, most of them smaller, have been published from Uganda, Thailand and Cambodia [3,9–12]. We report the largest cohort study so far, from Indonesia, which has one of the most rapidly growing HIV epidemics in Asia [13]. Most patients in Indonesia present with advanced disease, and early mortality is very high [14]. We determined the prevalence and clinical significance of serum cryptococcal antigenemia among ART-naïve patients with CD4 count below 100 cells/μL. We used a recently developed and reliable point-of-care test for detection of cryptococcal antigen [8], which would allow implementation of targeted preemptive treatment of cryptococcosis even in the most remote areas.

METHODS

Setting and design

The study was conducted in Hasan Sadikin Hospital, the top referral hospital for West Java Province, Indonesia (population: 42 million). Since December 2004, integrated in- and outpatient services are provided for HIV-infected individuals. Services include HIV counseling and testing, HIV treatment, and management of opportunistic infections. Since September 2007, patients are included into a cohort study and characterized prospectively using a standard questionnaire, physical examination, and laboratory examination. Baseline blood samples are archived for all patients. Patients on ART generally visit the clinic monthly, with scheduled clinical and laboratory follow up, including measurement of CD4 cell

count and plasma HIV-RNA [15]. Patients with symptoms suggesting central nervous system infection are referred to a neurologist for further investigations. Cerebrospinal fluid, if taken, is examined for *M. tuberculosis,* bacteria and cryptococci [16]. Cryptococcal meningitis is diagnosed if cryptococci are found in CSF, either with direct India ink staining or culture, or if cryptococcal antigen testing is positive. Cryptococcal meningitis is treated with oral fluconazole (800mg/day) or intravenous amphotericin B (0.7 – 1 mg/kg bodyweight) if available [16]. Cerebral imaging is rarely done in this setting and not supported by the government insurance for the poor, but lumbar puncture is performed routinely as indicated. In-hospital death is recorded in medical records, but the cause of death is often not recorded, and autopsy is not performed. Outpatients are classified as dead if reported by family or community organizations or confirmed by telephone calls from the clinic. Patients not returning to the clinic are traced by telephone calls. No home visits are done as confidentiality might be breached since many patients do not disclose their HIV status to others. Baseline data and follow up are recorded in an electronic database.

For this study, newly enrolled HIV-infected adult patients between November 2007 and October 2011 who were ART-naïve and had a CD4 count below 100 cells/μL were included. Subjects with a diagnosis of cryptococcal meningitis on admission were excluded. Patient data needed for this study including sex, age, CD4 cell count, plasma HIV-RNA concentration ('viral load'), body mass index (BMI), baseline history of illness, and follow up were retrieved from database of the HIV clinic. Tuberculosis (TB) at time of enrollment was defined as TB treatment started at some point between one month before and one month after baseline. The definition of cerebral toxoplasmosis was based on a physician's decision to start treatment with pyrimethamine and clindamycin for presumed cerebral toxoplasmosis. Oral candidiasis was diagnosed clinically. During follow up the time until initiation of ART was recorded, and clinical response was defined as alive, dead, lost to follow up, or transferred out. Length of follow up was counted from the day of inclusion until the date of death or the most recent date the patient was known to be alive. Patients were considered lost to follow up if they had not returned to the HIV clinic within 6 months if ART-naive, or 3 months if already taking ART, and the last date they visited the clinic plus one day was considered the date of loss to follow up.

Ethics statement

Anonymous blood samples were used from an already-existing hospital collection, collected as part of baseline laboratory assessment for patients included in the HIV cohort, and approved by the Ethical Committee of Hasan Sadikin Hospital/Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia (No. 114/FKUP-RSHS/KEPK/Kep./EC/2007). Informed consent had been asked prior to participation into the cohort, including consent to use archived samples, therefore no separate consent was asked for this particular study.

Laboratory examinations

For cryptococcal antigen testing, our hospital used latex-based antigen testing (CALAS, Meridian Bioscience, USA) until April 2011, and lateral flow assay (LFA, Immuno-Mycologics, USA), recently approved by the US Food and Drug Administration [8], since April 2011. For this study, serum cryptococcal antigen was measured retrospectively using serum samples archived at time of enrollment in HIV-care ('baseline'). Quantification of the concentration of cryptococcal antigen in all positive samples was done using LFA according to the manufacturers instruction. LFA titers were determined semiquantitatively by diluting samples in LFA diluent solution and assessing reactivity as described above. The highest sample dilution that produced a positive result as read by 3 observers was recorded as the LFA titer.

Data analysis and statistics

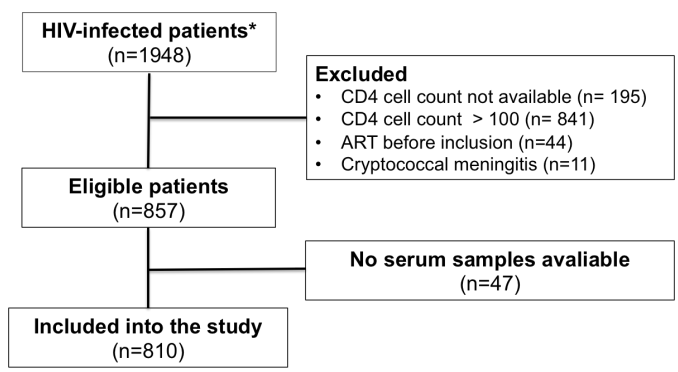
Baseline characteristics of patients with and without cryptococcal antigenemia were compared. Continuous variables are presented as mean (standard deviation) if normally distributed and median (interquartile range, IQR) if not normally distributed, and categorical variables as percentage. Differences between groups were compared using Chi-square test for proportions and Mann-Whitney U test for continuous variables, with p-values <0.05 considered statistically significant. Progression to the primary endpoints death and a composite endpoint of death or loss to follow up was examined by Kaplan–Meier estimates. Associations between cryptococcal antigenemia and these endpoints were examined using Cox regression. In multivariable Cox regression, we adjusted for possible confounding factors including CD4 cell count, hemoglobin, ART (as a time-dependent variable), oral candidiasis, toxoplasmosis

and TB treatment, age and gender. Multiple imputation (five times imputation) was performed for time of death (as outcome), WHO clinical stage, and body height and weight, with assumption that all predictors were missing at random. All variables including time of death were used as predictors. Variables with p-value < 0.100 in univariable analysis were included in the multivariable cox regression model, and the final model was determined by backward selection. Statistical analysis was done by SPSS version 18.0.2. The analysis presented used pooled data from the imputation, unless stated otherwise. Survival curves were drawn using GraphPad Prism version 5.0.

RESULTS

From 1948 patients registered in the HIV clinic during study period, 857 patients were eligible and 810 could be included in the study (**Figure 1**). Patients were mostly young males presenting with advanced disease and a very low CD4 cell count. Fifty-eight patients (7.1%) had a positive cryptococcal antigen test. There were no significant statistical differences in sociodemographic characteristics,

Figure 1. Patient flow



*All HIV-infected patients included in the HIV clinic cohort during study period (2007-2011)

clinical status, laboratory parameters, and symptoms at presentation between patients with and without serum cryptococcal antigen, except for WHO clinical staging as recorded in the medical records (**Table 1**). Titration of serum cryptococcal antigen using LFA showed a median titer of 1:80 (range: 1:1 – 1:2560). We found a moderate correlation between CD4 cell count and the titer of cryptococcal antigen (r=-.309, p=.056).

Table 1. Baseline characteristics of patients according to cryptococcal antigenemia status[§]

	All patients (n=810)	CrAg-positive (n=58)	CrAg-negative (n=752)	p-value
Sociodemographics				
Age – year (IQR)	30 (27-34)	30 (25-32)	30 (27-34)	.345
Male sex – %	76.3	72.4	76.6	.471
History of injecting drug use – %	59.7	62.2	59.5	.721
Clinical status				
WHO clinical stage 4 – %	66.0	81.1	64.8	.016
BMI < 18.5 kg/m2 – %	59.2	70.4	58.6	.225
Laboratory parameters				
CD4 cells/µL– median (IQR)	20 (7 – 45)	18 (4-37)	21 (7-45)	.327
HIV-RNA /mL – log (IQR)	5.2 (4.4-5.6)	5.0 (4.8-5.7)	5.2 (4.3-5.6)	.923
Hemoglobin in g/dL – median (IQR)	11.3 (9.6-12.9)	11.1 (9.5-12.5)	11.4 (9.6 – 12.9)	.322
Anemia* – %	68.9	75.9	68.4	.248
Positive HBsAg – %	7.7	2.2	8.1	.144
Positive anti-HCV – %	60.7	59.6	60.7	.875
Symptoms at presentation				
Fever – %	43.9	44.8	43.8	.884
Cough > 1 week – %	34.6	36.2	34.5	.789
Weight loss > 10% – %	58.9	58.6	58.9	.969
Co- infections at presentation				
TB treatment – %	19.2	17.2	19.3	.700
Cerebral toxoplasmosis treatment – %	9.3	12.1	9.0	.444
Oral candidiasis – %	56.7	66.7	56.0	.138

[§]Data were missing for TB treatment (n=1), Hemoglobin (n=19), weight loss (n=20), cough (n=21), fever (n=22), oral candidiasis (n=82), history of IDU (n=88), WHO clinical stage (n=115), HBsAg (n=187), anti HCV (n=195), BMI (n=271), HIV-RNA (n=720)

CrAg = cryptococcal antigen test

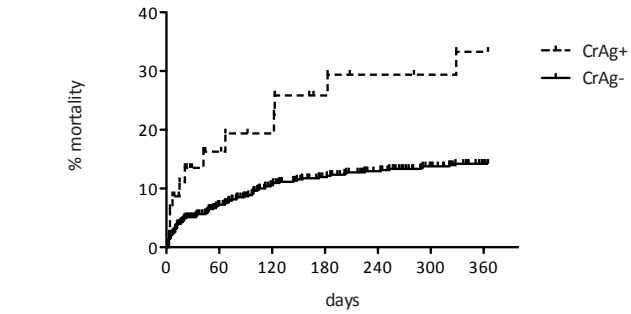
*Anemia was defined as ≤ 11.0 g/dL for females, and ≤ 13.0 g/dL for males

Patients were followed for a median 343 (IQR 21 – 1000) days (total observation: 1190 patient years). 561 patients (69.3%) started ART, after a median of 24 (IQR 14 – 42) days. After one year, 100 patients had died and 243 patients were lost to follow up; corresponding with 12.3% one-year mortality and 30.0% one-year loss to follow up. Loss to follow up was significantly higher among patients with a positive cryptococcal antigen test as compared to those with negative result (44.8% vs. 30.5%, p=0.011). Death and loss to follow up especially occurred during the first few months; 39 patients (39.0% of all deaths in one year) died within one month, and another 158 patients (65.0% of all patients loss to follow up in one year) did not return after their initial visit.

During follow up, 6 out of 58 patients (10.3%) with a positive cryptococcal antigen test were diagnosed with cryptococcal meningitis, while none of the 752 patients with a negative test did so. The cases of cryptococcal meningitis were diagnosed after a median of 91 days (range 17 – 219 days); three patients were diagnosed within 3 weeks after starting ART. Two out of six patients with confirmed cryptococcal meningitis died despite treatment with amphotericin B. The risk of developing cryptococcal meningitis did not correlate with the cryptococcal antigen titer (median titer 1:80 for those who developed meningitis vs. 1:40 for those who did not; p=0.813).

The date of death was missing for seven patients, for whom multiple imputations were performed. All variables had a similar distribution among those with missing data. A positive cryptococcal antigen test was clearly associated with increased mortality. Survival analysis revealed that cryptococcal antigenemia was strongly associated with one-year mortality (HR 2.57, 95%CI 1.43 – 4.60; p=0.002; **Figure 2a**). It is likely that some patients who were lost to follow up may actually have died, especially those who dropped out during the first few months. Indeed, more patients with a positive cryptococcal antigen test did not return after their initial visit to the clinic (44.8% vs. 30.5%; p=0.011), and more had reached the combined endpoint of death or loss to follow up after one year (HR 2.05, 95% CI 1.47 – 2.86; p<0.001; **Figure 2b**). Mortality remained significantly higher among patients with a positive cryptococcal antigen test after two (22.4% vs. 11.7%) and three years (24.1% vs. 12.9%).

Figure 2a. Death in one year of subjects with and without cryptococcal antigenemia

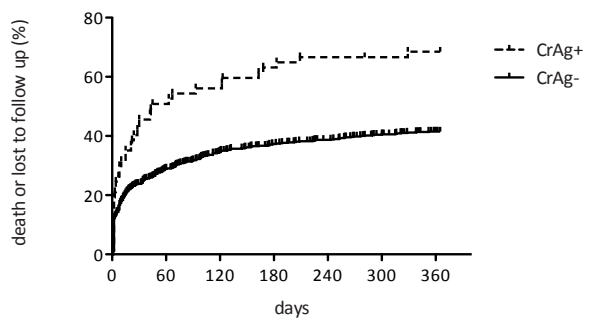


#subjects at risk							
CrAg positive	57	29	26	22	20	19	18
CrAg negative	746	515	467	443	425	404	385

(Hazard ratio 2.57 (95% CI 1.43 – 4.60), p = 0.002)

CrAg+ = positive cryptococcal antigen test; CrAg- = negative cryptococcal antigen test

Figure 2b. Combined death or loss to follow-up in one year of subjects with and without cryptococcal antigenemia



#subjects at risk							
CrAg positive	58	29	26	22	20	19	17
CrAg negative	752	515	467	443	425	404	385

(Hazard ratio 2.05 (1.47 – 2.86), p < 0.001)

CrAg+ = positive cryptococcal antigen test; CrAg- = negative cryptococcal antigen test

Besides cryptococcal antigenemia, other factors associated with one-year mortality included ART administration, low CD4 cell count, anemia at baseline, WHO clinical stage 4, BMI < 18.5 kg/m^2, a high plasma HIV-RNA, and oral candidiasis. After correction for other factors, cryptococcal antigenemia remained significantly associated with one-year survival (**Table 2a**) and the combined endpoint of death or loss to follow up (**Table 2b**).

ART was associated with a survival benefit. It reduced the risk of dying by 61% with every month of ART administration (p<0.001). CD4 cells count reduced the risk by 8.5% with every 10 cells increment (p<0.001). This benefit of having

Table 2a. Factors associated with one-year mortality [§]

	Dead n=100	Alive n=467	Univariable[#]		Final model[£]	
			HR (95% CI)	p value	HR (95% CI)	p value
ART (time dependent)	–	–	0.39 (0.25 – 0.60)	< 0.001	0.44 (0.27 – 0.71)	0.001
Positive Cryptococcal Ag – %	13.0	4.1	2.57 (1.43 – 4.60)	0.002	2.19 (1.78 – 4.06)	0.013
Male sex – %	72.0	78.4	0.74 (0.48 – 1.15)	0.181	–	–
Age in years, median	31	30	1.02 (0.99 – 1.05)	0.261	–	–
CD4 cells / µL, median	11	23	0.98 (0.98 – 1.00)	0.004	–	–
WHO stadium 4 – %	82.0	58.4	2.81 (1.37 – 5.79)	0.007	2.28 (1.17 – 4.43)	0.017
HIV-RNA in log/mL, median	5.45	5.16	2.34 (1.21 – 4.53)	0.011	–	–
Anemia* – %	83.0	63.5	2.59 (1.53 – 4.37)	< 0.001	–	–
Oral candidiasis – %	66.0	52.0	2.50 (1.54 – 4.05)	< 0.001	2.26 (1.38 – 3.65)	0.001
Cerebral toxoplasmosis – %	13.0	8.7	1.25 (0.93 – 1.69)	0.445	–	–
TB at admission – %	17.0	19.4	0.76 (0.85 – 0.99)	.300	–	–

Cox regression was performed using pooled data from multiple imputations. Data were missing for anemia (n=8), candidiasis (n=56), WHO clinical stadium (n=115),
BMI (n=115), HIV-RNA (n=473)

[#] Variables with p-value >0.100 were not included in multivariable analysis

[£] HIV-RNA and BMI were not included in the final model because of the many missing values. Candidiasis was not included in the final model because of the
possible interaction with WHO stadium

*Anemia was defined as < 11.0 g/dL for female, and < 13.0 g/dL for male

Table 2b. Cox regression analysis of composite end point death and loss to follow up in one year[§]

	Dead or lost n=363	Alive n=463	Univariable[#]		Final model[£]	
			HR (95% CI)	p value	HR (95% CI)	p value
ART (time dependent)	–	–	0.24 (0.19 – 0.31)	< 0.001	0.24 (0.19 – 0.32)	< 0.0001
Positive Cryptococcal Ag – %	10.3	3.9	2.05 (1.47 – 2.86)	< 0.001	1.57 (1.12 – 2.20)	0.009
Age in year, median	31	30	1.01 (1.00 – 1.03)	0.084	–	–
Male sex – %	78.2	78.4	0.81 (0.64 – 1.03)	0.090	0.75 (0.59 – 0.96)	0.022
CD4 cell count, median	11	23	0.99 (0.99 – 1.00)	< 0.001	0.99 (0.99 – 1.00)	0.002
WHO stadium 4 – %	75.8	59.0	1.79 (1.27 – 2.51)	0.002	1.62 (1.16 – 2.28)	0.007
HIV-RNA in log/mL, median	5.40	5.08	1.78 (1.18 – 2.70)	0.006	–	–
Anemia* – %	80.3	63.7	1.65 (1.28 – 2.13)	< 0.001	1.46 (1.13 – 1.91)	0.004
BMI in kg/m^2, median	17.19	18.08	1.49 (1.10 – 2.02)	0.011	–	–
Oral candidiasis – %	63.3	52.0	1.41 (1.11 – 1.78)	0.004	–	–
Cerebral toxoplasmosis – %	8.2	10.1	0.77 (0.64 – 0.94)	0.195	–	–
TB at admission – %	14.6	22.5	0.65 (0.48 – 0.88)	0.005	0.61 (0.45 – 0.83)	0.002

Cox regression was performed using pooled data from multiple imputations. Data were missing for anemia (n=8), candidiasis (n=56), WHO clinical stadium (n=115), BMI (n=115), HIV-RNA (n=473)

[#] Variables with p-value >0.100 were not included in multivariable analysis.

[£] HIV-RNA and BMI were not included in the final model because of the many missing values. Candidiasis was not included in the final model because of the possible interaction with WHO stadium

*Anemia was defined as < 11.0 g/dL for female, and < 13.0 g/dL for male

ART was significant for both cryptococcal antigen-positive and -negative cases, although the benefit was more prominent in the group of patients with cryptococcal antigenemia (risk reduction of 73.3% vs. 58.7%). Higher CD4 cells were only beneficial in those without cryptococcal antigen (risk reduction 9.1% with every 10 cells increment, $p<0.001$).

DISCUSSION

In this cohort of 810 patients with advanced HIV-infection but with no clinical suspicion of meningitis, 7.1% had a positive serum cryptococcal antigen test. Those with a positive result had a much higher chance of developing cryptococcal meningitis, and of early loss to follow up and death, also when corrected for possible confounding factors. To our knowledge, this is one of the largest cohorts of HIV patients examined so far, the first study from Indonesia, and one of the first to use the newly developed laminar flow assay that can easily be implemented in low-resource settings.

The prevalence of serum cryptococcal antigenemia in our cohort was comparable to previously published studies that have screened HIV patients for circulating cryptococcal antigen in the absence of signs suggesting meningitis. In Uganda, cryptococcal antigenemia was found in 5.8% of 377 patients with CD4 count below 100 cells/μL [9], and 8.8% of 295 similar patients in another study [11]. In South Africa, 7% of 707 patients screened were positive, including 12.5% of those with CD4 count below 100 cells/μL [6]. A smaller study among 131 ART-naïve patients in Thailand found cryptococcal antigen in 9.2%, especially among those with CD4 count below 100 cells/μL (n=85, 12.9%) [12]. Finally, in Cambodia, among a subgroup of 295 patients most of whom had CD4 count below 100 cells/μL but none of whom had signs of meningoencephalitis, 10.8% were positive [10].

In our study, one in every ten patients with a positive serum cryptococcal antigen test was diagnosed with cryptococcal meningitis during follow up. In fact, this number may have been much higher as early loss to follow up was very high and significantly associated with cryptococcal antigenemia. The majority of

meningitis cases occurred after initiation of ART, probably due to the 'unmasking' effect of ART [17]. Compared to a previous series from South Africa [6], meningitis in our cohort was diagnosed somewhat later (median 91 vs. 35 days). First, this may be due to the lower cryptococcal antigen titers in our cohort, even though we used a method that roughly produces five-times higher titers compared to the latex agglutination method [8]. The difference in titers might reflect the lower burden of cryptococcal infection in our setting compared to Africa [1]. Second, fluconazole use for oral candidiasis, possibly more common in Indonesia, may have delayed the development of meningitis.

The presence of serum cryptococcal antigen was associated with at least a two-fold higher risk of death during follow up, also after correction for possible confounding factors. Similar to the development of meningitis, our risk estimate for increased mortality may be an underestimate, as many cryptococcal antigen-positive patients who were classified as early loss to follow up may in fact have developed severe cryptococcal meningitis leading to death. Mortality associated with cryptococcal antigenemia appeared lower, and seemed to occur somewhat later in our series (median time to death 80 days) compared to a study in South Africa (median time to death 53 days) and Uganda (median survival 26 days) [3,9], but again this may have been biased by the high loss to follow up in the first months.

All patients in this cohort had an indication for ART, but 30% never started treatment, mostly because of early death or loss to follow up. We were not able to retrace those patients who were loss to follow up mainly because of fear of breaching confidentiality and stigmatization. However, in line with a systematic review and meta-analysis addressing loss to follow up among HIV patients [18], it seems likely that many have died.

This prevalence of cryptococcosis among asymptomatic patients found in our setting emphasizes the importance of screening of patients with low CD4 cell count for cryptococcal antigen, as has been advocated by experts in this field, and as was recently recommended by WHO [7,8,19,20]. This should preferably be done with simple and accurate point-of-care tests like the LFA method used in

this study [5,19]. After completing this study we have implemented screening for cryptococcal antigen for all ART-naïve patients with CD4 count below 100 cells/μL, followed by lumbar puncture for those with a positive result, and preemptive fluconazole treatment for those with a positive result but no meningitis. A recently published study from Uganda showed that primary prophylaxis of cryptococcal disease with fluconazole effectively prevented development of cryptococcal meningitis and cryptococcal-related death in HIV-infected individuals with no cryptococcal antigenemia, although all-cause mortality was not affected [21]. The WHO has recently recommended screening of circulating cryptococcal antigen in all ART-naïve adults with CD4 count below 100 cells/μL prior to ART initiation in settings where the prevalence of cryptococcal antigenemia is more than 3%, followed by preemptive anti-fungal therapy if the result is positive [11,20]. Such a targeted approach starting with cryptococcal antigen testing may be more cost-effective than universal prophylaxis for all patients with low CD4 cell count like in the Ugandan trial [21].

Our study suffers from several limitations. The outcome of patients who were lost to follow up could not be verified. In addition, we may have missed cases of subclinical or mild cryptococcal meningitis at baseline. Since cryptococcal antigen testing was done retrospectively, no neurologist was consulted and no lumbar puncture was performed if there were no obvious signs of meningitis. On the other hand, as part of an ongoing cohort study on meningitis in our hospital [16,22], physicians in our HIV clinic have a high vigilance for diagnosing meningitis. The study also suffers from missing data including baseline HIV-RNA concentration, and the unavailability of neuroradiology, and from the fact that fluconazole use (for possible other purposes) was not well recorded. However, despite these limitations, we feel that this large cohort with long follow up provides further evidence for the need to screen HIV-patients with low CD4 cell count for serum cryptococcal antigenemia, in an effort to reduce the high mortality of such patients. Future studies should be conducted to optimize screening and preemptive treatment of cryptococcosis.

ACKNOWLEDGEMENTS

We would like to thank Dr. Bayu Wahyudi, Director of Hasan Sadikin General Hospital, Bandung, and Prof. Tri Hanggono Achmad, Dean of Faculty of Medicine Universitas Padjadjaran, for encouraging and accommodating research. We also thank JD Sprague from Immuno-Mycologics, USA, for providing the LFA test strips for this particular study. ARG and RW are supported by fellowships from Radboud University Nijmegen Medical Centre, and RvC by a VIDI grant from the Netherlands Organization for Scientific Research Foundation.

Part of this data will be presented as an oral presentation at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin, 27 – 30 April 2013 (abstract number: 3044).

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Chapter 7

Perception of illness and health-seeking behavior in tuberculous meningitis patients in Indonesia: a qualitative study

ABSTRACT

Background

Tuberculosis (TB) meningitis, the primary manifestation of TB involving the brain, usually develops gradually over 1-2 weeks. The main factor associated with the high mortality of TB meningitis in late presentation. The majority of patients with TB meningitis in Indonesia present with very advanced disease characterized by loss of consciousness and focal neurological signs. We examined how perception of illness and health-seeking behavior may contribute to this late presentation.

Methods

Twenty consecutive TB meningitis patients and their relatives were subjected to in-depth interviewing in a referral hospital in Bandung, Indonesia about their perception of illness and health-seeking behavior prior to hospital admission.

Results

From the in-depth interviews, it appeared that meningitis patients and their relatives were not alarmed by the initial symptoms such as severe headache. In addition, when they finally went to health care facilities, medical personnel did not seem to appreciate the significance of patients' signs and symptoms, often sending them home. Communication between health care workers and patients seemed unsatisfactory as patients had virtually no understanding about their illness or the treatment. Delays in accessing appropriate treatment occurred both at the household health facility level. As a result, most patients were finally brought to hospital when they lost consciousness or developed other neurological complications.

Conclusion

A lack of awareness about meningitis among patients/laypeople and health professionals is likely to contribute to the late presentation and severe outcome of patients with TB meningitis in Indonesia.

INTRODUCTION

Tuberculosis (TB) is the most common cause of subacute meningitis, an infection involving the brain coverings, in Indonesia like in many other countries [1-3]. It has a very high mortality [1, 4-7], and the main risk factor for death is severity of disease at time of presentation. A study in Egypt revealed that patients presenting after 4 weeks of symptoms had a mortality of 80%, while mortality in patients presenting after 2 weeks was 40% [8]. In the largest study sofar in Vietnam, the average mortality was 16.7% among patients in stage I (with headache only), but 31.1% if patients present with stage II (focal neurological signs with or without altered consciousness), and 54.8% in stage III (more severe focal neurological signs or altered consciousness [4]. In the largest Indonesian cohort, more than three quarters of the TB meningitis patients presented with grade II or III of TB meningitis, and more than 50% of the patients died during six-months follow up [1, 9, 10]. It therefore seems likely that earlier presentation and treatment will improve the prognosis of patients with TB meningitis.

Many factors drive people to seek medical advice. Perceptions of illness as well as religious, philosophical or ideological beliefs of an individual determine how he or she perceives health, illness, severity of illness, etc., and this in turn affects their attitude and health-seeking behavior [11]. In this study, perception and health-seeking behavior of TB meningitis patients and their relatives were analyzed in order to understand reasons of late presentation and to find ways to increase awareness and decrease late presentation and treatment.

Subjects and methods

Setting and design

This was a cross-sectional study. The study was conducted in Hasan Sadikin Hospital, the top referral hospital for West Java Province, Indonesia (population: 42 million), between 6 July and 31 August 2012. Twenty patients aged 15 years or more who were consecutively admitted to the neurology ward with a clinical suspicion of TB meningitis were approached and included after giving informed consent. Family members who were involved in the decision for hospital admission were also approached to participate. This study was approved by

the Ethical Committee of Hasan Sadikin Hospital/Medical Faculty of Universitas Padjadjaran, Bandung, Indonesia. Informed consent was asked to patient or closest relatives if the patient was unconscious. Consent was also asked to relatives to participate in this study.

Examinations

Patients underwent physical and laboratory examinations according to standard diagnostic procedures in the hospital [1]. Diagnosis of meningitis was made using clinical signs, cerebrospin fluid (CSF) findings, and a TB meningitis scoring system as recently developed by a panel of experts, with values ranging from 0 – 14 [5]. Definite TB meningitis was diagnosed if CSF microscopy, culture or PCR were positive for *M. tuberculosis*. Probable TB meningitis is diagnosed with a score $\geq$ 10, and possible TB meningitis with a score of 6 – 9. The neurological status of patients was classified according to a modification of the British Medical Research Council (BMRC) as grade I (Glasgow Coma Scale, GCS, of 15 with no focal neurologic signs); grade II (GCS 11-14, or GCS 15 with focal neurologic signs); or grade III (GCS $\leq$ 10) [4]. HIV testing was performed with consent from patients or their relatives in case of unconscioussness of the patient.

Interviews were conducted in a quiet room or at the bedside after the patients were admitted to the neurology ward, within the first 3 days of hospitalization. If the patients were not capable of giving answers due to their medical condition, relatives were interviewed in the presence of the patient. The interview template was developed prior to recruitment and pretested in three patients. This led to minor changes. The final template was used for the in-depth interviews. Results of the interview were then transcribed verbatim.

Data analysis and statistics

Baseline characteristics of patients were collected and tabulated. Continuous variables are presented as means (SD) if normally distributed and median (interquartile range, IQR) if not normally distributed, and categorical variables as percentage. Qualitative data was analyzed using thematic analysis based on interview transcripts.

RESULT

Twenty patients were interviewed. Most patients were male (n=14, 70%), and the median age was 33 years. Patients mostly presented with neck stiffness (90%), headache (85%), fever (25%) and lowered consciousness (70%). Two patients had experienced seizures, and on examination weakness or paralysis of arms or legs was found in 70% of patients. The median time from the first complaint to the time of presentation at hospital was 24 days. One patient (5%) was diagnosed as having TB meningitis grade I, 18 (90%) as grade II and one (5%) as grade III. At time of admission, 16 patients (80%) were classified as having probable, and 4 (20%) as having possible TB meningitis. Microbiological examination later confirmed TB meningitis in nine (45%) patients. Nineteen patients underwent HIV serology examination, and one was positive.

Perception of patients and relatives about the disease

None of the patients or their relatives was able to mention the name the disease meningitis, and none had heard of it, or had associated this illness with tuberculosis. Some symptoms such as headache, seizure, muscle cramp, hypertension, cough, fever, stomachache, cholesterol and typhoid fever were mentioned; but no one associated this condition with TB. As for etiology of disease, no one associated it with bacterial infection. Half of the patients and relatives associated this condition with emotional stress and having too many problems in life, while the rest associated this with common cold or mild illness. One patient said to his father before getting sick:

> *"Let it only be me who knows what (my illness is), and what is happening to me. I don't want to make everyone busy. Let it be me who suffers from this" (H., male)*

The statement was then interpreted by the father that his son was ill because he had too many things on his mind which made him anxious and which led to accessing primary health care.

Many patients complained about having recurrent headaches, but they correlated the headaches with emotional stress. The state of decreased level

of consciousness that was suffered by most patients was also correlated to headache. One relative perceived that this condition had something to do with head trauma

> *"... He had stumbled from second floor when he was 4 years old, and when he was at the secondary school he also had accident with another head injury. He always has problem with his head ... " (mother of P, male)*

The majority of patients and relatives believed that there was a natural cause for this illness, and they did not think that the condition was caused by supernatural phenomena such as curses, black magic, or the like. However, they still doubted it, that they also went to religious authorities to ask for some remedies to overcome it.

Social effect of the disease

Patients and relatives perceived that this illness made their lives harder, especially from a lower socioeconomic level. Most patients (60%) were the breadwinners, and their illness made them unable to earn money for the family. The fact that most of them had no permanent jobs made the condition worse. However, the patients did not mention financial constraints as a cause for delay in seeking treatment since they were covered by government insurance for the poor. Socially, their illness did not affect their relation with relatives, neighbors, and friends. The disease was not perceived as contagious or shameful.

Perceived expected outcome

Despite the bad clinical condition at time of hospital admission, almost all relatives believed that the patients would get well, when they saw the patients improving after hospitalization. There was a fear though, that if the disease became protracted, it would make their lives difficult since no one would earn money for the family.

Health-seeking behavior

More than 80% patients firstly took over-the-counter medicine (mostly analgesics) when they had early symptoms of the disease. When they felt that these over-the-counter pills had no effect, they went to a primary health service or a private

practice (in Indonesia, it is a common practice that medical doctors run a private practice after office hour, or even run a small clinic by their own. Midwives and nurses also provide such services). Almost 50% patients even went to a district hospital, but all were sent home. Most of the time these health providers either in primary health service or referral hospital did not explain anything about the condition of the patients, and even when the patients or relatives asked about it only vague definitions were mentioned like "stomach ache", "sign of typhoid fever", "head problem", and "lung problem". On the other hand, patients were also reluctant to ask when the doctor did not open the communication about the illness showing that there may have been an issue in power relationship between doctor and patients. One subject said that the medical doctor in his primary health care did not tell anything about the disease but only ordered a chest radiography without further explanation. The communication issue is depicted in the following quotes:

> *"I received many kinds of medicine. But they did not say what the disease was. (I didn't ask further) because I was so panic that I was trembling. Imagine, she was on the rice field and suddenly she just collapsed" (husband of Patient A, female)*

> *"From a clinic, I was given a prescription to be given to this private hospital. The doctor referred my son to the internist when he should've been referred to the neurologist. I was scolded by the doctor at the hospital because of it when actually that was the fault of the clinic's doctor, not mine" (mother of patient P, male)*

> *"I didn't receive the conclusion (of the disease), even I have not received the result of the X-ray test yet. So the communication with the doctor was not really good, I even had not met the doctor so I decided to change (the hospital)" (husband of Patient O, female)*

All patients received medication from the health service providers, but they either did not get any information about the drugs they were taking, or did not understand the information they were given. In the interview, none of the patients could mention the name and the use of the drugs they received at the primary

health care; they only remembered that they got three of four drugs, and some still remembered the color of the drugs. In two-thirds of patients (n=13), relatives self-referred the patients to hospital, mostly because they noticed a decreasing level of consciousness of the patients, and one because the patient got a seizure. At some points all patients had come to primary health care providers, none of whom seemed to recognize the condition as serious illness, as as none had referred the patients to hospital. Ten patients (50%) went to different doctors before they finally came to Hasan Sadikin hospital. A statement from one relative may explain the process more clearly:

> *"After having stomachache for several weeks, we noticed that our son got fever. I already asked him to let me take him to a doctor, but he refused. He said it was only a stomachache. Then I asked a medical doctor to visit my son at home. He told us that my son got typhoid fever and gave him medicine. I have done what the doctor told me; our son should get a full bed rest, but we did not see any improvement. Then I took him to a hospital, and he was hospitalized. He got infused, but the following day I saw that he could not move his right leg. Then a neurologist paid a visit, and he told us that our son needed to be taken to other hospital to get his head scanned. We took him for scanning and we then took him back to our previous hospital. On Thursday, after seeing the result, the neurologist said that this case needed a consultation to neurosurgery. Then I was told that our son needed to be treated in ICU, but since the hospital does not have ICU available, on Sunday the patient was moved to here (Hasan Sadikin hospital). We reached the hospital at 3 pm, and at 7 pm all the laboratory examination were completed. Then someone came, asking permission to take some fluid from the low back of the patient, and we need to wait until 4 at dawn because he needed to stay immobile until around 6 hours (after lumbar puncture procedure)." (mother of patient F, male).*

In addition, one of the patient's relative made a statement that she was the one who took the patient to the hospital:

> *"I was the one who had the initiative (to take the patient to the hospital) because I was anxious, I had taken him to that doctor for many visits but no improvement at all" (wife of Patient H, male)*

Besides medical treatment, some patients and their relatives reported asking for spiritual help with holy water, "bekam" (a traditional method of healing that involves sucking out 'dirty' blood from the patient's body by bleeding), or other religious rituals.

DISCUSSION

In this study, we found that most of patients presented late to the hospital not merely due to lack of knowledge of the patients and relatives, but also because health professionals did not seem to recognise the importance of symptoms. All patients visited health providers before admission, almost half of them even at some points went to referral hospital but were sent home. Two third of hospital admission was self-referred.

The early symptoms of meningitis were usually not perceived as serious, and this probably led to delay in seeking medical advice. Previous studies estimate that around 70-90% of all self-recognized episodes of illness are dealt with exclusively outside the formal health care system, in which the "popular" and "folk" sectors (self-treatment, family care, self-help groups, religious practitioners, heterodox heales, and so forth) provide most of the care [12].

In addition, patients and their relatives did not consider infection as a cause of headache. The study shows that headache is mostly referred as an effect of emotional stress, hence no physical treatment was sought. When the symptom persisted patients mostly used over-the-counter drugs, because headache is not culturally perceived as a sign of serious illness. This is in line with what Kleinman said that cultural belief affects how we present symptoms, when and to whom we go for care [12]. Since the process of illness and health seeking behavior begins with symptom perception and appraisal [11], one can understand that patients in this setting do not seek medical intervention when they have a headache.

When symptoms got worse, patients went to primary health care providers. However, primary health care providers did not seem to recognize the patient's complaints as a sign of serious illness, and therefore only prescribed symptomatic treatment. This might be caused by the medical provider's inability to diagnose the disease in an early stage, and the patients' inability to explain their symptoms clearly. Interestingly, in a study in the UK, when general practitioners were faced with patient's complaints without a clear ascertainable medical disorder, they often considered symptoms to be trivial, not an appropriate reason for a doctor's visit, and a waste of the doctor's time [11]. This finding is also similar to what was found in a health seeking behavior study related to acute bacterial meningitis in Malawi. This study showed that patients were sometimes viewed as too demanding when they come repeatedly with the same complaint [13]. This explains why patients went to different doctors, and finally to hospital as self-referrals.

The communication between patient and doctor seemed unsatisfactory, as most of the patients could not state the diagnosis the primary health care provider had made, nor the the name and the use of the medicine prescribed to them. This needs further study as communication is a major determinant of appropriate use of health facilities [14]. Culture definitely influences how health providers communicate with patients. From the interviews it seemed that in Indonesia, the doctor-patient relationship is still more of an 'adult-child' rather than 'adult-adult' relationship. This is important because 'adult-adult' relationship increase respect, autonomy, accountability, trust and humanity [15].

This present study also highlights how doctor's delay contributes to late diagnosis and treatment of TB meningitis. This is similar to a study involving pulmonary TB patients in Jogjakarta, Indonesia, and a study in bacterial meningitis in Malawi [13], which also identified patient and as well as provider's delay as a cause of treatment failure [16]. It is worrying that very few of the patients in our study were referred to hospital by health providers, while most were eventually brought to hopsital when they lost consciousness.

Our study had some limitations. Firstly, it was based on anamnesis of reported historical events, which is prone to recall bias. To minimize bias, we did not probe patients or relatives with leading questions. The fact that some interviewees were not the ones who made decision to take the patients to hospital added further bias. Selection bias might also influence the result. No subject mentioned economic constraints as a cause of delay in hospital presentation. This might be due to selection bias, since patients with no government insurance for the poor might never reach the hospital.

Despite these limitations, this qualitative study revealed that delay in seeking medical advice was not merely caused by patients' ignorance, but also by unawareness among health providers. This study shows that both patient's and doctors' delay contribute to late presentation and poor outcome of tuberculous meningitis in Indonesia. Further study is needed to discover health care provider's knowledge, attitude, and practice related to meningitis diagnosis and treatment, and to develop effective strategies to reduce delay and improve early diagnosis and treatment.

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Chapter 8

Summary and General Discussion

SUMMARY OF RESEARCH FINDINGS

This thesis addresses the etiology, diagnosis, outcome and treatment of adult meningitis in Indonesia. The studies were conducted in Hasan Sadikin Hospital, Bandung, the third referral hospital for West Java province, Indonesia between December 2006 and August 2012.

In **Chapter 2** we carefully characterized and followed a cohort of adult patients presenting with meningitis. We found that the vast majority of patients present with subacute meningitis. HIV had high prevalence (24%), was newly diagnosed in more than 70% of the patients, and associated with very low CD4 cell counts. An extensive microbiological work-up showed that bacterial meningitis was rare. The most common diagnosis was TB meningitis (80%), while cryptococcal meningitis was found in one-third of HIV patients. Approximately half of patients died, 41% within the first month of follow up, with HIV infection as the strongest risk factor for death.

In our initial cohort, TB meningitis was confirmed in 50% of cases, in line with experience elsewhere which shows that bacteriological confirmation of TB meningitis is difficult. Culture is slow and often negative, and microscopy has an even much lower sensitivity. In **Chapter 3** we evaluated in-house real-time PCR (rt-PCR) targeting insertion sequence IS*6110* among 230 consecutive adult meningitis patients. IS*6110*-PCR of cerebrospinal fluid (CSF) samples had a much higher sensitivity than microscopy of ZN-stained slides, and this in-house rt-PCR identified more cases of TB meningitis than CSF culture, many of them with a somewhat milder presentation. IS*6110*-PCR was negative in all CSF samples from non-meningitis control patients.

In our initial cohort, approximately 50% of HIV-infected patients either had TB or cryptococcal meningitis, leaving the other half without a definite diagnosis. In **Chapter 4** we examined if toxoplasmosis accounted for some of these cases. In the absence of CT or MRI of the brain, toxoplasmosis is generally not considered as part of the differential diagnosis of meningitis. Among 64 HIV-infected patients with clinically suspected meningitis, CSF PCR for *Toxoplasma gondii* was

positive in one third. Clinically, in the absence of neuroimaging, toxoplasmosis was difficult to distinguish from TB or cryptococcal meningitis, although CSF abnormalities were less pronounced. Without specific toxoplasmosis treatment (as this study was done retrospectively on archived samples) patients with a positive *T. gondii* PCR had a roughly two-fold higher mortality compared to those with a negative PCR.

Even when diagnosed and treated promptly, TB meningitis generally has a poor outcome. Intensified antibiotic treatment might possibly improve outcome, especially since rifampicin and some other TB drugs do not penetrate well into the brain. In **Chapter 5** we examined intensified antibiotic treatment containing a higher dose rifampicin intravenously and/or moxifloxacin, a fluoroquinolone with high potency against *M. tuberculosis*, besides standard dose INH, pyrazinamide and adjuvant corticosteroids. Sixty patients were randomized in a phase II open label randomized clinical trial. Standard oral rifampicin showed poor penetration into the CSF, but a 33% higher dose administered intravenously led to three times higher rifampicin concentrations in blood and CSF. Moxifloxacin penetrated well into CSF. Intensified treatment regimens did not result in increased toxicity. Finally, high dose rifampicin was associated with an approximately 50% reduction in 6-month mortality (35% vs. 65%).

A key factor in the poor outcome of meningitis in Indonesia is the severity of disease at time of presentation. Among HIV-infected patients with low CD4 count, screening of cryptococcal antigen prior to ART administration has been proposed by experts and recently advised by the World Health Organization (WHO) to prevent the development of cryptococcal meningitis and subsequent death. This is especially true in countries with high burden of cryptococcal meningitis (sub-Saharan Africa). In **Chapter 6** we investigated the prevalence and clinical significance of cryptococcal antigenemia in Indonesian patients. Among 810 ART-naïve HIV patients with CD4 count less than 100 cells/μL, 7.1% had a positive cryptococcal antigen, and cryptococcal antigenemia at baseline was strongly associated with the development of cryptococcal meningitis, early loss to follow-up, and early death.

Also for TB meningitis, the late presentation clearly contributes to high mortality in our setting. In **Chapter 7** we assessed health-seeking behavior of patients with TB meningitis. From in-depth interviews it appeared that meningitis patients and their relatives were not alarmed by the initial symptoms such as severe persistent headache. In addition, when they finally went to primary health care facilities, health providers usually did not appreciate the significance of typical signs and symptoms, and often sent them home. As a result, most patients were self-referred when presenting at the hospital, mostly with loss of consciousness and focal neurological signs. These data indicate a lack of awareness about meningitis among patients/laypeople as well as health providers.

GENERAL DISCUSSION

Clinical presentation, diagnosis, and outcome (Chapters 2, 3, and 4)

Table 8.1 shows the research questions related to clinical presentation, etiology, diagnosis and outcome of meningitis. The table also contains the main findings with the implications for practice or further research.

Clinical presentation and HIV

Clinically, our patients were mostly young males, possibly reflecting the higher susceptibility of men for TB, or the rise of HIV, which in Java is mostly driven by young males injecting drugs [1,2]. Patients mostly had a subacute presentation, usually with headache and fever, and often with altered consciousness (45%) and focal neurological signs (30%), reflecting a more advanced course of disease. HIV prevalence was unexpectedly high (25% in the initial study; 18.9% among 410 patients recruited since year 2006), with two-thirds of patients not aware of their HIV status until hospital admission. Available CD4 cell counts were very low (median of 22 cells/mL), in line with the late detection of HIV in Indonesia (median of 37 cells/mL in outpatient clinic in the same setting) [3].

Our research findings have had immediate implications for patient care. Provider initiated testing and counseling (PITC) for HIV is performed to all patients immediately after admission, usually at the emergency room. The result of HIV testing also determines the flow of CSF samples for microbiological testing (**Figure 8.1**)

Table 8.1. Clinical presentation, etiology, diagnosis and outcome of meningitis

Clinical presentation, etiology, diagnosis and outcome		
Questions	**Findings**	**Implications**
• What are the main pathogens causing adult meningitis in Indonesia? (Chapter 2)	• TB and cryptococcal meningitis are the main causes, bacterial meningitis is extremely rare	• Clinical and laboratory flowchart for meningitis patients was established and introduced. Empiric treatment now includes TB
• What is the prevalence of HIV infection and what is its effect on outcome of meningitis? (Chapter 2)	• One fourth of adult meningitis patients are HIV-infected. HIV increases mortality by more than two-folds	• Provider-initiated testing and counseling for HIV is performed immediately to patients presenting with subacute meningitis
• What is the performance of IS6110 rt-PCR for diagnosis of TB meningitis? (Chapter 3)	• IS1610 rt-PCR is highly specific and more sensitive than CSF culture and microscopy	• IS6110 rt-PCR is technically demanding and cannot be implemented easily in routine care. Automated Xpert TB and MODS should be tested
• Are there any other possible infectious agents in HIV-infected patients presenting with subacute meningitis? (Chapter 4)	• Toxoplasma PCR was positive in CSF in one third of archived samples from HIV-infected meningitis cases • Mortality among patients with undiagnosed toxoplasmosis was very high	• Toxoplasmosis should be included in the differential diagnosis of subacute meningitis, especially if neuroradiology is not available • Empiric anti-toxoplasmosis treatment should be evaluated in HIV-infected meningitis patients

Microbiological diagnosis

In our initial study, a definite cause of meningitis was found in 55% of patients. Confirmation of cryptococcal meningitis (introduced as part of these studies) is straight forward, but bacteriological confirmation of TB meningitis was much lower (56%). Different from studies in Vietnam and Argentina that revealed positive results up to 60% among HIV positives [4,5], the positivity rate in HIV positive patients in our cohort was much lower (32.3%), possibly due to methods used for bacteriological confirmation at the time of the study (direct

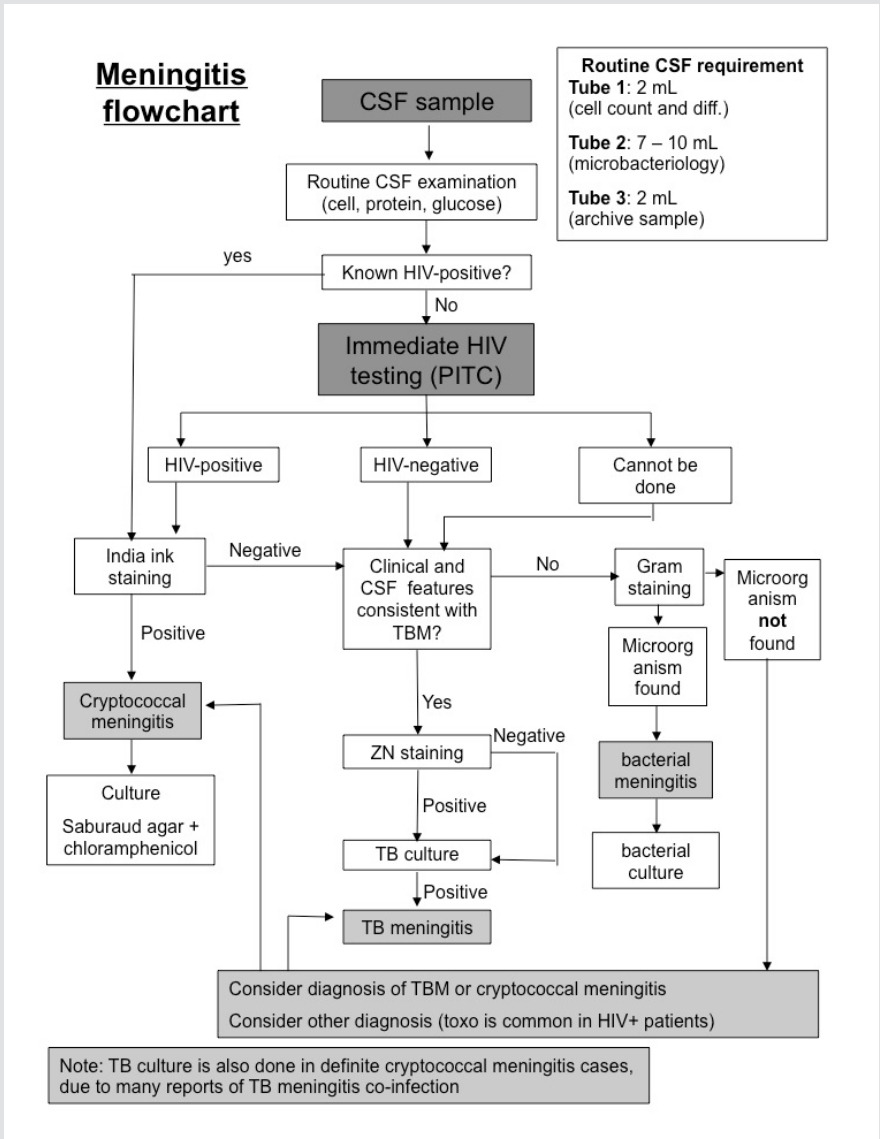
staining and solid/liquid culture only). Molecular testing (rt-PCR) was specific and had a positivity rate which was 6 times higher than microscopy and 1.5 times higher than culture [6]. Interestingly, very few patients (less than 3%) in our setting present with acute bacterial meningitis, different from series from other Asian countries where acute bacterial meningitis is diagnosed in around 30% of patients [7–9]. It could be that patients with acute bacterial meningitis never reach our hospital, either because they die earlier or because they receive antibiotic treatment elsewhere.

These findings have led to the development of a diagnostic algorithm for patients with subacute meningitis in our hospital (**Figure 8.1**), and for a change in empiric treatment, which now always contains TB drugs.

In patients with advanced HIV infection, cerebral toxoplasmosis is usually considered when patients present with subacute or acutely developing confusion, with or without focal neurological deficits [10]. Confirmation of diagnosis is usually achieved by finding space occupying lesion(s) in cerebral imaging. If neuroimaging is not performed, cerebral toxoplasmosis is usually not considered in this group of patients. Our hypothesis that toxoplasmosis might mimic subacute meningitis in our setting was confirmed by the finding of positive *T. gondii* PCR in one third of HIV-infected patients with meningitis, all of whom had toxoplasma IgG in their blood. Toxoplasmosis was similarly frequent with TB and cryptococcal meningitis, and could not be distinguished easily. Because of these results, in the absence of CT or MRI of the brain, we recommend that toxoplasmosis should be part of the differential diagnosis in patients presenting with clinical signs and symptoms of subacute meningitis [4,11,12], especially if cryptococcal or TB infection cannot be confirmed by CSF microscopy. Further, empiric treatment for toxoplasmosis should be considered in such patients.

The high proportion of non-definite meningitis cases, especially in HIV-infected patients underlines the need to search for other possible causative agents. We ruled out neurosyphilis [11], which is common among HIV patients in the western world. Aiming for more bacteriological confirmation, we evaluated in-house IS6110 TB-PCR. Real-time PCR had a 100% specificity and a high positivity rate

Figure 8.1. Meningitis flowchart



(68%), higher than other TB diagnostic modalities, higher than often reported in literature [13–15], probably due to the use of large CSF samples used [11], and the use of IS*6110*, which has multiple copies present in the genome of *M. tuberculosis* complex, as the PCR target [16]. Although this PCR was reliable in a research setting, it proved too difficult and technically demanding to implement in daily practice. The recently developed geneXpert (XpertTB), a very robust and closed PCR [17], should be evaluated as an alternative. To the best of our knowledge, the use of GeneXpert in TB meningitis has only been studied in small numbers of meningitis patients in Germany and India [18,19]. At present we are also busy evaluating microscopic observed drug susceptibility testing (MODS) as a rapid culture system for TB meningitis.

Improving outcome: HIV, late presentation and other operational issues

In our initial cohort, approximately 50% patients died within 6 months, most of them in the first month. HIV infection and late presentation increased mortality, as well as a positive CSF *T. gondii* PCR among those with HIV. In the absence of toxoplasmosis treatment, not less than 83% of patients died within 2 months, compared to 64% of patients treated for TB meningitis, and 46% for cryptococcal meningitis [20].

This extreme mortality in HIV-infected patients emphasizes the need for earlier detection and treatment of HIV. Unfortunately, HIV is mostly diagnosed very late in Indonesia, and even when diagnosed, many patients remain without treatment [21]. Earlier detection and treatment of HIV using the 'universal test and treat' approach, where every adult gets tested annually and gets immediate treatment irrespective of CD4 count, would decrease mortality and morbidity resulting from late diagnosis as well as significant cost savings in the longer term [22]. Of course this approach poses conceptual, ethical, programmatic, and financial issues [23], and operational research is needed to implement such measures [24]. At a more simple level, recognition of simple signs of HIV, like chronic diarrhea and oral candidiasis, and targeted counseling and testing of risk groups such as prisoners has shown great benefit in our setting in Bandung [25–27].

Another approach should target late presentation of meningitis, as it strongly correlates with higher mortality, as shown in a study in Vietnam where mortality was 16.7% in grade I, 31.1% in grade II and 58.8% in grade III TB meningitis [28]. Less than 10 % of our TB meningitis patients present with grade I TB meningitis, all the others with more advanced stages. In-depth interviews showed considerable delay both at the level of patients and health providers. Both patients and health care providers should be made aware of the initial signs of meningitis to increase early detection and treatment of meningitis. Operational research should be conducted to limit delay once patients admit to hospital, and increase timely start of antibiotic treatment, as has been shown for other diseases [29]. Further studies are needed to examine the benefit of timely diagnosis and/or empiric treatment meningitis patients in settings like ours, which possibly include treatment for tuberculosis and toxoplasmosis.

Treatment (Chapter 5)

Table 8.2. summarizes the research questions, findings and implications of studies in this thesis on treatment of TB meningitis.

Intensified antibiotic treatment

Our findings from the phase II clinical trial suggest a clear benefit of high dose rifampicin. Standard dose rifampicin results in a very low concentration of rifampicin in CSF. Administration of oral rifampicin in this study results in a lower plasma concentration when compared to administration of similar dose to pulmonary TB patients in the same setting [30], possibly due to lower absorption when rifampicin is given through a nasogastric tube or when patients are severely ill. Higher dose of rifampicin, given intravenously, significantly increased drug exposure both in serum and CSF. AUC_{0-6} 78.7 mg.h/L, C_{max} plasma 22.1 mg/L, and concentrations in CSF 0.60 mg/L that were resulted from administration of this higher dose of rifampicin exceed minimal inhibitory concentration (MIC) in liquid medium (0.2 – 0.4 mg/mL) [31], and considered adequate for treatment of TB meningitis.

As to moxifloxacin, this drug penetrates well into CSF. With standard dose (400 mg), its AUC_{0-6} 15.1 mg.h/L, C_{max} 3.9 g/L, and drug concentration in CSF 1.52

Table 8.2. Treatment of TB meningitis in this thesis

Treatment		
Questions	**Findings**	**Implications**
• Is intensified treatment of TB meningitis safe?	• Treatment regimens including high dose rifampicin iv and standard and high dose moxifloxacin did not lead to more drug toxicity	• Intensified treatment should be evaluated in larger phase 3 trials
• Is intensified treatment associated with better drug exposure in blood and CSF?	• Standard rifampicin is associated with very low drug exposure in the CSF • Moxifloxacin has good penetration • Intensified treatment leads to much higher drug concentrations	• Pharmacokinetics of rifampicin i.v. and an even higher dose of oral rifampicin should be compared • Rifampicin high dose and moxifloxacin standard dose throughout the intensive phase
• Does intensified treatment lead to better treatment results?	• High dose rifampicin improves neurological outcome and significantly increased patient survival	• Intensified treatment should be evaluated in larger phase 3 trials • Adjuvant treatment should be further explored as mortality remains high

g/L were considered favorable for treatment of TB meningitis [32]. Doubling the dose of moxifloxacin gives rise to proportional increase in those parameters. Of note, the fact that rifampicin reduces concentration of moxifloxacin by 30% when administered together [33] might support the use of higher dose of moxifloxacin.

Increasing the dose of rifampicin and moxifloxacin did not seem to increase toxicity, in agreement with data for the use of a higher dose of rifampicin for tuberculosis [30] and other indications and with the few data available for high-dose moxifloxacin [34,35].

Most importantly, mortality was much lower in patients treated with rifampicin high-dose i.v. (cumulative 6-month mortality 34% vs. 65%), and this was not due

to confounding. Longer administration of high dose rifampicin might further reduce the mortality. Moxifloxacin did not seem to affect mortality, but this phase II trial was actually not powered to detect differences in survival.

One immediate question for implementation of the findings of this study is the use of intravenous preparation of rifampicin. Oral administration (rather than intravenous) of rifampicin would simplify intensified treatment, as it is more readily available worldwide. A bioavailability study comparing 600 mg i.v. rifampicin with an even higher dose oral rifampicin is need to define the optimal oral dose. A phase III multicenter randomized clinical trial comparing intensified and standard treatment will be the next step before proposing change of international treatment guidelines.

Adjuvant treatment

Corticosteroid as adjuvant in TB meningitis treatment reduces mortality, especially in HIV-negative patients but do not prevent development of neurological complications [28,36], and longer follow up revealed that the benefit was only seen until 2 years [37]. Recent evidence suggests that the effect of corticosteroids is dependent on genetic variation, opening the way for tailored steroid treatment in the future [38]. Aspirin might be another adjuvant option, as it may reduce stroke, a common complication of TB meningitis [39]. Other treatment modalities, such as neurosurgical interventions for treating increased intracranial pressure, as well as more aggressive treatment for accompanying metabolic conditions might help to reduce morbidity and mortality from this deadly and devastating disease.

Prevention (Chapter 6 and 7)

Two studies were performed in the field of prevention, one for cryptococcal meningitis among ART-naïve patients, and one addressing health-seeking behavior of TB meningitis patients. The questions, findings and implications of research findings are listed in Table 8.3.

The significant prevalence of cryptococcal antigenemia among ART-naïve HIV patients in our setting warrants routine screening. Screening of patients with

Table 8.3. Prevention of meningitis in this thesis

Prevention		
Questions	**Findings**	**Implications**
• What is the prevalence and clinical significance of serum cryptococcal antigenemia among HIV patients with CD4 cell counts below 100/mL? (Chapter 6)	• High prevalence of cryptococcal antigenemia among ART-naïve patients, higher early mortality and drop out among patients with positive result	• Cryptococcal antigen screening has been implemented in HIV clinic following the study • Pre-emptive treatment for antigen-positive patients should be examined
• What factors caused delay in presentation of meningitis patients in Indonesia? (Chapter 7)	• Unawareness among patients and providers in diagnosis of TB meningitis	• Further study to examine knowledge, attitude and performance of health providers towards meningitis, and ways to education lay people

low CD4 cell counts for cryptococcal antigenemia has been advocated by experts in this field [40–42], and has also been recommended by WHO since late 2011 [43]. The use of simple and accurate point-of-care tests like the LFA method [41,44] is preferred. Patients who are positive should probably get a proper neurological examination and lumbar puncture. The high early mortality and loss to follow-up suggest that we may have missed some (sub)clinical cases of cryptococcal meningitis. If meningitis is excluded, those with asymptomatic cryptococcal antigenemia should receive pre-emptive treatment [41,43], as this proved a significant risk factor for subsequent meningitis and death. Further operational research is needed to optimize this strategy in our setting.

As mentioned above, late presentation is a strong risk factor for poor outcome. Patient as well as provider's delay has been identified as a cause of failures in pulmonary TB control in a study in Indonesia [45], and a qualitative study in our cohort revealed that delay in seeking medical advice was not merely caused by patient's ignorance, but also by unawareness among health providers. Further steps should be taken to examine this in more depth and to develop strategies to counteract this problem.

FURTHER RESEARCH PRIORITIES

In the past 6 years we have learned a lot about the clinical management of meningitis in Indonesia. One of the most striking conclusions is the high proportion of TB meningitis. However, although the research has had a big impact on diagnosis, we can still only get bacteriological confirmation of TB meningitis in around 50%, and mostly through culture, which is too slow to guide initiation of treatment. Similarly, intensified treatment containing high-dose rifampicin seems very promising, and is likely to be implemented in some forms in the coming years. But despite these advancements, the mortality of meningitis remains very high. The problems addressed in this thesis are not specific for Indonesia; around the world, diagnosis and treatment of TB meningitis remain a huge challenge. And in HIV-infected patients, cryptococcal meningitis and cerebral toxoplasmosis pose a big burden. Surely, much more need to be done to improve care of meningitis patients in Indonesia and elsewhere. In addition, many questions regarding the pathophysiology of meningitis remain unanswered.

Therefore, we hope to address two important questions in future studies.

- Why do some patients develop TB meningitis and not lung or asymptomatic TB infection?
- How can the poor prognosis of meningitis be improved?

Based on these questions and in light of our previous studies we can define three priorities: basic sciences, operational research, and a new clinical trial for TB meningitis, as would now briefly explain below.

Basic sciences

It is not known why some patients develop TB meningitis and not lung TB or asymptomatic TB infection. Both human and bacterial genetic factors may be involved, but the exact mechanisms are unknown. Variation in certain host genes is associated with TB. This has also been the focus of earlier studies in Bandung, using a cohort of more than 1,100 Indonesian pulmonary TB patients and 1,100 healthy controls. This allowed to confirm or disprove associations in

TIRAP [46], a locus on chromosome 11p13 [47], TLR 8 [48], and autophagy genes [49] among others.

The particular clinical phenotype of TB (pulmonary TB, TB meningitis, etc.) may also be genetically determined, but this has not been properly examined as only three genetic studies have included TB meningitis, the most severe manifestation of TB [50–52]. Since TB meningitis is associated with more severe forms of acquired immunodeficiency (e.g. caused by advanced HIV infection or strong immunosuppressive drugs), one might hypothesize that the same holds true for genetic risk factors: compared to pulmonary TB, mutations leading to more pronounced loss of host immunity might be 'needed' for development of TB meningitis. However, this has not been studied. The degree of evolutionary adaptation of certain *M. tuberculosis* lineages might also affect the clinical phenotype of active TB [53], with certain *M. tuberculosis* lineages overrepresented among TB meningitis patients, but this has only been studied in a single study from Vietnam [50].

The interaction between human and mycobacterial genotypes, and the relation with the disease 'phenotype' requires further study. We have now established a large cohort of TB meningitis patients in Bandung, which will allow us to conduct such studies to increase our understanding of the pathogenesis of TB meningitis.

Operational research

In dealing with the problems in meningitis, operational studies should be conducted as outlined in the previous sections. In the field of diagnostics, GeneXpert MTB/RIF assay, a novel diagnostic tool that integrates rapid diagnosis of TB and detection of rifampicin resistance [17], and other (much cheaper) methods such as MODS should be evaluated [54,55]. TB meningitis caused by drug-resistant mycobacteria has a far worse outcome than disease caused by susceptible organisms, but timely confirmation of drug-resistance in TB meningitis is problematic [56–58]. Fortunately, our preliminary data show low prevalence rates of drug resistance among TB meningitis (Lidya Chaidir, unpublished data). Still, methods for rapid evaluation and risk stratification should also be evaluated.

The use of uniform case definition for TB meningitis has been proposed by a committee of experts, in order to make the result of studies from different setting comparable [59]. However, implementation of the scoring system in daily routine is somewhat difficult, and cut-off points for including patients into clinical diagnosis of possible/probable TB meningitis requires further validation among settings [60]. Other operational studies should focus on late presentation. The knowledge, attitude and practice of health providers towards this topic should be examined and appropriate interventions should be designed. In the field of treatment for meningitis other than TB, operational research should be done to optimize diagnosis and treatment of HIV-associated meningitis, i.e. implementation of anti toxoplasmosis treatment and screening and treatment of cryptococcosis. Often, diagnostic delay seems not only caused by patients' ignorance, but also by lack of awareness among health care providers. Increasing awareness of the disease is the first step to improve patient management, which can be done both to lay people and health care providers.

In general, we hope that dissemination and implementation of our operational findings in diagnosis and treatment, and our clinical algorithms to other hospitals in Indonesia, will help improve care for meningitis patients.

Defining an optimal treatment regimen – need for further trials

There seems to be two possible approaches to improve treatment of TB meningitis. First, as shown in our phase II clinical trial, intensified antibiotic treatment with high dose rifampicin should be further explored. The use of equivalent oral dose of the intravenous rifampicin in the trial will make the regimen more practical and easy to deliver, and give less financial burden to health system. Second, more effective adjuvant treatment should be developed. In a landmark trial in 2004, Thwaites et al proved the benefit of adjuvant corticosteroids for TB meningitis, with a clear reduction in mortality (RR 0.69, 95% CI 0.52 – 0.92, p=0.01) [28]. However, no significant effects were noted on neurological disability, and the benefit of dexamethasone was only seen up to two years of follow-up [37]. Interestingly, stratification according to a functional SNP in the promoter region of the *lta4h*, a gene involved in eicosanoid metabolism, reveals differences in the effect of corticosteroids. Adjunctive dexamethasone only showed survival

benefit in patients with the pro-inflammatory *lta4h* genotype [38]. Replication of this finding in other cohorts would explain some of the variability in trials evaluating the beneficial effect of dexamethasone and would open the way for individualized corticosteroid treatment of TB meningitis.

Another possible adjuvant is aspirin. Stroke is a common complication of TB meningitis, typically located in the basal ganglia, mostly in the middle cerebral arterial territory and associated with poor outcome [61,62]. Aspirin appeared to reduce mortality and neurological outcome in a trial from India [39], although this trial was flawed by its lack of blinding, relative small size, incomplete corticosteroid treatment, and high dropout from the aspirin group. Besides its inhibition of platelet aggregation, aspirin may harbour direct antimycobacterial effects [63], may modulate antituberculous drugs [64,65] and has immunomodulatory effects [66,67], and effects on the eicosanoid metabolism implicated in susceptibility and outcome of TB infection.

It seems rational to combine these two approaches, intensified antibiotics and low-dose aspirin, in a large randomized clinical trial. Intensified treatment was proven to be very effective by itself, and aspirin is unlikely to antagonize this effect. In addition, combining two or more drugs in developing TB treatment is encouraged by Stop TB Partnership, and in keeping with new regimen development paradigm to shorten the development of TB drug that traditionally requires many years [68,69]. If we would be able to conduct (and fund) such a trial, it should preferably be multi-site and blinded. The outcome of such a trial would have an important impact on treatment of TB meningitis.

Concluding remarks

This thesis is a result of a longstanding multidisciplinary and international collaboration. In Indonesia, the infrastructure to conduct clinical trials and pharmacological researches is not fully established yet, and experience of conducting such studies is limited. More collaboration among Indonesian researches is needed, along with improvement of infrastructure and scientific conducts. Partnership with other researchers from different disciplines and other countries may help to empower Indonesian researchers to engage in this type of work.

This thesis is the continuation of the work in the field of TB and HIV conducted by my Indonesian colleagues Bachti Alisjahbana, Ida Parwati, Rovina Ruslami, Rudi Wisaksana and others who have conducted research in the field of TB and HIV, including operational, bacteriological, and pharmacological research. Their efforts and the longstanding academic collaboration between Indonesia and the Netherlands in this particular field have helped establish the studies in this thesis, which I hope again will be a 'stepping stone' for future researches.

The findings in this thesis provide some important data regarding the etiology, diagnosis and outcome of meningitis in Indonesia. In addition, they shed light on the likely benefit of intensified antibiotic treatment for outcome of TB meningitis, and on the prevention of meningitis. Clearly, more effort is needed to help improve patient management and increase our basic understanding of meningitis.

I am grateful that I was able to engage in this research in the last 6 years, and I hope I can contribute to future studies in collaboration with professionals in Indonesia and abroad.

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Ringkasan

RINGKASAN

Ringkasan hasil penelitian

Tesis ini membahas etiologi, diagnosis, luaran dan pengobatan meningitis pada pasien dewasa di Indonesia. Penelitian yang disebutkan dalam tesis ini dilakukan di Rumah Sakit Umum Pusat dr. Hasan Sadikin, Bandung, yang merupakan rumah sakit rujukan provinsi Jawa Barat pada kurun waktu Desember 2006 hingga Agustus 2012.

Bab 2 membahas karakteristik pasien kohort yang terdiri dari orang dewasa yang datang untuk dirawat dengan gejala meningitis. Pada kohort ini kami mendapati bahwa sebagian besar pasien yang datang menderita meningitis subakut. Prevalensi HIV pada kelompok pasien ini tinggi (24%), dan lebih dari 70% pasien dengan HIV-positif baru mengetahui status HIV mereka saat dalam perawatan ini. Jumlah sel CD4 pada pasien-pasien ini sangat rendah. Pada kohort ini, diagnosis klinis yang paling sering ditegakkan adalah meningitis TB (80%); dan pada kelompok pasien HIV-positif, meningitis kriptokokus dijumpai pada satu dari tiga pasien. Meningitis bakterialis jarang ditemukan, meskipun telah dilakukan pemeriksaan mikrobiologi yang cukup menyeluruh terhadap sediaan cairan serebro spinal (CSS) pasien-pasien ini. Separuh dari pasien dalam kohort ini meninggal dalam waktu 6 bulan, dan kematian paling banyak terjadi pada satu bulan pertama (41%). Infeksi HIV merupakan faktor risiko kematian yang paling kuat.

Diagnosis pasti pada kelompok pasien dengan diagnosis klinis meningitis TB pada umumnya baru didapatkan setelah beberapa pekan, yaitu setelah kultur bakteri tumbuh. Tidak seperti TB paru, diagnosis segera melalui pemeriksaan mikroskopis (pewarnaan Ziehl Nielsen) jarang mendapatkan hasil positif karena sedikitnya jumlah kuman di dalam CSS. Secara umum diketahui bahwa konfirmasi bakteriologis meningitis TB sulit didapatkan. Hal ini disebabkan oleh karena kultur TB memerlukan waktu yang lama dan sering menunjukkan hasil negatif, sementara pemeriksaan mikroskopis langsung mempunyai sensitivitas yang lebih rendah. Hal ini menjadi masalah, karena diagnosis pasti yang terlambat dapat membuat penanganan penyakitnya menjadi terlambat juga.

Karena itu pada **Bab 3** kami mengevaluasi kegunaan *in-house real-time PCR (rt-PCR)* yang menggunakan *insertion sequence* IS*6110* sebagai primer. Penelitian dilakukan terhadap bahan pemeriksaan CSS dari 230 pasien meningitis yang dikumpulkan selama 3 tahun. Pemeriksaan IS*6110*-PCR dengan menggunakan bahan pemeriksaan CSS menunjukkan sensitivitas yang jauh lebih tinggi dibanding pemeriksaan mikroskopis langsung, dan dapat mengidentifikasi lebih banyak kasus meningitis TB dibandingkan dengan kultur, bahkan pada kasus dengan gejala klinis yang lebih ringan. IS***6110***-PCR menunjukkan hasil negatif pada semua bahan sediaan CSS yang berasal dari pasien non-meningitis.

Pada kohort penelitian kami, sekitar separuh pasien HIV menderita meningitis TB atau kriptokokus, sementara separuh yang lain tidak memiliki diagnosis pasti. Pada **Bab 4** kami menyelidiki kemungkinan toksoplasmosis sebagai penyebab keadaan klinis pada pasien-pasien ini. Pada praktek sehari-hari, tanpa adanya hasil pemeriksaan CT-scan atau MRI, toksoplasmosis umumnya tidak menjadi diagnosis banding meningitis. Dari 64 pasien HIV yang diperiksa, kami mendapatkan hasil PCR toksoplasma positif pada sepertiga kasus. Secara klinis, pasien dengan PCR toksoplasma positif sulit dibedakan dari pasien meningitis TB dan kriptokokus, walaupun kelainan CSS-nya lebih ringan. Tanpa pengobatan spesifik untuk toksoplasmosis (penelitian ini dilakukan retrospektif terhadap bahan pemeriksaan yang tersimpan), pasien dengan PCR toksoplasma positif meninggal dua kali lebih banyak daripada pasien dengan hasil PCR negatif.

Meningitis TB biasanya memiliki luaran yang buruk, bahkan jika diagnosis dan pengobatan bisa dilakukan dengan segera. Pengobatan antibiotika yang lebih intensif menggunakan obat baru atau dengan menaikkan dosis obat yang penting mungkin akan memperbaiki luaran ini. Pada **Bab 5** kami mendiskusikan hasil penelitian farmakologi obat TB untuk meningitis TB dengan menggunakan rifampisis dosis tinggi intra vena dikombinasikan dengan moksifloksasin oral. Moksifloksasin merupakan obat golongan florokuinolon yang mempunyai potensi tinggi terhadap ***Mycobacterium tuberculosis.*** Sejalan dengan kebijakan pemberian empat jenis obat untuk penanganan TB, obat TB lain yaitu INH, pirazinamid tetap diberikan dalam dosis standar. Kortikosteroid telah menjadi bagian dari protokol pengobatan meningitis TB, dan tetap diberikan pada pasien

yang masuk pada penelitian ini. Enam puluh pasien meningitis TB menjalani randomisasi untuk mendapatkan satu dari beberapa kemungkinan kombinasi rifampisin dan moksifloksasin. Hasil pemeriksaan kadar obat dalam darah dan CSS menunjukkan bahwa penetrasi rifampisin ke dalam CSS sangat buruk pada dosis standar yang diberikan secara oral, namun penaikan dosis 33% melalui vena meningkatkan konsentrasi rifampisin sampai 3 kali lipat baik di darah maupun di CSS. Moksifloksasin dapat menembus CSS dengan sangat baik. Pemberian rejimen intensif ini tidak meningkatkan toksisitas obat. Rifampisin dosis tinggi juga berhubungan dengan perbaikan angak kematian dalam follow up 6 bulan hingga 50% (35% vs. 65%).

Salah satu penyebab utama keluaran buruk meningitis di Indonesia adalah beratnya derajat penyakit saat datang berobat ke rumah sakit. Hal ini bisa dicegah dengan mengenali penyakit secara lebih dini atau mencegah sebelum penyakit muncul. Berdasarkan temuan-temuan penelitian terdahulu, WHO pada akhir tahun 2011 merekomendasikan pemeriksaan penapisan kriptokokus pada pasien HIV dengan jumlah sel CD4 yang sangat rendah sebelum pemberian obat anti retroviral. Penapisan ini dapat mencegah timbulnya meningitis kriptokokus, dan diketahui dapat mencegah kematian. Hal ini terutama berlaku pada negara-negara yang mempunyai banyak kasus meningitis kriptokokus (negara-negara sub-Sahara di Afrika dan Asia Tenggara). Pada **Bab 6** kami menyelidiki prevalensi dan aspek klinik dari adanya antigen kriptokokus di dalam darah pengidap HIV dengan jumlah CD4 < 100 dan belum mendapat terapi ARV di Indonesia. Dari 810 pasien yang memenuhi kriteria inklusi, 7.1% mempunyai antigen kriptokokus di dalam darahnya, dan adanya antigen ini pada pemeriksaan awal sangat berhubungan dengan kejadian meningitis kriptokokus dan tingginya angka *loss to follow up* dan kematian.

Hal serupa juga didapatkan pada kasus meningitis TB. Keterlambatan datang berobat telah terbukti menjadi salah satu penyebab tingginya angka kematian. Pada **Bab 7** kami menilai persepsi dan perilaku mencari pengobatan pada pasien yang dirawat dengan diagnosis meningitis TB. Dari wawancara mendalam terhadap 20 pasien dan keluarganya, didapatkan bahwa pasien dan keluarga tidak menyadari bahwa nyeri kepala hebat yang menetap itu merupakan gejala awal

meningitis. Namun demikian, saat mereka datang berobat ke fasilitas pelayanan kesehatan primer, penyedia pelayanan yang ada umumnya juga tidak menyadari bahwa gejala yang ada merupakan tanda kelainan yang berat dan karenanya tidak melakukan rujukan. Tidak jarang keluarga pasien yang langsung membawa pasien ke rumah sakit saat mendapati pasien mengalami penurunan kesadaran atau kejang-kejang. Hal ini menunjukkan adanya kekurangan kewaspadaan akan meningitis baik pada level pasien atau penyedia pelayanan kesehatan.

Samenvatting

SAMENVATTING

Samenvatting van de onderzoeksbevindingen

Dit proefschrift behandelt de etiologie, diagnostiek, de uitkomsten en de behandeling van meningitis bij volwassenen in Indonesië. De studies die aan het proefschrift ten grondslag liggen, zijn tussen december 2006 en augustus 2012 uitgevoerd in het Hasan Sadikin Hospital, Bandung, het tertiaire verwijscentrum voor de provincie West-Java van Indonesië.

In **hoofdstuk 2** beschrijven we een cohort van volwassen patiënten die zich klinisch presenteerden met meningitis. Dit cohort werd zorgvuldig gekarakteriseerd en gevolgd. We stelden vast dat de overweldigende meerderheid van patiënten zich met subacute meningitis presenteerde. In deze studie toonde HIV een hoge prevalentie van 24%. HIV werd als nieuwe diagnose vastgesteld in 70% van de patiënten en was geassocieerd met erg lage aantallen CD4-positieve cellen. Een uitgebreide microbiologisch vervolgonderzoek toonde dat acute bacteriële meningitis zeldzaam was. De meest voorkomende diagnose was tuberculeuze meningitis (80%). Bij HIV-patiënten, werd in een derde van de gevallen cryptokokkenmeningitis gevonden. Ongeveer de helft van alle patiënten overleed, 41% binnen de eerste maand van follow-up, waarbij HIV-infectie de belangrijkste voorspeller voor overlijden was.

In ons oorspronkelijke cohort werd de klinische diagnose tuberculeuze meningitis bacteriologisch bevestigd in 50% van de gevallen. Dit is in lijn met ervaring elders die laat zien dat bacteriologische bevestiging van tuberculeuze meningitis moeilijk is. Het kweekproces gaat langzaam en is vaak fout-negatief. Microscopie heeft zelfs een nog lagere sensitiviteit. Als aanvulling op deze gebrekkige diagnostische mogelijkheden, evalueerden we In **hoofdstuk 3** een in-house real-time PCR (rt-PCR) gericht op de *M. tuberculosis* insertie IS*6110* in 230 opeenvolgende volwassen meningitispatiënten. De IS*6110* –PCR op liquor cerebrospinalis monsters had een veel hogere sensitiviteit dan microscopie van Ziehl-Neelsen gekleurde coupes. Deze in-house rt-PCR stelde meer gevallen van tuberculeuze meningitis vast dan conventionele kweek van liquor cerebrospinalis, met name in die gevallen die zich milder presenteerden. De

IS*6110*-PCR was negatief op alle liquormonsters van controlepatiënten die geen meningitis hadden.

In ons oorspronkelijke cohort had van de HIV-geïnfecteerde patiënten de helft ofwel tuberculeuze ofwel cryptokokkenmeningitis, en bleef de andere helft aanvankelijk zonder definitieve diagnose. In **hoofdstuk 4** hebben we onderzocht of toxoplasmose verantwoordelijk zou kunnen zijn voor enkele van deze gevallen. De praktijk leert dat als een CT of MRI van de hersenen niet beschikbaar is, toxoplasmose op basis van louter de kliniek slechts zelden overwogen wordt in de differentiaaldiagnose van meningitis. Retrospectief werd onder 64 HIV-geïnfecteerde patiënten bij wie meningitis vermoed werd op basis van de klinische presentatie, een PCR op liquor cerebrospinalis voor *Toxoplasma gondii* uitgevoerd die positief was in een derde van de gevallen. Het was ingewikkeld om op klinische gronden, in de afwezigheid van neuroradiologie, toxoplasmose te onderscheiden van tuberculeuze of cryptokokkenmeningitis, al waren bij toxoplasmose de afwijkingen in de liquor minder uitgesproken. Zonder dat specifieke behandeling voor toxoplasmose werd ingesteld – deze studie werd immers retrospectief uitgevoerd op opgeslagen samples –, toonden patiënten met een positieve PCR voor *T. gondii* grofweg een tweemaal hogere mortaliteit dan patiënten met een negatieve PCR.

Zelfs als tuberculeuze meningitis direct vastgesteld en behandeld wordt, heeft het in het algemeen een slechte uitkomst. We veronderstelden dat een geïntensiveerde antibiotische behandeling het resultaat mogelijk zou kunnen verbeteren omdat rifampicine en enkele andere tuberculostatica een slechte penetratie naar de hersenen kennen. In **hoofdstuk 5** hebben we dit onderzocht in een gerandomiseerde, open label fase II klinische trial met zestig patiënten. Patiënten ontvingen de standaarddosering isonazide, pyrazinamide en adjuvante corticosteroïden en werden gerandomiseerd voor wel of niet een hogere dosering rifampicine intraveneus en/of moxifloxacine, een fluoroquinolon met een hoge effectiviteit tegen *M. tuberculosis.* Standaard orale rifampicine liet een slechte penetratie in de liquor cerebrospinalis zien, maar een 33% hogere dosering die intraveneus werd toegediend leidde tot driemaal hogere rifampicine concentraties in het bloed en liquor. Moxifloxacine penetreerde goed in de liquor. Een geïntensiveerd behandelschema leidde

niet tot toegenomen toxiciteit. Ten slotte: een hoge dosering rifampicine was geassocieerd met een ongeveer een halvering van de 6-maanden mortaliteit (van 65% naar 35%).

Van cruciaal belang voor de slechte uitkomst van meningitis in Indonesië was de ernst van de ziekte op het moment van presentatie. Voor HIV-patiënten met een laag CD4-getal, is door experts en recent door de Wereldgezondheidsorganisatie voorgesteld te screenen op cryptokokkenantigeen voordat met antiretrovirale therapie (ART) gestart wordt. Dit om te voorkomen dat zich een cryptokokken-meningitis ontwikkeld waaraan patiënten kunnen overlijden. Dit is in het bijzonder relevant voor landen waarin cryptokokkenmeningitis een hoge ziektelast kent (sub-Sahara Afrika). In **hoofdstuk 6** hebben we de prevalentie en klinische relevantie van het voorkomen van cryptokokkenantigeen in bloed onderzocht in Indonesische patiënten. Van de 810 ART-naïeve HIV-patiënten met een CD4-getal van minder dan 100 cellen/µL, werd 7,1% positief getest voor cryptokokkenantigeen. De aanwezigheid van cryptokokkenantigeen in bloed was sterk geassocieerd met het ontwikkelen van cryptokokkenmeningitis, vroege loss to follow-up en vroeg overlijden.

Voor meningitis die wèl tuberculose als oorzaak had, speelde een late presentatie een duidelijk bijdragende rol aan de hoge mortaliteit in onze klinische setting. In **hoofdstuk 7** hebben we in kaart gebracht in welke mate waarin patiënten met tuberculeuze meningitis op zoek gaan naar zorg. In diepte-interviews bleek dat meningitispatiënten en hun naasten niet gealarmeerd waren door de oorspronkelijke symptomen zoals een weerbarstige hoofdpijn. Bovendien, als patiënten zich uiteindelijk tot de faciliteiten in de primaire gezondheidszorg wendden, werden ze vaak naar huis gestuurd door de zorgverleners die de typische tekenen en symptomen vaak niet op waarde schatten. Dit had tot gevolg dat de meeste patiënten uiteindelijk zonder verwijzing in het ziekenhuis binnenkwamen, meestal met gevorderde symptomen zoals bewustzijnsverlies en focale neurologische verschijnselen. Deze gegevens laten zien dat er een gebrek aan bewustzijn over tuberculeuze meningitis bestaat onder zowel patiënten/leken als zorgverleners.

List of publications

List of Publications

1. The effect of HIV on adult meningitis in Indonesia: a prospective cohort study. Ganiem AR, Parwati I, Wisaksana R, van der Zanden A, van de Beek D, Sturm P, van der Ven A, Alisjahbana B, Brouwer AM, Kurniani N, de Gans J, van Crevel R. *AIDS*. 2009;23(17):2309-16.
2. Validation of real-time *IS6110* PCR for diagnosis of TB meningitis in a cohort of adult patients in Indonesia. Chaidir L, Ganiem AR, van der Zanden A, Muhsinin S, Kusumaningrum T, Kusumadewi I, van der Ven A, Alisjahbana B, Parwati I, van Crevel R. *PLoS ONE*. 2012;7(12): e52001.
3. Cerebral toxoplasmosis presenting as subacute meningitis in HIV-infected patients; a cohort study from Indonesia. Ganiem AR, Dian S, Indriati A, Chaidir L, Wisaksana R, Sturm P, Melchers W, van der Ven A, Parwati I, van Crevel R. *PLoS NegI Trop Dis*. 2013;7(1):e1994.
4. Intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis: an open-label, randomized controlled phase 2 trial. Ganiem AR, Ruslami R, Dian S, Apriani L, Achmad TH, van der Ven A, Borm G, Aarnoutse RE, van Crevel R. *Lancet Infect Dis*. 2013;13(1):27-35.
5. Asymptomatic serum cryptococcal antigenemia is strongly associated with mortality among HIV-infected patients in Indonesia. Ganiem AR, Indrati A, Wisaksana R, Meijerink H, van der Ven A, Alisjabana B, van Crevel R. Submitted for publication.

Acknowledgements

Acknowledgements

This journey began with taking Reinout van Crevel for a short visit to see TB meningitis patients in our neurology ward in Bandung, arranged by my teacher dr. Dede Gunawan back to year 2005. This short visit continued with more discussions and then visits to the Netherlands for preparing this PhD, courses, setting up the field work, coordination with many people, writing and submitting papers, until I reached this day. No word is concise enough to wrap the whole journey I have gone through to attain this PhD, except *alhamdu lillah*. Today I have completed my PhD program at the Radboud University Medical Center, Nijmegen, the Netherlands. This would not have happened without His help.

Perhaps the most important people to thank are our patients and their family, without them these papers would not exist. Their willingness and trust has made it possible to do the work. I wish I could give more than my sincere thanks.

Naturally I would start with my mentors. I would like to express my highest gratitude to my promotor, Prof. Andre van der Ven, for giving me the opportunity to participate in this PhD program. Andre, thank you very much for your trust. And thank you for giving me one more chance to continue this once suspended journey.

To Reinout van Crevel, my co-supervisor. I could not thank you enough for teaching and coaching me throughout this journey. Thank you for pushing me to the limit. Your ability to see things straightforwardly always astonishes me. Thank you for abundant time and effort you have spared for me during my PhD time despite your busy work. I owe you a bunch.

To Rovina Ruslami, my co-supervisor. Thank you very much for being there for the scientific discussion and preparation, and also the not-so-important *curhat* and *curcol* (now you know the difference, don't you?) sessions, that were treated with similar enthusiasm and seriousness. I enjoyed working with you. I do hope this work of pharmacology in TB meningitis may give contribution to the improvement of patient care.

I would like to express my thankfulness to the people in Bandung who have helped me in one way or another to make this day. To Prof. Cissy Kartasasmita, dr. Rizal Chaidir, and Dr. Bayu Wahyudi, the former and current Directors of Hasan Sadikin hospital, thank you for allowing me doing my research in the hospital. I hope that I could help achieving the hospital mission to be the leading hospital in infectious disease in Indonesia. I believe that operational as well as basic research activities in our institution will have a huge impact to increase health service in our hospital, as well as at the national level.

During my research, I received great support from the Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. Therefore I would like to thank former Dean Dr. Eri Surachman and current Dean Prof. Tri Hanggono Achmad, for their support during my research. I hope I can help building the research infrastructure in the faculty to achieve one of the goals through research and collaboration.

To the manuscript committee: prof. Burger, prof. van Soolingen, and prof. van de Beek, thank you for reading and approving this doctoral thesis. Diederik, I have enjoyed very much the discussions. I hope I can learn more about meningitis from you, and maybe do research together in the future.

I indebt vey much to dr. Dede Gunawan who had introduced me to and 'pulled' me into this world of neuro-infection. To the head of Neurology Department, dr. Nani Kurniani, and all my seniors and colleagues in the department dr. Yustiani Dikot, dr. Reggy Panggabean, dr. Adelina Yasmar Alfa, dr. Djadjang Suhana, dr. Thamrin Sjamsudin, dr. Nurdjaman Nurimaba, dr. Henny Anggraeni, dr. Siti Aminah, dr. Yusuf Wibisono, dr. Anam Ong, dr. Suryani Gunadharma, dr. Andi Basuki, dr. Nushrotul Lailiyyah, dr. Uni Gamayani, dr. Sobaryati, dr. Sofiati Dian, dr. Aih Cahyani, and dr. Lisda Amalia, thank you for your support and understanding. Especially for taking over my responsibility when I had to leave the duties (that happened many times!) for working visits abroad.

To the train locomotive, Bachti Alisjahbana, who was the first Bandung PhD graduated from Nijmegen in the field of TB and Prof. Ida Parwati, who is always

willing to help and has solved so many problems in microbiology, I extend my great gratitude. May we always work together, especially in the field of TB and HIV. And to the successors of this extending train: Arjan van Laarhoven, Sofiati Dian, Ekta Lachmandas ... welcome aboard.

During my early time in the Netherlands, I spent several months in the Amsterdam Medical Center, under supervision of Jan de Gans who generously accepted me not only at the office, but also in the privacy of his house. Thank you, Jan for boarding me in your home. I also thank Elisabeth Jens who always created the homey feeling with rice at dinners, the talk and the coffee (decafeinated!), and the ride along the IJselmeer on weekends.

To all other PhD candidates along the way: Shelly Iskandar, Rudi Wisaksana, Merrin Rutherford, Adhiatma Siregar, Erni Nelwan, Herman Kosasih, Lidya Chaidir, Hinta Meijerink, Susan van Santen, Meta Michels, Khutso Mothapo, Ajeng Pratiwi, who had been (or are still) at some time in their lives a resident of the "PhD room" at route 456 of Radboud Hospital, I thank you for the atmosphere. Wishing you all the best.

To the secretaries of International Health office in Nijmegen, especially Mieke Daalderop, thank you for always willing to help fixing big things as well as small things with sheer generousness.

To the residents in neurology, nurses, field doctors (Regina Loprang, Mia Yasmina, Laila Mahmudah, and Ela Hayati), and pak Kris the social worker, thank you for helping me with the field works. Without your help in screening, recruiting, and doing follow up to our patients, I believe I will not be able to finish following any single patient.

To my colleagues in Clinical Pathology Department of Hasan Sadikin Hospital and microbiology team of Health Research Unit of Faculty of Medicine Universitas Padjadjaran: dr. Agnes Indrati, dr. Asep Zainuddin, Teh Lies, Witri, Neng Rina, mbak Yanti, kang Soni, teh Jessi, Inri; thank you very much for your continuous support. Without your hard work I would not be able to write my papers.

To the co-authors both in Nijmegen and Bandung: Patrick Sturm, Anne-Marie Brouwer, Adri van der Zanden, Willem Melchers, Diba Basar, Isma Novitasari, Reynie Purnama Raya, I am very grateful to have the opportunity to work with you. Thank you very much for the contribution on the actual work as well as reading and writing the papers.

I also would like to express my thankfulness to colleagues in Teratai Clinic (dr. Nirmala, dr. Yovita, dr. Merry, dr. Ajeng, dr. Intan) and people from the former IMPACT (Yusni, Irma, Arthur, Kuri, Boyke, Rozar, Dindin, Berry, Yani, Mawar, Aang, and many more) who have helped me to make this day happened. Kiki Irmawati is thanked for their help with the data collection. Nopi is thanked for quick statistic tutorials every time in need one.

To my friends Annisa, Lika, Darmawan, Rio thank you for the continuous support and the time spent listening to my grumbles. Herlan is thanked for translating my idea into a beautiful cover design.

To my Indonesian family in the Netherlands, pak Dedi dan ibu, Gozali, Rizal "satunya lagi", Fauzan, thank you for always putting a taste of Indonesia during my stays.

For my family, I would start with thanking my belated parents, Umi and Abi for teaching and caring for me and for providing me the highest possible education, and for encouraging me to learn and learn and learn. Blessed are your souls.

Last but not least, I owe very much to my big bunch of brothers and sisters (along with all in-laws, nieces, and nephews) who are always at my disposal and always support me in happy and hard times.

Fundings

The works in this thesis has been financially supported by (1) a PhD-fellowship from Radboud University Nijmegen; (2) funds from the project "IMPACT" (Integrated Management of Prevention, Care and Treatment of HIV/AIDS), a collaborative research and implementation program of Universitas Padjadjaran, Bandung, Indonesia, Maastricht University and Radboud University Nijmegen the Netherlands, and Antwerpen University, Belgium which is funded by the European Commission (SANTE/2005/105-033); (3) SPIN mobility program from KNAW, the Netherlands, 2010, project number 07-MP-10; (4) Research grant year 2011 from Universitas Padjadjaran, Bandung, Indonesia, under ANDALAN-scheme; and (5) Staff Exchange program of Directorate General of Higher Education year 2011, under PHKPHPD-scheme.

About the author

About the author

Ahmad Rizal Ganiem (Rizal) is an Indonesian, born in Bandung, West Java, Indonesia, on 26th of May 1966. He graduated as medical doctor from Faculty of Medicine Universitas Padjadjaran Bandung in 1990. He worked as emergency doctor in hospitals in Bandung for almost 2 years before serving in military service for 6 years as battalion doctor in Ambon, the Moluccas, and in military district hospital in Papua. Since 1999 he did his training in Neurology in the same faculty for 4 years, and after the graduation he continues working in Neurology Department of Hasan Sadikin Hospital. His interest in neurology infectious disease has been nurtured since his neurology residency when he realized that despite the abundant cases of infectious diseases, this field was neglected.

In 2005 he started to establish connection with a group of Indonesian PhD candidates doing researches on pulmonary TB in Bandung with the collaboration of Radboud University Nijmegen the Netherlands. In 2007 he joined IMPACT (Integrated Management of Prevention and Control and Treatment of HIV) program – a five-year program funded by EU – and worked for the neurology part of the program. From 2009 he received PhD fellowship from Radboud University Nijmegen, the Netherlands. During this program he has had opportunity to get trainings both on clinical and research. He also joined numerous international and national congresses to gain knowledge and share experience with scientific community.

Besides working as a neurologist in Hasan Sadikin hospital, he also coordinates the service in the hospital HIV clinic and serves as a member of the institutional research board.

Rizal now lives in Bandung and does photography as a hobby.