TUBERCULOUS MENINGITIS;

REDUCING THE BURDEN OF DISEASE BY IMPROVING DIAGNOSIS AND TREATMENT

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ABSTRACT

Notwithstanding available diagnosis and treatment, tuberculosis (TB) is the most persistent pandemic known to modern man. Of all forms of tuberculosis, tuberculous meningitis (TBM) is the most lethal. The true burden of disease is unknown, but of the people who reach treatment facilities, approximately 30% die, despite therapy. The onset of disease is insidious and diagnosis is often only made after neurological compromise, a factor associated with increased mortality. Diagnosis is further complicated by lack of sensitivity of currently available conventional microbiological techniques. Moreover, recommended treatment regimens are derived from pulmonary TB regimens and not specifically adjusted to treat the infection in the brain, thus neglecting the differential ability of antimycobacterial drugs to cross the blood-brain barrier (BBB). Similarly, drug resistant infection is mostly detected late, because it depends on culture confirmation of this slow growing organism and optimal treatment regimens are unknown. In the context of intracranial infection, drug resistance is even more precarious, with high rates of mortality reported.

This thesis addresses these pressing issues of diagnosis and treatment of TBM. The overarching aims of the studies performed in this thesis are: 1. To improve diagnosis of TBM by evaluating the performance of a novel molecular diagnostic test; Xpert MTB/RIF, 2. To improve treatment of TBM by evaluating an intensified antituberculosis treatment regimen. 3. To improve management of drug resistant TBM by exploring factors associated with drug resistance and evaluating response to intensified treatment.

The Xpert MTB/RIF test was able to rapidly confirm a diagnosis of TBM with a sensitivity of 59.3% (n=108/182 (95% confidence interval(CI): 51.8-66.5)) compared to clinical diagnosis of TBM. Specificity was 99.5% (95% CI: 97.2-100). Particular advantages are the high sensitivity of the test in HIV positive patients and early detection of rifampicin resistance. Among HIV co-infected patients, sensitivity was 78.8% (n=52/66, (95% CI: 77.6-79.7)). Xpert MTB/RIF performance compared favourably with performance of commonly used classical staining techniques in most settings (1-

60%). This represents a significant advance in the early diagnosis of TBM and in particular of rifampicin resistance, which is considered a key drug in treatment regimens. Intensified antituberculosis treatment with higher dose rifampicin (15mg/kg) and additional levofloxacin, however, did not improve outcome in our cohort of 817 HIV infected (n=349) and uninfected (n=468) TBM patients. 113 and 114 patients died in the intensified treatment and placebo arm respectively (hazard ratio(HR), 0.94; 95% CI: 0.73 -1.22, p=0.66). Overall 9-month mortality was 28%, which is still unacceptably high. Predictors of death are HIV infection, disease severity grade on presentation and infection with multidrug resistant (MDR) mycobacteria, defined as at least resistance to rifampicin and isoniazid. The overall mycobacterial resistance rate found in this population was high; 45% of isolates showed resistance to at least one of four first line antituberculosis drugs, 27% were isoniazid resistant and MDR was detected in 5%. Patients with drug resistant infection did not have different presenting symptoms, but were more likely to have a history of previous TB treatment. Early intensified treatment did appear to be beneficial to outcome, in particular in HIV uninfected patients with isoniazid resistance (HR 0.11; 95%CI: 0.01-0.91, p=0.04). Of the 15 patients with MDR TBM, early detection of rifampicin resistance by Xpert MTB/RIF led to a successful early switch to second line therapy in four patients, who all survived up to nine months of follow up. In previous studies, prior to the availability of Xpert testing and secondline drugs, MDR TBM was uniformly lethal.

Early diagnosis and treatment with effective antituberculosis drugs remains the most crucial aspect of management of this devastating condition.

DECLARATION

Other than the support received outlined in the acknowledgements, the work described in this thesis is my own effort and has not been submitted for a degree or other qualification to this or any other university.

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ABBREVIATIONS

AFB	Acid-fast bacilli
ADA	Adenosine deaminase
ALT	Alanine transaminase
MTD	Amplified Mycobacterium Tuberculosis Direct Test
ADH	Antidiuretic hormone
ARV	Antiretroviral
ART	Antiretroviral treatment
AIDS	Aquired immunodeficiency syndrome
AUC	Area under the curve
AST	Aspartate transaminase
BCG	Bacillus Calmette–Guérin
BBB	Blood-brain barrier
BMRC	British Medical Research Council
CRF	Case record form
CDC	Centers for disease control and prevention
CNS	Central nervous system
CSW	Cerebral salt wasting
CSF	Cerebrospinal fluid
cfu	Colony forming units
CT	Computerised tomography
CI	Confidence interval
QTc	Corrected QT interval
CFP-10	Culture filtrate protein-10
Cs	Cycloserine
DSMC	Data and Safety Monitoring Committee
DNA	Deoxyribonucleic acid
DoH	Department of Health
DST	Drug susceptibility testing
DIH	Drug-induced hepatitis
EBA	Early bactericidal activity
ESAT-6	Early secretory antigenic target-6
E	Ethambutol
EMB	Ethambutol
Eth	Ethambutol
XDR	Extensively drug resistant
FLAIR	Fluid Attenuation Inversion Recovery
FDA	Food and drug administration
FIND	Foundation for Innovative New Diagnostics
KatG	Gene encoding catalase-peroxidase
inhA	Gene encoding the isoniazid target
GEE	Generalized estimating equations
HR	Hazard ratio
HCMC	Ho Chi Minh City
HTD	Hospital for tropical diseases
HIV	Human immunodeficiency virus

IMiD3	Immunomodulatory drug 3
ITT	Intention to treat
IFNγ	Interferon gamma
IGRA	interferon gamma release assay
IQR	interquartile range
i.m.	Intramuscular
i.v.	Intravenous
H	Isoniazid
INH	Isoniazid
INH-r	Isoniazid resistant
Km	Kanamycin
LTA4H	Leukotriene A_4 hydrolase
Lfx	Levofloxacin
LCTB	LightCycler Mycobacterium Detection kit
LJ	Lowenstein-Jensen
MRI	Magenetic resonance imaging
MS	Mass spectrometry
MRC	Medical Research council
MODS	Microscopically observed drug susceptibility
MIC	Minimal inhibitory concentration
MoH	Ministry of Health
MDR	Multidrug resistant
MGIT	Mycobacteria growth indicator tube
MOTT	Mycobacteria other than tuberculosis
M.tuberculosis	Mycobacterium tuberculosis
MTB	Mycobacterium tuberculosis
NAT2	N-acetyltransferase- 2
NTP	National TB program
NPV	Negative predictive value
INH-s+RIF-s	No isoniazid or rifampicin resistance
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NTM	Non-tuberculous mycobacteria
NMR	Nuclear magnetic resonance
NAAT	Nucleic acid amplification test
NRTI	Nucleoside reverse transcriptase inhibitors
OR	Odds ratio
o.d.	Once daily
OPC	Outpatient Clinic
Pas	Para-aminosalicylic acid
Cmax	Peak serum concentration
PP	Per protocol
PBMC	Peripheral blood mononuclear cells
p.o.	Peroral
PD	Pharmacodynamic
PK	Pharmacokinetic
PCR	Polymerase Chain Reaction
PPV	Positive predictive value
PEPFAR	President's emergency plan for AIDS relief
Pto	Protionamide
Z	Pyrazinamide

RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
R	Rifampicin
RIF	Rifampicin
RIFMDR	Rifampicin monoresistance or multidrug resistance
RRDR	Rifampicin resistance defining region
RIF-r	Rifampicn resistant
SAE	Severe adverse event
SAP	Statistical analysis plan
S	Streptomycin
STR	Streptomycin
SIADH	syndrome of inappropriate ADH secretion
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
TIA	Transient ischaemic attack
TST	Tuberculin skin test
TB	Tuberculosis
TBM	Tuberculous meningitis
TNFα	Tumor necrosis factor alpha
US	United States
ULN	Upper limit of normal
VNTR	Variable number tandem repeat
WCC	White cell count
WHO	World Health Organization

CHAPTER 1

1 INTRODUCTION

"Yea, I have known inflammations, Imposthumes, whelks, scirrhus Tumors growing to the Meninges, with the Skull, and other Diseases of an evil conformation, excited in the Membranes of the Brain; by which at first for a long time, frequent headache, and most cruel, and then afterwards a sleepy and deadly distemper hath been induced; the cause of the Disease not detected, but after Death by the Anatomy; and indeed it is to be suspected that inveterate and pertinacious pains in the Head, which return, and daily become more tormentive, in spight of all Remedies depend upon some such invincible Cause."

Thomas Willis (1621-1675), from "De Anima Brutorum" (1672)[1]

1.1 Tuberculosis

Tuberculosis (TB) has caused more deaths through the last 200 years than any other infectious disease

Figure 1.1), and has been with us since ancient times, with evidence found in in 9,000 year-old mummies[2, 3].

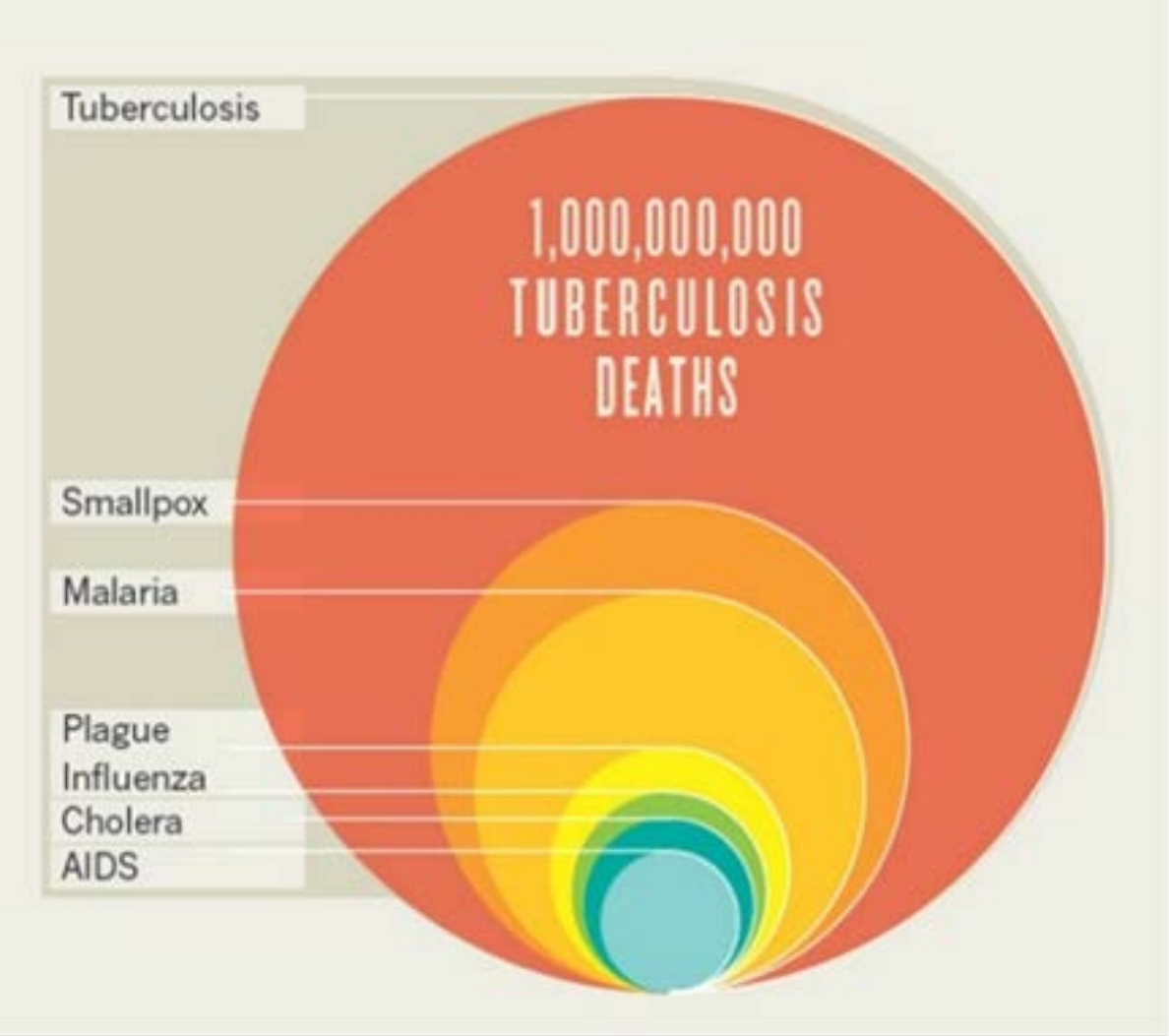


Figure 1.1 Deaths from infectious diseases in the past 200 years

(from T. Paulson, Nature 2013[3], reprinted with permission)

TB is a chronic granulomatous disease caused by the bacterium *Mycobacterium tuberculosis (M.tuberculosis)*. The term "tubercle" (from: tuberculum, "small lump" in Latin) in the context of consumptive ("wasting") disease was first coined by a Dutch anatomist, Franz de le Boë (1614-1672). He found tubercles in the lungs of most consumptives. Before the discovery of the pathogen in 1882 by Robert Koch, the spectrum of diseases caused by the mycobacteria were known by many names including: consumption, phtisis (from Greek "phtinein" to waste away), scrofula (swelling of lymphnodes, especially in the neck), Pott's disease (tuberculous spondylitis, named after a British orthopedic surgeon Percivall Pott, in the 18th century, but found in Egyptian mummies and art as early

as 1000 BC) or the “King’s evil”, as in the middle ages, it was thought that royal touch could cure the disease. The European TB epidemic, was named “the white plague” in the 17th century. The causative agent was discovered in 1882 by Robert Koch (1843-1910). The discovery was met with euphoria, as new hopes were raised of a cure.

1.1.1 THE EPIDEMIOLOGY OF TUBERCULOSIS

More than a century later, in 2014 there were an estimated 9.6 million new cases of active TB and 1.5 million deaths; therefore, there is one new TB case every 3 seconds and more than two TB-related deaths every minute[4]. Twenty-two high-burden countries account for 80% of all TB cases, with India and China alone contributing almost 40% (26% and 12% respectively). The TB incidence per 100,000 population varies dramatically, from less than 10 per 100,000 in developed countries such as Japan, the United States, Western Europe and Australia, to rates exceeding 1,000 per 100,000 in South Africa and Swaziland[4, 5] (Figure 1.2). Overall, it is estimated that just 64% of incident TB cases were notified to National TB Programmes in 2013[5].

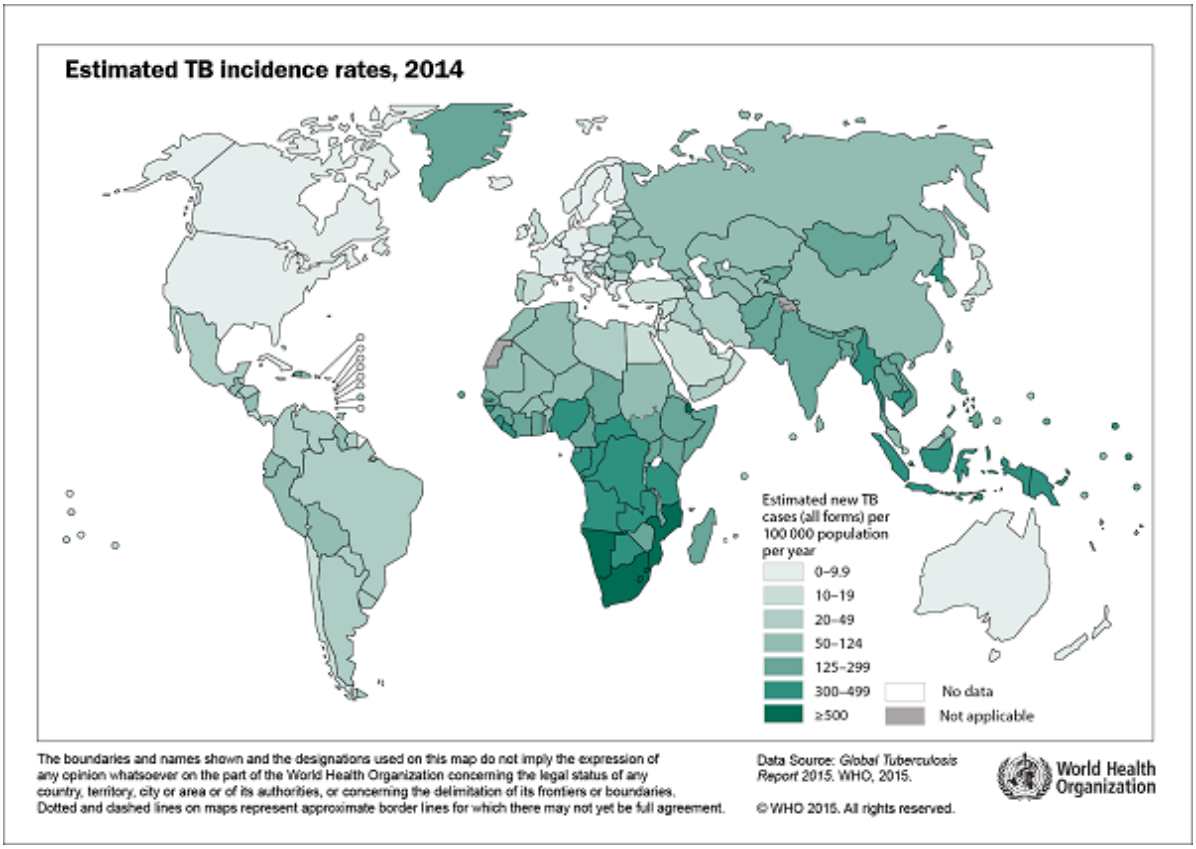


Figure 1.2 WHO estimated TB incidence rates in 2014

(Source: WHO[4], reprinted with permission)

In high burden settings TB has its peak incidence in early adulthood, affecting the most economically productive age-groups. Whilst in low burden countries, TB is more common in the elderly; also in immigrant populations and the socially destitute. In the United States (US) 63% of the 9,945 TB cases (a rate of 3.2 cases per 100,000 persons) reported in 2012 were among immigrants; with case rates 11 times higher than among US-born citizens. (http://www.cdc.gov/tb/statistics/reports/2012/default.htm). TB notifications are usually higher among men than women in a ratio of approximately 2:1. Various theories have been proposed to account for this difference including differences in smoking rates, occupational lung damage, social networking patterns and immune function. It is likely that the causes are multifactorial and include potential detection bias in settings where women have greater difficulty in accessing health care. A systematic analysis for the global burden of disease study 2010, including mortality data from 187 countries from

1980-2010 ranked TB as the tenth leading cause of death globally. Of the 52.8 million deaths of all causes globally in 2010, 1.2 million were attributable to tuberculosis[6].

Infection with human-immunodeficiency virus (HIV) greatly increases the chances of an individual developing active TB following exposure, or of having reactivation of latent disease, with the probability increasing as immunosuppression advances[7]. For an HIV uninfected individual with latent TB there is a 10% lifetime risk of developing active TB disease, while for those with HIV there is a 10% annual risk[8]. 1.2 million (13%) of the incident TB cases in 2014 were in people living with HIV/AIDS and 74% of these were in sub-Saharan Africa. TB is the leading cause of death among HIV-infected patients, with an estimated one in three HIV-related deaths attributed to TB[4].

Young children with TB are generally less infectious and due to the difficulty of confirming a TB diagnosis in this age group, data has not been systematically collected on the TB disease burden suffered by children and many are treated without notification. However, since 2010 countries have been encouraged to report age disaggregated data to the World Health Organization (WHO) for children less than 5 years and 5-14 years of age. Despite being limited by poor case ascertainment and incomplete reporting, WHO estimates in 2014, 140,000 children died of TB of which 55,000 were HIV infected[4, 9]. The contribution of TB to child mortality is undetermined, particularly in TB endemic areas. Recent estimates are that approximately 1 million incident cases occur among children every year[10], while the contribution of TB to under-5 mortality is likely to be underestimated in TB endemic areas, especially among children dying from pneumonia, malnutrition and meningitis[11]. Pooled analysis of autopsy studies identified TB in ~10% of 811 children (both HIV-infected and -uninfected) who died from respiratory disease in five African countries[12]. Of the estimated 1.3 million deaths in children attributed to pneumonia in 2011, most occurred among young children living in TB endemic areas[13]. Apart from its contribution to “pneumonia deaths”, TB may also be the underlying cause in a substantial number of children dying from meningitis, presumed sepsis, HIV/AIDS or severe malnutrition.

Smoking, diabetes and other co-morbidities increase susceptibility to active TB. The increasing prevalence of diabetes, particularly in developing Asian countries such as India and China has focused attention on the link between diabetes and TB susceptibility and in 2011 WHO issued guidelines for the integrated management of TB among diabetes patients[14]. It has been predicted that global diabetes prevalence will increase by 69% by 2030, with 80% of prevalent cases in the developing world[15]. Individuals living with diabetes have a 2-3 times higher risk of developing active TB; around 10% of TB cases globally are now linked to diabetes[14]. The Stop TB Strategy was launched in 2006 and now aims to eliminate TB (defined as <1 case/million population) by 2050. (http://www.who.int/tb/features_archive/global_plan_to_stop_tb/en/). Efforts towards elimination are challenged by the HIV pandemic and the increasing prevalence of drug resistant strains of *M.tuberculosis*.

1.1.2 THE PATHOGEN

TB is caused by bacteria of the Mycobacterium tuberculosis complex, mostly *M.tuberculosis* , but rarely also *M.canetti*, *M.microti*, *M.africanum*, and *M.bovis*[16]. Mycobacteria are non-motile, non spore-forming, aerobic, rod-shaped bacteria of 2-4 μm in length and possess a unique lipid-rich cell wall which gives the ‘acid-fast’ property by which they are known (acid-fast bacilli, or AFBs) and renders them resistant to many disinfectants and antibiotics (Figure 1.3). They can be divided into slow growing or rapid growing species.

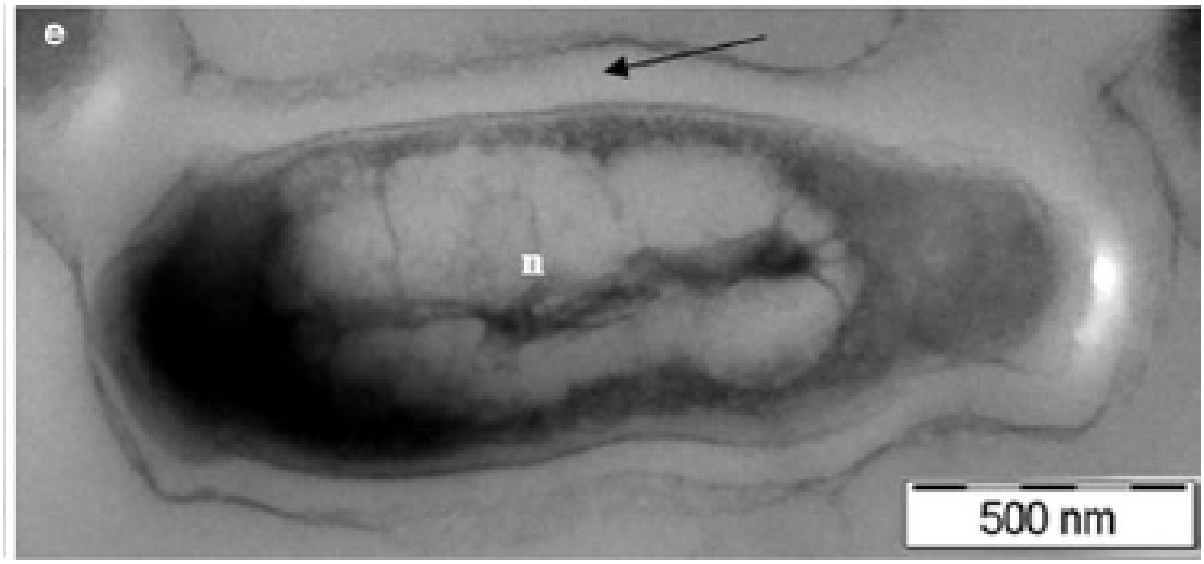


Figure 1.3 Transmission electron microscope image of Mycobacterium tuberculosis

The black arrow indicates the thick myolic acid layer. The n. indicates the nucleide (from: V, Srinivasan et al., 2014[17], reprinted with permission)

M.tuberculosis is slow-growing, non-pigmented and appears as cream coloured 'breadcrumbs' on culture, often also described as 'rough, tough and buff'[18] (Figure 1.7). Other mycobacteria are variously described by the synonymous terms non-tuberculous mycobacteria (NTM), mycobacteria other than tuberculosis (MOTT) and atypical mycobacteria. NTM management is complex and poorly standardized due to differences in disease presentation and available treatment options. The only other major human pathogen of the mycobacteria genus is *M.leprae*, which causes leprosy[19].

The whole genome of *M.tuberculosis* (laboratory strain H37Rv) was sequenced in 1998[20]. Subsequent sequencing of clinical strains from around the world has illuminated pathogen diversity, evolution and spread[21]. Six major geographic lineages of *M.tuberculosis* have been identified: the Euro-American, Indo-Oceanic, East-Asian (including Beijing strains), West-African 1 and 2, and East-African-Indian. Many studies have attempted to identify lineage-specific differences in clinical virulence and/or transmissibility, but results have been conflicting. These different findings may be the result of differences in the particular strains used for comparison, variation in host genetics, environmental influences or different study methodologies.

Some strains (eg. Beijing and Haarlem strains) have been associated with increased drug resistance. This may result from intrinsic factors such as increased genetic mutation rates, intrinsic drug tolerance, lower fitness cost associated with resistance-conferring mutations[22], or from environmental factors that facilitated its emergence and spread. Current typing methods such as spoligotyping, IS6110 restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR) have value for outbreak investigations and studies of population transmission, but do not offer any information to guide treatment. Advances in the speed and cost of whole genome sequencing will soon supersede other typing techniques and would be far more informative, facilitating detailed transmission mapping and proving information on likely drug-resistance to guide clinical management[21, 23-29].

1.1.3 DISEASE PROGNOSIS

TB is a curable disease. The fact that it remains the most pressing public health problem for a significant proportion of the world, despite the availability of a cure and knowledge on prevention of transmission shows how medicine can fail without commitment at all levels of society. The distribution of the TB pandemic painfully demonstrates the inequalities in health care delivery globally. Over 95% of cases and deaths are in low and middle income countries. In general, prognosis of outcome is dependent on a multitude of factors: host factors (genetic variance, co-morbidities, HIV-coinfection, treatment adherence, access to healthcare) and pathogen factors (pathogen virulence, drug-resistance) and the site of the infection (pulmonary or extra pulmonary). The principle factors in a favourable outcome are early recognition, drug susceptibility and appropriate treatment. Without treatment, the case fatality for sputum culture positive (HIV negative) patients is estimated to be 70%, in contrast with sputum culture negative patients for whom it is estimated to be 20%[30]. The treatment success rate (either cured or finished a full course of treatment) for newly diagnosed sputum positive TB patients reported for the US in 2011 (according to WHO) was 78%. For new smear negative and extrapulmonary TB, treatment success rate is 85% (http://www.who.int/gho/tb/epidemic/treatment/en/).

TB is the most common cause of death among HIV patients, estimated to cause 26% of AIDS related deaths. The treatment success rate globally for all new TB patients without HIV was 87%, in contrast with a 73% success rate for new TB patients with HIV[31]. The most lethal form of TB is TBM, which, when treated, has a mortality of approximately 25% in HIV negative patients and can exceed 60% in HIV positive patients. Half of TB meningitis survivors will suffer neurological sequelae[32, 33].

Drug resistant TB carries a higher mortality than drug susceptible TB. Of the 480,000 multidrug resistant (MDR, defined as resistance to at least rifampicin and isoniazid) estimated cases, only about a quarter, 123 000 patients, were detected and reported and 111,000 started MDR treatment[4]. Treatment outcome data was available for 70,000 patients enrolled on treatment in 2012, only 50% successfully completed treatment and 16% died, the remaining had treatment failure, were lost to follow up or had no data available[4]. Of the 2,685 extensively drug resistant (XDR, defined as resistance to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin)) cases for whom outcome data were available, 30% died, and only 26% were successfully treated[4].

1.2 Tuberculous meningitis

The earliest descriptions of intracranial tuberculosis date back to the 17th century. Physicians frequently used the term “acute hydrocephalus” or “dropsy of the brain” for a condition in children in which the etiology was unknown, but presented with fever, headache, vomiting and rapid death. Some of the early historical descriptions vividly illustrate the despair of both patient and doctor and are in line with our current understanding of disease pathogenesis; *Nec minus a phlegmone et abcessu quam hujasmodi meningitis et tuberculis, cephalgiae lethales et incurabiles oriuntur (Sometimes the headaches, fatal and incurable, follow abscesses and swellings of the envelopes of the brain, as well as placques and tubercles of these membranes) (Thomas Willis, 1672[1]).* Willis was far ahead of his time as it was not until 150 years later that the tubercles found upon autopsy were regarded as a

distinguishing aspect of the clinical syndrome, which was only then proposed to be a tubercular meningitis.

The term tubercular meningitis was first used in 1836 by P.H. Green in the Lancet. Green introduced the term tubercular meningitis to describe the condition of the cerebral membranes, which were affected by tubercular lesions in nine-tenths of the cases in a series of 45 children, who at the same time had tubercular deposits in the lungs or the abdomen. Green argued that these findings were a more essential characteristic of the disease than the accumulation of cerebrospinal fluid (CSF)[34, 35].

1.2.1 PATHOGENESIS

The disease was still thought to develop in a manner analogous to other meningitides, through haematogenous spread and direct seeding into the subarachnoid space, until a meticulous serial autopsy study of 82 patients by Rich and McCordock in 1933 provided the basis of our current understanding of TBM pathogenesis. In guinea pigs and rabbits, Rich and McCordock could only provoke inflammation of the meninges by the direct inoculation of bacilli into the central nervous system (CNS), and not by peripheral injection and consequent haematogenous spread. Key findings were that, in humans, after autopsy, firstly, the tubercles found in the brain were seldom of the same age as those found in other organs. Second, vasculitis found in the cerebral vessels was a process originating from the adventia inward, rather than coming from the lumen. Which led to the conclusion that TBM was more likely to be the result of an existing focus within the brain, rather than caused by direct haematogenous spread of bacilli. These findings led them to form a coherent hypothesis in which they postulated that, after inhalation of the pathogen a short-lived bacteremia followed, during which bacilli spread throughout the body and seeded the surface of the brain, forming small granulomas, known as Rich foci. These can exist without causing symptoms for an unknown period but may rupture and upon release of the mycobacteria, meninges become inflamed giving rise to a multitude of possible pathological consequences in the CNS[36].

Incorporating more recent pathological studies (which are unfortunately scant) Donald and colleagues[37], have proposed the following classification of pathogenesis in TBM:

(1) The haematogenous dissemination of bacilli from the primary complex establishes a cortical or meningeal focus. Soon after its establishment this proceeds to caseate and discharge its contents into the sub-arachnoid space. In young children this haematogenous dissemination is particularly likely to take the form of miliary tuberculosis.

(2) In a small minority of cases haematogenous dissemination may establish a caseating focus in the choroid plexus or in the walls of the ventricles from which TBM may develop.

(3) Haematogenous dissemination at the time of primary infection, or later, establishes a cortical or meningeal focus. This is initially controlled, but may at any time thereafter undergo caseation and discharge its contents into the subarachnoid space.

(4) Rarely, a caseous process extends from adjacent structures such as the vertebrae or middle ear to involve the CNS.

As a result of the infection, a dense gelatinous fibrinocellular leptomeningeal exudate is formed. Microscopically this exudate contains small and large mononuclear cells, including epithelioid cells, which also act as macrophages. The exudate typically centres around the interpeduncular fossa. When substantial, the exudate may extend anteriorly to the suprasellar region, and it may extend through the prepontine cistern and surround the spinal cord and cerebellum, often into the Sylvian fissures. It can envelope and compress cranial nerves and arteries. Vasculitis may develop giving rise to ischaemic events. Hydrocephalus can develop by blockage of CSF circulation when exudates cover the choroid plexus, the basal subarachnoid cisterns around the midbrain and pons or when tuberculoma cause narrowing of the aqueduct and third ventricle[38, 39]. Exudate, vasculitis, and hydrocephalus can cause changes in brain parenchyma. “Border-zone encephalitis” describes a tissue reaction commonly seen in brain tissue adjacent to zones of thick adherent exudate. The brain tissue softens, showing signs of oedema, perivascular infiltration and microglial reaction[39, 40]. Hydrocephalus, raised

intracranial pressure and vascular events are central to brain parenchyma damage and consequent morbidity and mortality[41, 42].

1.2.2 BURDEN OF DISEASE

The burden of extra-pulmonary TB is tightly associated with the general pandemic and accounts for more than 10% of all TB cases[14]. Of these, about 5% are forms of tuberculosis in the CNS. These estimates are crude since diagnosis of extra-pulmonary TB in general and CNS-TB in particular, is challenging and according to WHO definitions, a patient that has both signs of pulmonary and extra-pulmonary TB should be classified as having pulmonary TB[14].

In endemic settings tuberculosis is often the leading cause of childhood bacterial meningitis [43]. In 2009, 7% of all annual cases of bacterial meningitis and septicemia in the UK were caused by TB, being the third leading cause after meningococcal and pneumococcal disease[44]. With the introduction of Meningococcal C (1999) and pneumococcal (2006) vaccines in the routine immunization schedule in the UK (and in the absence of an effective TB vaccine), TBM may well become the leading cause in the UK.

In high TB-burden countries, young children aged 0-4 are mostly affected, however rarely younger than three months [45]. The age range from 5-15 is often referred to as “the favoured age” as this population has the lowest rate of tuberculosis of all forms[46]. In countries with a low prevalence of TB, most cases of TBM are in adults. Commonly reported risk factors are alcoholism, diabetes, recent corticosteroid use, malignancy and immunosuppression[47]. The emergence of HIV has dramatically changed the dynamics of the TB pandemic.

Mortality and morbidity

Mortality among TBM cases remains high, and varies depending on age, risk group (HIV), stage of disease upon diagnosis, drug-sensitivity of the infecting organism, time between onset of symptoms and initiation of effective antibiotics and the sophistication of healthcare infrastructure and facilities.

Mortality in children ranges between 10-20%[45, 46, 48, 49]. Neurological sequelae are frequent and reported in more than half of surviving children. In a retrospective survey of 554 children in South Africa, 74% of patients suffered long term disabilities or death (13%), including hearing or vision impairment (14 and 16% respectively), motor deficits (44%) and cognitive impairment (77%)[50]. Untreated TBM is uniformly fatal.

In 1948 the British Medical Research Council (BMRC) first published a classification of TBM patients according to the severity of disease (Table 1.1), which has been modified over the years to a comprehensive grading system, with slight variations. The grading definitions used in the studies for this thesis are listed in Table 1.2

.

Table 1.1 TBM grading according to the BMRC 1948

Grade	Description
Early	Patients with mainly non-specific symptoms, with little or no clinical signs of meningitis, with no pareses, in good general condition, and fully conscious. Diagnosis established mainly on findings in cerebrospinal fluid.
Advanced	Patients obviously extremely ill, deeply stuporose or comatose, or with gross pareses
Medium	Patients in a condition between those of the first two groups

BMRC= British Medical Research Council

Table 1.2 Modified BMRC TBM grading

BMRC grade	Diagnostic criteria
Grade1	Glasgow coma score 15, no focal neurology
Grade 2	Glasgow coma score 11-14 OR Glasgow coma score 15 with focal neurology
Grade 3	Glasgow coma score ≤ 10

BMRC=British Medical Research Council

In Vietnam, for HIV uninfected adult patients receiving adjunctive corticosteroid therapy overall mortality is approximately 30% [51]. Mortality increases with delayed presentation and advanced stage of disease (Table 1.3). As in children, in adults, permanent neurological disability affects over half of the survivors[51]. Co-infection with HIV has a major impact on mortality. In Vietnam, between 2005-2007, overall mortality, in antiretroviral (ARV) naïve patients was 57.5%[33].

Table 1.3 Mortality in TBM

Mortality % (n dead/n)	**HIV negative** (n=274) *****	**HIV positive** (n=252) †
Grade 1	16.7 (15/90)	40.0 (32/80)
Grade 2	31.1 (38/122)	57.1 (56/98)
Grade 3	54.1 (34/62)	75.0 (57/74)
Overall	**31.8 (87/274)**	**57.5 (145/252)**

**including 274 TBM patients in a randomized controlled trial (RCT) on dexamethasone adjunctive treatment, only included patients who received adjunctive dexamethasone. 44 patients were HIV infected, of which 27 died (61.4%)[51]*
†RCT including 252 HIV infected patients randomized, 126 in the immediate antiretroviral treatment (ART) group and 126 in the deferred ART group[33].

In HIV infected children in India 6-month mortality has also been reported to be substantially higher than in HIV negative children (36% vs. 10%) [52]. These mortality figures reflect the severe immune-suppression in these HIV infected patients, illustrating the importance of commitment of policymakers to invest in accessible integrated TB/HIV care.

The influence of HIV

HIV patients are more likely to develop extra-pulmonary forms of tuberculosis, including TBM, and have a higher mortality. Diagnosis of TB can be more challenging in HIV patients due to a less specific presentation, wider differential diagnosis and lower sensitivity of sputum smear microscopy. However, in contrast, CSF smear is more likely to be positive in HIV infected individuals with TBM due to higher bacillary loads[53]. Patients with low CD4 levels and smear negative results, may have atypical CSF findings, immunological tests are not reliable and neuroimaging may not reveal typical lesions and includes a wider differential diagnosis[54]. Polypharmacy may prove problematic, with higher toxicity from combined ART and antituberculosis regimens and drug interactions. ART naïve patients who commence ART treatment during their treatment of TBM may present with immune reconstitution inflammatory syndrome (IRIS), which may be particularly detrimental if it presents intra-cranially[55].

Long term disability

Very little is known about long-term outcome and disability for both children and adults. Anti-tuberculosis chemotherapy has been unsuccessful in completely preventing long-term sequelae; in many cases diagnosis may be too late but in others significant neurological damage occurs subsequent to initiation of treatment. Especially in children, neurocognitive impairment can jeopardize development, education, quality of life and place a great burden on families, schooling and medical systems. A recent long-term follow-up study on a South African cohort of pediatric TBM patients who were severely ill on presentation (grade II or III), reported only 20% of children to be functionally normal at follow-up (median 6 years after TBM treatment completion). The main areas of functional deficit were cognitive impairment (80%), poor scholastic progress (43%), and emotional disturbance (40%). A smaller proportion of children (25%) had evidence of motor impairment[56]. In a Danish nation-wide, population based cohort study with up to 30 years follow up, TBM was associated with an almost two-fold increased long term risk of dying compared to a background population (mortality risk ratio (MRR) 1.79 (95% CI: 1.09–2.95)). In this study the underlying cause of long-term death was most frequently TBM itself rather than secondary to the most commonly reported neurological sequelae[57].

Neurological sequelae most frequently described in adults are cognitive impairment, motor deficits, cranial nerve palsy and optic atrophy[58]. A 5-year follow up on Vietnamese adults with TBM who took part in a randomized controlled trial on the effect of dexamethasone on survival, could only demonstrate an overall benefit of dexamethasone up to two years following treatment. Five years after treatment completion there was no difference in overall survival or disability outcome in both groups. In the group receiving dexamethasone 48.4% (versus 52.7% in placebo group) of patients had died at 5 years (31% at nine months), 6.8% (versus 7.4% in the placebo group) were severely disabled, 17.2% (versus 14.8% in placebo group) had intermediate disability and only 27.6% (versus 25.1% in the placebo group) had good outcome[59]. However, the beneficial effect of dexamethasone was preserved at 5 years for patients with grade I TBM at presentation, demonstrating that, contrary to

preceding medical wisdom, patients with grade I TBM are the group who gain the greatest benefit from corticosteroids.

Immunization BCG protection for TBM

The controversies surrounding the protection that the Bacillus Calmette–Guérin (BCG) vaccine confers to adult pulmonary and meningeal tuberculosis still exist. In adults reported efficacy against all forms has ranged from as high as 80% to zero. It is proposed that the vaccine establishes immunity by inducing effector memory T-cells in the lungs that gradually wane after 10-15 years, rather than building a longer lasting "central memory"[60]. Various theories have been proposed for the differences observed in efficacy, including differences in the circulating *M.tuberculosis* strains, BCG vaccine strains, or pre-immunization exposure to environmental mycobacteria. Nonetheless consensus on the benefit of prevention of severe forms of childhood TB including TBM and miliary TB is established. The vaccine is thought to be 52% to 86% protective against developing the severe complications of childhood tuberculosis such as miliary tuberculosis and tuberculous meningitis[61]. It has been estimated that the 100.5 million BCG vaccinations given to infants in 2002 would have prevented 29,729 cases of TBM in children during their first 5 years of life, or one case for every 3,435 vaccinations, and 11,486 cases of miliary tuberculosis, or one case for every 9,314 vaccinations. Based on these data it is considered a cost-effective intervention in South-East Asia, Africa and the Western Pacific, where TB infection rate and vaccine coverage are highest[61]. In BCG vaccinated children in India who do develop TBM, the clinical spectrum of disease does not seem to be ameliorated[62]. The BCG is the most widely used vaccination globally.

1.2.3 DRUG-RESISTANCE

WHO estimates 20% of *M.tuberculosis* strains worldwide are now resistant to at least one first-line drug [63]. Multidrug resistant (MDR)TBM is resistance to at least rifampicin and isoniazid, the two most effective first-line agents. MDR-TBM has been associated with very high mortality in both children and adults[64-66]. Drug resistance in TBM is rarely diagnosed in time to make appropriate

treatment adjustments. The difficulties of access to rapid TB drug susceptibility testing in much of the world are compounded by the rarity of a positive isolation of *M.tuberculosis* from the CSF. Drug resistance prevalence among TBM cases will generally follow similar patterns to those observed regionally for pulmonary TB.

Isoniazid resistance in the absence of concomitant rifampicin resistance is more prevalent than MDR-TB; 8.1% of *M.tuberculosis* strains found in new cases of TB globally are now resistant to isoniazid, compared to 7% in 2010[4, 63].Since isoniazid is the most effective drug in decreasing the bacterial load in the first two days of anti-TB treatment, early recognition of resistance is of great importance, particularly in TBM cases where rapid killing of bacilli is likely to be crucial. In Vietnam, isoniazid-resistance alone or combined with resistance to other drugs was found in a third of culture positive samples in adult HIV patients with TBM, with 4.3% MDR TBM[67]. In a cohort with predominantly HIV-negative patients, isoniazid-resistance was found in 37.1% of samples of which 21% were MDR (overall MDR-rate was 5.6%)[64]. Mortality from MDR TBM was 100% in the absence of available second-line therapy. In children with a culture proven diagnosis of TBM, MDR-TB or rifampicin mono-resistance was identified in five per cent of cases in South Africa. Multi-drug resistance, not surprisingly, was associated with very high mortality (83%)[68].

Isoniazid resistance without rifampicin resistance also has a significant impact on mortality. An initial analysis of a cohort of TBM patients in Vietnam failed to find a significant impact of isoniazid resistance (+/-streptomycin resistance) on mortality[69], but a larger study in Vietnamese HIV positive patients showed a significant reduction in survival (adjusted hazard ratio (HR), 1.78, 95% confidence interval (CI), 1.18 to 2.66) among patients infected with isoniazid resistant, rifampicin susceptible strains compared to those with fully susceptible isolates[67]. In a retrospective cohort study in the US, researchers also found a significant increase in risk of death associated with isoniazid resistance (odds ratio (OR) 1.61, 95% CI 1.08 to 2.40) [70]. Among Vietnamese HIV positive adult patients, the adjusted HR for mortality of MDR-TBM patients compared to patients with isolates susceptible to all agents or streptomycin monoresistant was 5.21 (95% CI, 2.38 to 11.42). Management of drug resistant TBM will be further discussed in chapter 5.

1.2.4 CLINICAL PRESENTATION

General symptoms on presentation

TBM typically presents in a subacute manner. Presenting signs, symptoms, CSF findings and frequencies are shown in Table 1.4. In adults the majority of patients present with fever, headache, nuchal rigidity, vomiting, meningism, abnormal mental stage and occasionally photophobia [71-73]. Weight loss, night sweats, lethargy and cough have also been reported [74]. The mean duration of symptoms is typically more than 5 days.

Table 1.4 Presenting findings in adults and older children with TBM

	FREQUENCY/RANGE
SYMPTOM	
Headache	50-80%
Fever	60-95%
Vomiting	30-60%
Photophobia	5-10%
Anorexia/Weightloss	60-80%
CLINICAL SIGN	
Neckstiffness	40-80%
Confusion	10-30%
Coma	30-60%
Cranial nerve palsy	30-50%
VI	30-40%
III	5-10%
VII	10-20%
Hemiparesis	10-20%
Paraparesis	5-10%
Seizures adults	5%
Children	50%
CEREBROSPINAL FLUID FINDINGS	
Clear appearance	80-90%
Opening pressure>25cm H2O	50%
Leucocyte count (x10(3)/ml)	5-1000
Neutrophils	10-70%
Lymphocytes	30-90%
Protein (g/l)	0.45-3.0
Lactate (mmol/l)	5.0-10.0
Glucose CSF/Blood<0.5	95%

(from: Thwaites et al., British Infection Society guidelines 2009[71], reprinted with permission)

Typical findings upon neurological examination are sixth cerebral nerve palsy (present in up to 40% of patients) (Figure 1.4) but also the third and seventh nerve are often involved (5-20%).

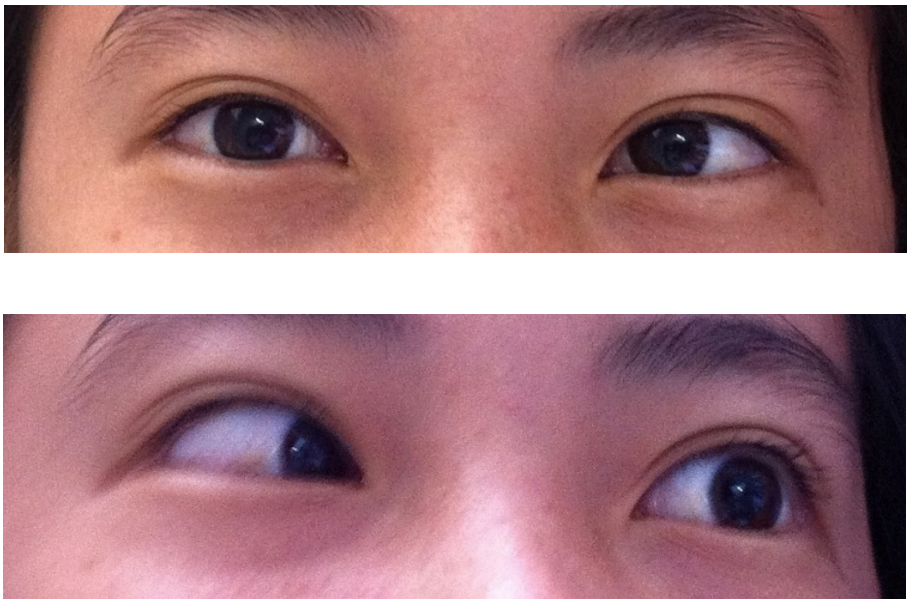


Figure 1.4 Sixth nerve palsy in a TBM patient

A female TBM patient with grade 2 disease, showing a sixth cranial nerve palsy on the left, a typical finding in TBM

Hemiparesis and paraparesis (frequently with urinary retention) may be present upon presentation (in 5-20% of patients)[71, 72], though hemiparesis may also develop during treatment secondary to infarction. Seizures are rarely a presenting symptom in adults, however in children are reported in around 50% of patients[49, 50]. Visual disturbances, decreased vision, diplopia may have a variety of causes including primary involvement of optic nerve by tuberculous lesion leading to optic neuritis, optochiasmatic arachnoiditis, tuberculoma in the chiasmatic region or in the optic pathways[75]. More often visual disturbances are secondary to raised intracranial pressure or ethambutol toxicity. Tuberculoma can cause a wide array of neurological symptoms associated with space occupying lesions, depending on their location within the CNS[76] (Figure 1.5). Urinary retention is common which may indicate spinal cord involvement. Movement disorders are associated with basal ganglia involvement, mostly tremor, but also chorea, ballismus and myoclonus have been reported[72].

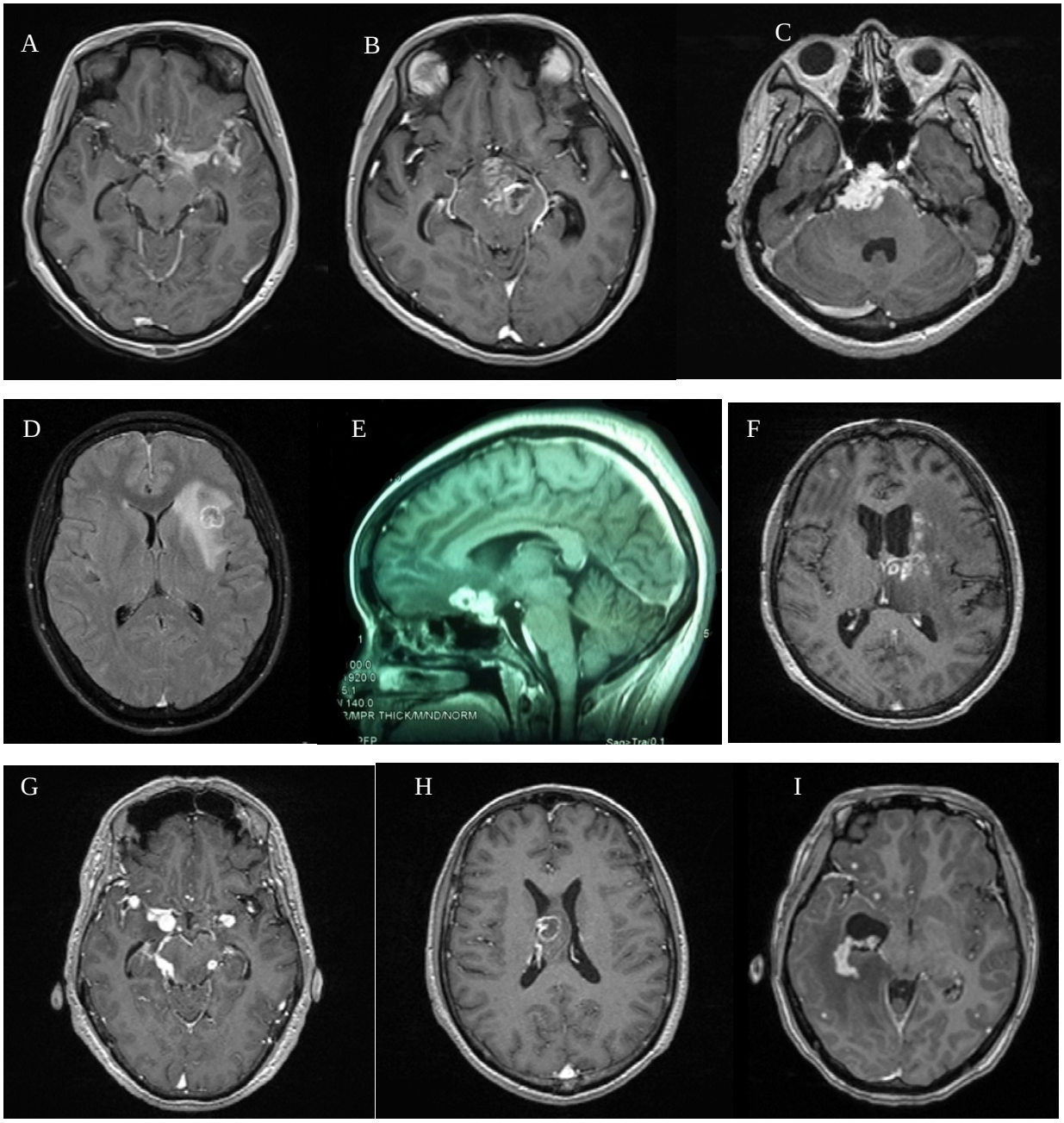


Figure 1.5 Tuberculoma of the brain in TBM

Gadolinium enhanced T1 and T2 MRI images of nine different patients with TBM
A. 29-year old female patient with scan showing basal meningeal enhancement and ring enhancing lesion (tuberculoma), mainly on the left side. B. 48-year male patient with multiple enhancing lesions in the brainstem, extending from the midbrain to the pons. C. An 18-year old male patient with tuberculomatous thickening of the basal meninges with lesions extending into the brainstem. D. A T2 FLAIR scan of a 28-year old female patient with multiple tuberculoma throughout the brain, this section showing a ring enhancing lesion in the left capsula externa with surrounding oedema. E. A 24-year old female with visual field disturbances, MRI revealing enhancing lesions involving the optic chiasm, extending to the hypothalamus and frontal lobe. F. A 29-year old male patient with multiple ring enhancing lesions in the left basal ganglia (involving the head of caudate nucleus, and thalamus) and capsula interna with surrounding oedema and associated enlargement of both lateral ventricles. G. A 40-year old male patient with solid and ring enhancing lesions of the basal meninges. H. A 53-year old male patient with a ring enhancing lesion and thickening of the septum pellucidum. I. A 36-year old female patient with an extensive enhancing lesion throughout the enlarged right lateral ventricle with extensive surrounding oedema and multiple ring enhancing lesions in the brain in both hemispheres.
FLAIR=Fluid Attenuation Inversion Recovery

The initial symptoms of tuberculous meningitis may be non-specific, but within the context of prolonged symptoms (more than 5 days), a previous history of tuberculosis or a chest X-ray consistent with recent or past tuberculosis infection, this history must raise heightened suspicion with the treating physician. In many textbooks, TBM is described as chronic or subacute meningitis, however this terminology is misleading. Once a patient with TBM seeks medical care they should be treated as a medical emergency as with any other form of meningitis.

HIV associated TBM

The emergence of HIV has changed the epidemiology of tuberculosis and particularly the clinical outcome of disease. HIV infected patients are more likely to develop extrapulmonary forms of the disease. TBM is considered an AIDS defining condition. Research studies do not suggest that HIV greatly alters the clinical presentation of TBM, especially in patients with higher CD4 counts, the clinical presentation may mirror the one seen in HIV negative individuals. Patients with lower CD4 counts, may have a more atypical course of disease, with less specific and more subtle signs, wider differential diagnosis, rendering diagnosis more challenging[54]. Therefore, HIV infected patients may present later during the course of disease, with altered consciousness, and consequently more often in the advanced stages of disease[53]. HIV patients are more likely to have impaired cognition, generalised lymphadenopathy and hepatosplenomegaly[52]. A retrospective study comparing clinical presentation and outcome in children with and without HIV infection surprisingly found that HIV uninfected children were more likely to present with a decreased level of consciousness; this may be related to the poor immune response in immunocompromised children. Similar to adults, HIV infected children had a longer history of being unwell, poorer nutritional state, more commonly had accompanying hepatosplenomegaly, lymphadenopathy and otorrhea[77]. Despite the similarity in presentation outcome is significantly worse for HIV infected adults and children with tuberculous meningitis.

Paradoxical treatment response

Paradoxical responses during anti-tuberculosis treatment are frequently reported, despite appropriate chemotherapy against susceptible bacilli. This can be encountered in all tissues, but most often in the lungs, lymph nodes and the brain[78]. In the brain, tuberculoma may develop or enlarge during treatment for pulmonary TB, TBM or miliary TB. These are either discovered on routine brain imaging or accompanied by worsening of symptoms, signs of a space occupying lesion or convulsions. This generally occurs within one to four months of starting treatment, often after initial improvement. Antituberculous therapy should be continued, the addition of systemic corticosteroids may be considered[79]. Within the context of a sound diagnosis and microbiological confirmation of a susceptible pathogen, paradoxical response can be diagnosed clinically; however incorrect diagnosis, drug-resistance and cerebral infarction may be alternative causes of deterioration despite treatment. Early recognition of these alternative causes is important as they warrant urgent intervention.

Vascular events

In TBM, vascular events are most often ischaemic in nature. Vascular involvement is more frequently seen in chronic meningitides than in treated acute bacterial forms of meningitis[80]. Other infective causes of stroke in tropical regions may include malaria, syphilis, Chagas disease, cysticerosis. Events can go unnoticed, as they occur silently or in severely ill patients already in deep coma. The most common signs of TBM associated stroke are mono- or hemiplegia, but also may present as lowered consciousness, disorders of movement, seizures, cranial nerve palsies, papilloedema and decerebration[41]. Clearly neurological deterioration also can have its origin in a tuberculoma, cerebral oedema or infiltrating exudate[81]. Unlike hypertensive or atherothrombotic stroke, transient ischaemic attacks (TIAs) and lacunar lesions are rare in tuberculous meningitis[41]. Patients may present with symptoms of stroke, but more often develop stroke later in the course of disease, typically during the first weeks of treatment[41, 82]. It is not currently possible to predict which patients will develop stroke and it is associated with higher mortality and morbidity. Initial imaging studies may not be sensitive enough to detect the early changes of an ischaemic event. Anti-thrombotic therapies, such as aspirin and dipyridamole which prevent or reduce the incidence of

stroke may improve outcomes in TBM and warrant further clinical study. Results of a phase 2B randomized controlled trial looking at safety of two different doses of aspirin (81mg and 1000mg) as adjunctive treatment in TBM are underway (clinical trials registration number at clinicaltrials.gov; NCT02237365)

1.2.5 DIAGNOSIS

Heinrich Quincke began to popularize the lumbar puncture for both therapeutic and diagnostic purposes in the late 19th and early 20th century. With the discovery of *Mycobacterium tuberculosis* (*M.tuberculosis*) by Robert Koch in 1892 and the development of X-rays by Wilhelm Rontgen in 1895, diagnosis before death was achievable although effective treatment remained elusive. Although effective treatment is now available, the mainstay of diagnosis has remained largely unchanged over a century.

Cerebrospinal fluid analysis

Fundamental to diagnosis is the lumbar puncture and consequent cerebrospinal fluid (CSF) analysis. CSF pressure is raised (>20cmH_20) in approximately 50% of adults and 40-75% of children (>10cm H_20). Typically, the CSF is "straw" colored. The results can be equivocal. The white cell count (WCC) is generally lower (10-1000 cells/µl) than in bacterial meningitis, predominantly lymphocytic, with a low serum to CSF glucose ratio (<50%). Lactate is usually raised reflecting intracerebral metabolic disturbances or ischemic processes. Raised total protein levels (>0.5 g/l) are an indication of blood brain barrier disruption or increased intracerebral production of immunoglobulins. In children, cerebrospinal fluid modifications are similar to adults; however, smear and culture are less sensitive (Table 1.5).

Table 1.5 Typical cerebrospinal fluid findings in TBM in adults

Typical CSF findings	Adult TBM
Opening pressure	Normal or moderately increased
CSF appearance	Clear, colourless or yellow ('straw-like')
White cell count	50-1000/ µl
% lymphocytes*	50-100%
Protein	1.5-5.0 g/l
CSF/serum glucose	<50%
CSF lactate	3-10 mmol/l
Sensitivity ZN smear†	1-10%
Sensitivity liquid culture†	~65%
Sensitivity Xpert MTB/RIF†	~60%

**In early disease, or in HIV-infected patients, neutrophil predominance may be noted*
†Against a clinical diagnosis as the gold-standard
CSF=cerebrospinal fluid

In HIV-positive patients, atypical findings are encountered in a considerable proportion of patients, which may lead to delay in diagnosis and treatment. Normal levels of lactate, glucose, protein and WCC are more often reported in HIV, even completely normal CSF findings can be found particularly in patients with severe immunosuppression (CD4 count<50 cells/µl). In the context of HIV, neutrophils often predominate in the CSF cell population[55].

Essential to the search for acid-fast bacilli is the volume of the CSF sample, the time spent on microscopic examination and the efficiency with which the microbiology, biochemistry and hematology laboratories use the precious CSF sample. Increasing the volume (to a minimum of 6ml) and slide examination-time to a standard time (preferably 30 minutes) can improve the yield to more than 60% of clinically diagnosed cases[83]. Particularly for pediatric patients it can be difficult to obtain large volumes of CSF but drawing of larger volumes should be encouraged to improve the confirmation rate, where not contra-indicated. It should be remembered that the major safety issue relates to the decision on whether to perform a lumbar puncture, the volume of CSF then taken is of secondary consequence. Hence having made the decision to perform a CSF it is only ethical to then take a volume of CSF that will give a good chance of improving the care of the patient. The CSF should be concentrated prior to examination of the deposit, either by centrifugation or filtration[84]. Direct smear examination of CSF is rarely positive.

Microbiological diagnosis

Early diagnosis is crucial to a favorable outcome from TBM. CSF microscopy is still central to diagnosis, as the presence of acid-fast bacilli (AFB) confirms the diagnosis and results are available quickly (Figure 1.6). The ancient Ziehl-Neelsen (ZN) stain is simple and rapid; however, although highly specific in TBM, the sensitivity reported is highly variable and generally is very low (1-60%)[83].

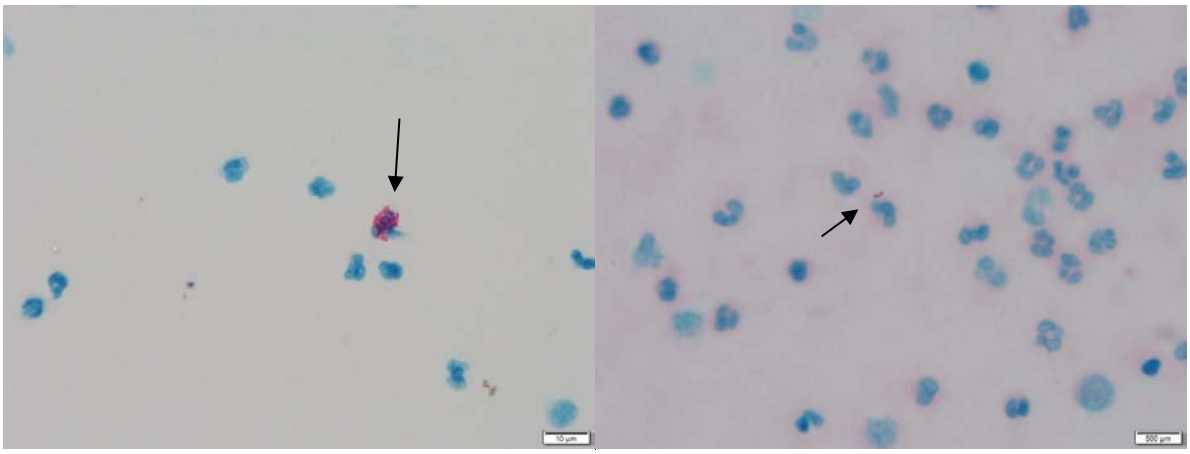


Figure 1.6 Ziehl-Neelsen staining of CSF

Left panel: arrow indicating typical cording pattern of intracellular mycobacteria, with clearly recognizable pink coloration of carbolfuchsine of acid-fast bacilli. Right panel: arrow indicating single acid-fast bacilli, darker coloration
CSF= cerebrospinal fluid

TBM cannot be excluded by smear or any of the other currently available diagnostic modalities. Typically, the decision to treat is made on clinical grounds. Several clinical algorithms exist to aid clinicians in diagnosis[85].

Without the context of a suggestive clinical history, TBM can mimic other chronic meningo-encephalitides. Notably in HIV infected patients the chief obstacle in clinical practice is the distinction from other chronic forms of meningitis in, particularly cryptococcal meningitis, cerebral toxoplasmosis, cytomegalovirus encephalitis and CNS lymphoma. Since diagnostic yield from smear is generally low, careful exclusion of other diagnoses is imperative. As soon as suspicion is raised, history taking and additional investigations should be directed towards the exposure to TB and ruling out other treatable causes.

Mycobacterial Culture

Liquid culture is currently considered the 'gold standard' of TBM diagnosis, although compared to a clinical case definition, sensitivity is approximately 70%[86]. As for CSF smear, sensitivity of culture of *M.tuberculosis* from the CSF is increased by using a larger volume of CSF, which should be concentrated prior to inoculation of the deposit wherever possible. Previously, Lowenstein-Jensen (LJ) media (Figure 1.7) and later agar media (Middlebrook 7H10, 7H11) were recommended, however liquid culture techniques show increased sensitivity and more rapid turn-around times for the isolation of mycobacteria and should be used where possible. Commercial liquid culture systems include BACTEC MGIT 960 system (Becton Dickinson Microbiology Systems, Sparks, Md), and MB/BacT system (BioMérieux, Durham, N.C) and have reduced the time to results for both isolation and drug susceptibility testing of mycobacteria[87]. CSF cultures generally become positive between 10 and 21 days on commercial liquid culture systems, although late positives may occur after 35 days due to the low bacillary load[88, 89].

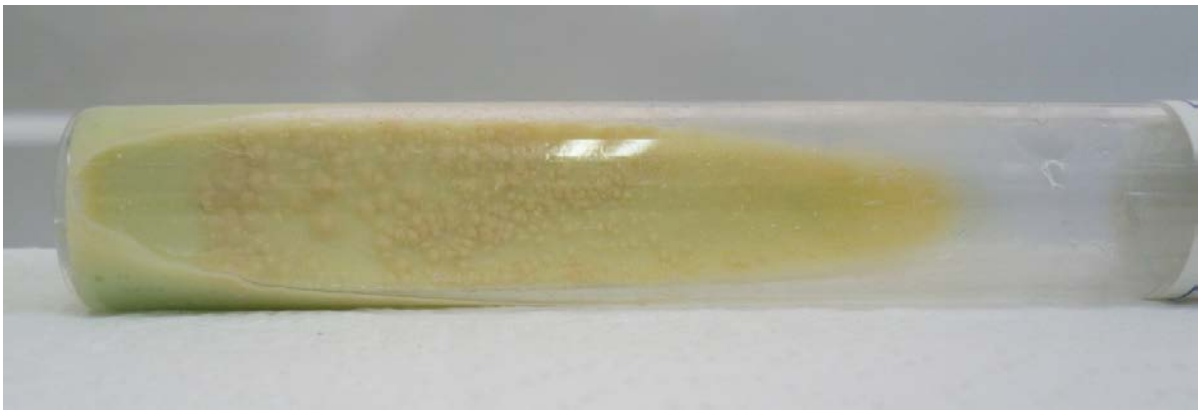


Figure 1.7 Mycobacterial colonies on solid LJ medium

LJ= Lowenstein-Jensen (Image: courtesy of dr. Dang Thi Minh Ha)

The microscopically observed drug susceptibility (MODS) assay is a non-commercial liquid culture technique with minimal technical requirements that can detect both mycobacteria and drug resistance[90]. (Figure 1.8) In TBM, MODS culture has shown comparable sensitivity to MGIT for CSF culture, however with a median turnaround time of 6 days versus 15.5 days[88]. This study did not evaluate direct drug susceptibility testing using MODS or MGIT. MODS is increasingly used in

low resource countries for the diagnosis of pulmonary MDR-TB, however it is still not widely used for TBM, although that should change. WHO has endorsed both commercial and non-commercial liquid culture systems for TB diagnosis[91].

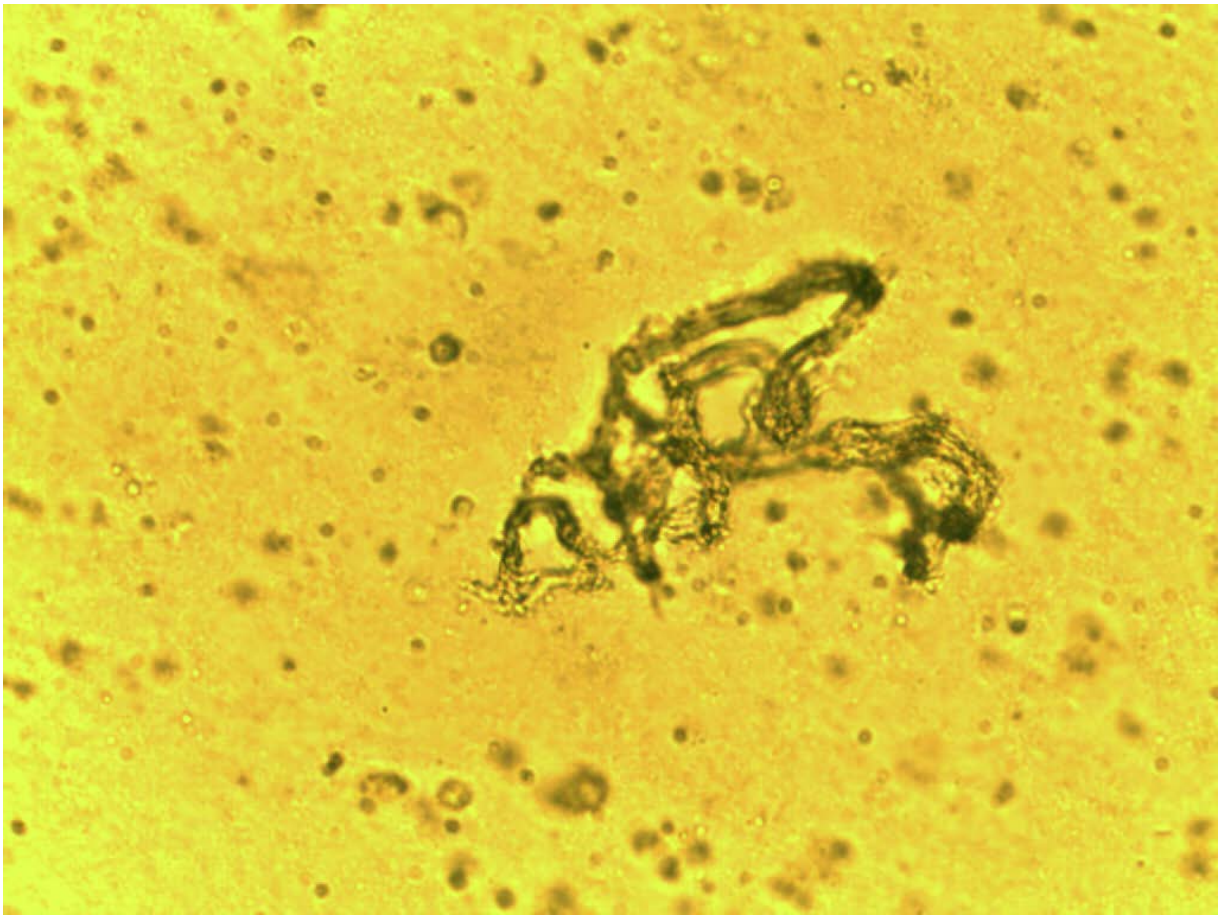


Figure 1.8 Typical cording of Mycobacterium tuberculosis on MODS assay

(Image: courtesy of dr. Dang Thi Minh Ha)

Chest X-ray

The chest X-ray may reveal active or previous infection with *M.tuberculosis*. In children signs of primary infection may be noted on chest X-ray. In adults the chest X-ray is often normal, but all typical lesions can be found including apical scarring, calcified Ghon complex, upper lobe infiltration, nodular and cavitating disease. Miliary TB is frequently associated with TBM (Figure 1.9), found on chest X-ray in 25-50% of adults and 15-25% of children with TBM[92, 93].

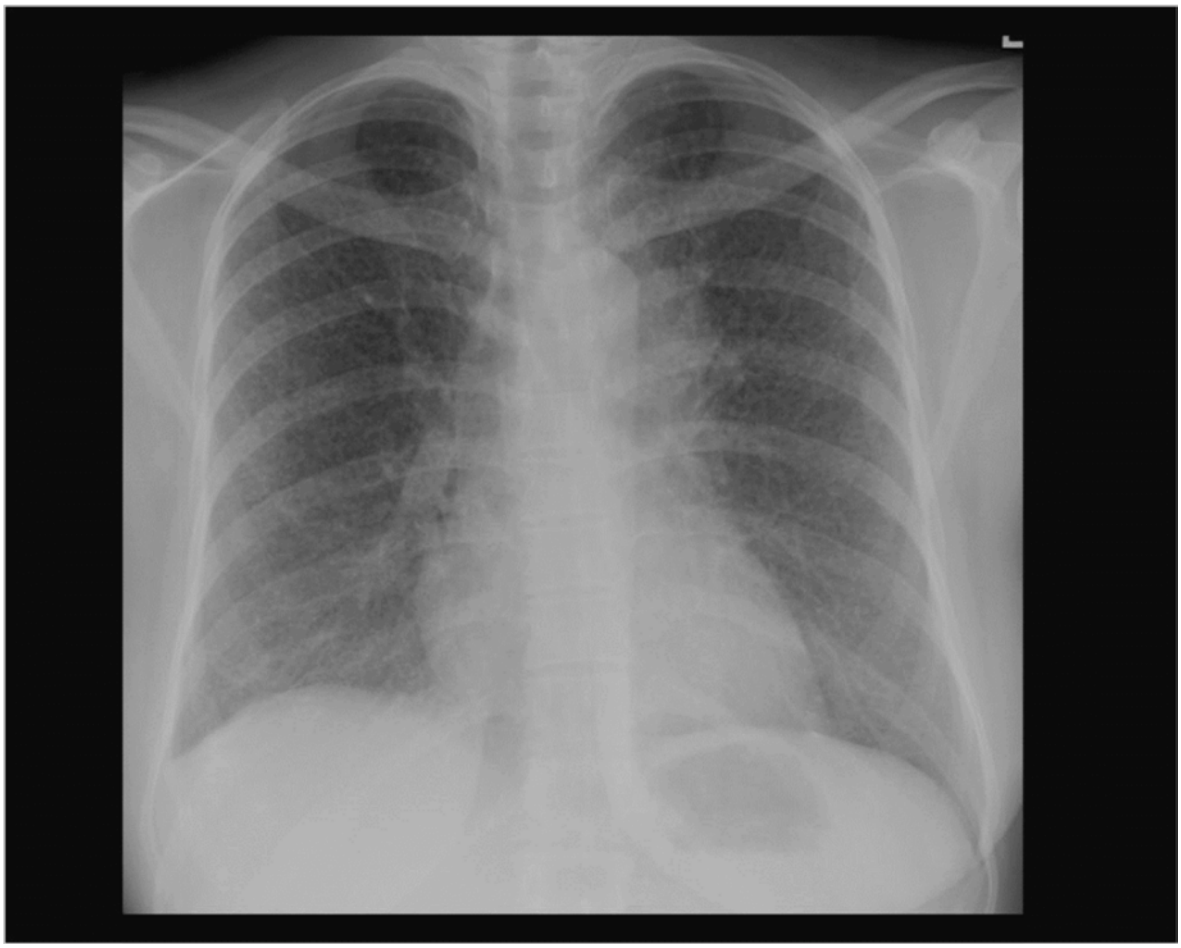


Figure 1.9 Miliary TB on a chest x-ray

Chest X-ray of a female TBM patient, showing a typical fine-grained miliary pattern throughout all lung fields

CNS imaging

Radiological results can add additional evidence for diagnosis, while also potentially serving as the missing link between epidemiology, etiology and pathophysiology, especially given the paucity of post-mortem studies. Presenting findings are pluriform and during treatment progressive changes may appear. Contrast enhanced MRI is the modality of choice since it has a higher resolution over CT-scanning. However, in many endemic settings, MRI is too expensive, or not readily available.

The typical initial findings are basal meningeal enhancement, hydrocephalus, and tuberculoma (Figure 1.10A, B and C respectively). Hydrocephalus is the most common finding. Hydrocephalus is also seen in bacterial meningitis, although less frequent[94].

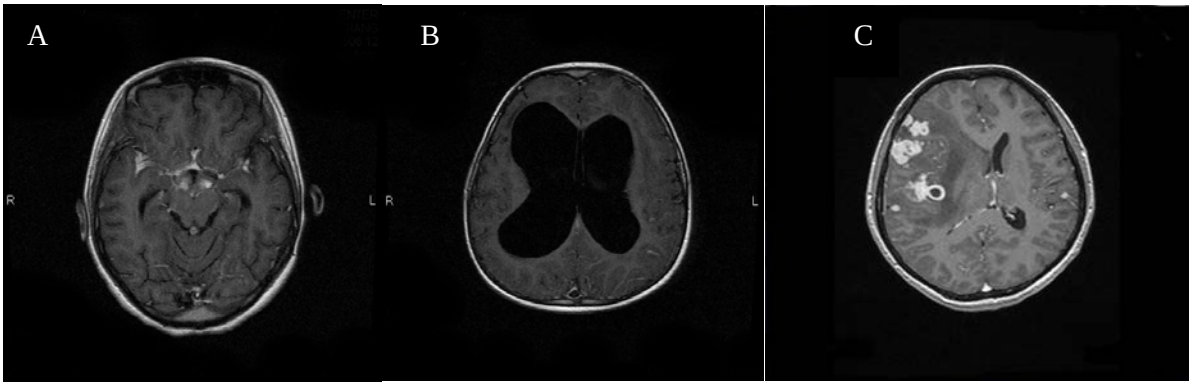


Figure 1.10 Typical TBM findings on MRI imaging upon disease presentation

Panel A: T1 weighted gadolinium enhanced MRI of an adult patient with TBM showing enhancement of the basal meninges. Panel B: T1 weighted gadolinium enhanced MRI of a pediatric patient with TBM showing severe hydrocephalus. Panel C: T1 weighted gadolinium enhanced MRI of a TBM patient, showing multiple tuberculoma and surrounding oedema with midline shift in the right hemisphere.

Ischemic events most commonly occur during treatment, rather than being a presenting sign, mostly located in the basal ganglia (Figure 1.11), which is in line with the severe basal infection, exudates formation and consequent vasculitis. In a serial MRI study dexamethasone reduced the proportion of patients that developed infarcts during treatment, however the difference was not statistically significant, possibly due to insufficient sample size[82].

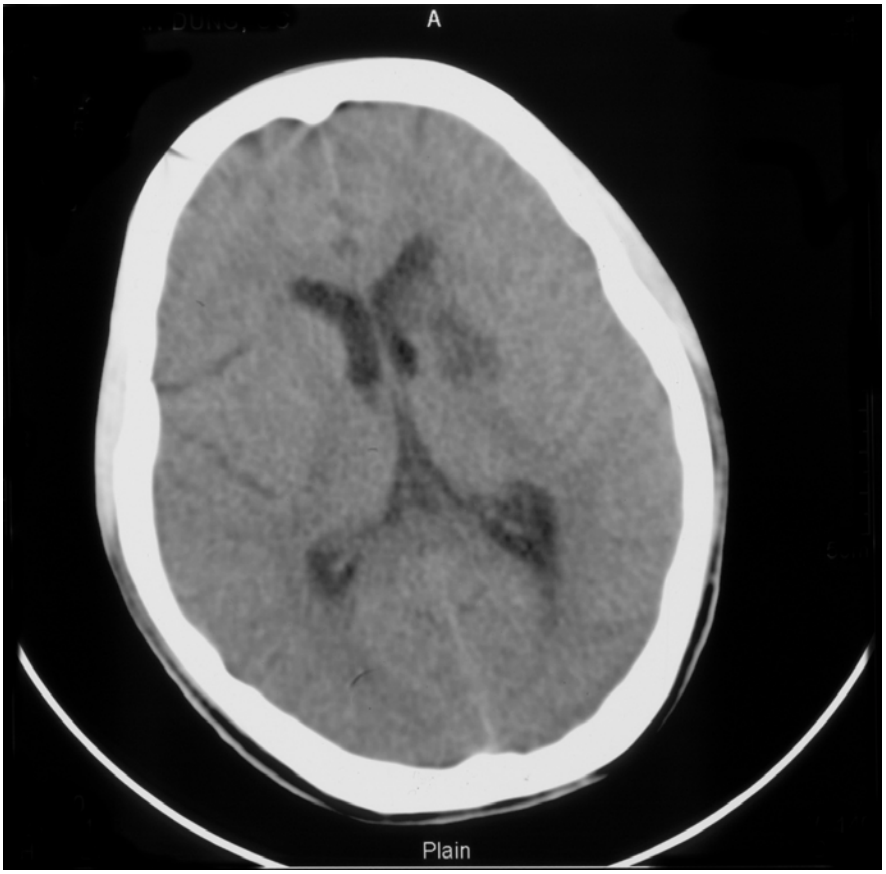


Figure 1.11 Infarct in basal ganglia on non- contrast enhanced CT scan

Tuberculin skin testing

The Mantoux tuberculin skin test (TST) is performed by intracutaneously injecting a small dose of purified protein derivative. After 48-72 hours an induration of 15mm or more is considered positive in all persons[95]. The value of this diagnostic test is highly dependent on the background prevalence of TB, age of the patient and the co-infection with HIV. Up to 10% to 15% of immunocompetent children with culture-documented TB do not initially show TST reactivity. Sensitivity can be decreased by host factors, such as young age, poor nutrition, immunosuppression, other viral infections (such as measles, varicella, and influenza), recent TB infection, and disseminated TB diseases[96].In culture proven TBM patients in Egypt only 19% of patients had tuberculin positivity on admission. The yield improved when the test was repeated after 60 days, with 62% positivity[97]. Severe immunosuppression will also suppress skin-test reactivity[98]. False-positive TST results may

also occur in BCG vaccinated individuals and those exposed to environmental nontuberculous mycobacteria [96]. In the UK Heaf-testing was preferred to Mantoux. It follows the same principles as the Mantoux test, however a special “Heaf gun”, with multiple small needles is used, which is thought to be less painful in children, and has an easier read-out[99].

Diagnostic development

Various immunoassays have been evaluated for TBM with varying performance. In 2011, the WHO issued a first negative recommendation against using existing commercial serological assays for the diagnosis of pulmonary TB following a systematic evaluation[100]. Immunoassays are unsuitable to distinguish acute infection from previous exposure. Cross-reactivity of antibodies may further decrease specificity. With the advent of nucleic acid amplification tests (NAATs), diagnostic development has been turned away from immunological tests. Commercial NAATs have shown potential to work as a rapid ‘rule-in’ diagnostic for TBM, with high specificity (98%), but low sensitivity (56%)[101, 102]. A more recent review highlights the promise for multiplex PCR techniques, which have favourable sensitivity compared to commercial NAAT (sensitivity 71-94%, specificity 88-100%)[103]. However, these NAATs require high standard laboratory conditions with rigorous quality control. Xpert MTB/RIF (Cepheid, Sunnydale, CA, USA) is the most recently endorsed diagnostic test for TB by the WHO in 2010. Key advantageous properties of this test are that the technique can be learned by relatively unskilled staff, the machine can be used in decentralized settings, it has a turnaround time of 2 hours and the closed disposable cartridge system reduces the risk of contamination. It also is able to detect rifampicin resistance on captured bacilli, which is considered a surrogate marker for multi-drug resistance (MDR). Disadvantages are the price of individual test cartridges (currently at least 10 USD), and their limited shelf life. The machine needs regular maintenance and continuous power supply[104].

Diagnosis of TBM is discussed more extensively in the following chapter (chapter 2), in which we have evaluated the performance of Xpert MTB/RIF for use on cerebrospinal fluid of patients with TBM.

1.2.6 TREATMENT

Treatment of patients with TBM is complex, and can be divided in four major areas. First, the core is formed by anti-tuberculosis treatment. Early initiation of anti-tuberculosis treatment, before the onset of coma, greatly increases chances of survival. Secondly, adjunctive immunomodulatory treatment is currently recommended for all grades and ages. Thirdly, supportive therapy consists of intra-cranial pressure control and management of other secondary complications, such as hyponatremia. Finally, for HIV-positive patients, ART should be initiated or continued, with particular care for drug-drug interactions and IRIS.

Anti-tuberculosis treatment

The first-line TB agents recommended in global guidelines for the treatment of TBM are rifampicin (R), isoniazid (H), pyrazinamide (Z), ethambutol (E) and streptomycin (S). Currently, WHO recommends 2 months treatment with 4 first-line drugs in the intensive phase, followed by a continuation phase with at least rifampicin and isoniazid for 4-10 months[14]. The regimens recommended for TBM are derived from short course treatment for pulmonary TB. However optimal drug doses and duration of treatment for TBM have not been established by large clinical trials. The brain should be considered as a distinctive compartment, to which a therapeutic regimen should be geared, taking into account pharmacokinetic (PK) and pharmacodynamic (PD) evidence. Of the first-line agents used in the regimen, rifampicin, ethambutol and streptomycin have poor ability to penetrate the blood-CSF barrier[105-108]. Rifampicin is a key constituent of TBM treatment, and used throughout treatment. Resistance to rifampicin is associated with very high mortality in TBM, a finding which emphasizes the importance of rifampicin in effective treatment[64]. Recently, small studies have shown that higher doses of rifampicin in TBM regimens are associated with more favorable levels in CSF and improved survival[109]. Fluoroquinolones are an attractive candidate in the treatment of TBM, as they are active against *M.tuberculosis*, are well tolerated, with extensive safety data, relatively little resistance found in *M.tuberculosis* and good penetration in the CSF[110, 111]. A CNS tailored antituberculosis treatment regimen will be evaluated in chapter 3 and 4.

In pulmonary MDR-TB the WHO guidelines recommend the addition of an injectable drug (amikacin, kanamycin or capreomycin) and a fluoroquinolone (moxifloxacin or levofloxacin) and at least 2 other agents to which the mycobacterium is susceptible[63, 112]. There are no guidelines that specify treatment for MDR-TBM. Of the second-line agents, ethionamide, prothionamide and cycloserine are able to penetrate the CSF and may be effective in TBM[63, 113].

Adjunctive immunomodulatory treatment

The rationale of adjunctive anti-inflammatory therapy is based on the hypothesis that a substantial part of the pathological morbidity in TBM is caused by an inappropriate inflammatory response to *M.tuberculosis.* The use of corticosteroids in TBM has been the subject of numerous clinical trials in the past decades. These have been systematically reviewed by the Cochrane collaboration, whose meta-analysis concluded that corticosteroids improved outcome in HIV negative adults and children with TBM. Since then, corticosteroids are generally recommended to be included in treatment regimens of TBM[114]. However the benefit in HIV positive patients remains to be established[115]. Data on the benefit of corticosteroid on long-term survival is lacking. 5-year follow-up data from a large Vietnamese trial, which included 545, predominantly HIV negative, adults, suggested that the benefit is preserved in the subgroup of grade 1 TBM patients. However, in the higher severity groups, grade 2 and grade 3, there was no difference in survival in the two treatment arms (dexamethasone or placebo). The additional deaths that occurred in those severe groups were associated with neurological sequelae[59]. In countries with improved neuro-rehabilitation programs, those later deaths may be prevented, hence it is recommended to give corticosteroids to patients in all grade groups[14] (WHO IDSA, UK guidelines). The different types of corticosteroid (methylprednisolone, prednisolone or dexamethasone), dose or duration have not been compared in large clinical trials; therefore, it is advisable that regimens are used similar to those that showed a survival benefit in the published literature[51, 115-117].

More recently it has been reported that the immune response can be modulated through the leukotriene A_4 hydrolase (LTA4H) gene. Indirectly this gene regulates tumor necrosis factor alpha (TNFα) levels, and promoter polymorphisms can lead to either a hyperinflammatory state, inadequate

inflammation or an intermediate response. Patients with TBM, who were homozygous for the LTA4H-high polymorphism (T/T), had higher levels of TNFα and high leukocytes in CSF. Among patients not receiving glucocorticoids, mortality was highest among patients with a T/T genotype. However, these patients showed the greatest reduction in mortality in the group treated with adjunctive dexamethasone, suggesting that the dexamethasone may reduce excessive inflammatory response in this group. By contrast, patients who were homozygous for the polymorphism causing low expression of LTA4H (C/C) showed increased mortality when treated with dexamethasone, probably due to further suppression of an inadequate immune response. The patients who were heterozygous at this locus (C/T) had the lowest mortality and the use of dexamethasone did not appear to influence mortality in this group[118].

These results suggest that disease severity in humans can be caused either by an exaggerated or a deficient immune response, and perhaps more importantly, that treatment with corticosteroids may only benefit those with a certain predisposition to hyper inflammation and may be detrimental in those with deficient immune responses. This needs prospective assessment and if proven may necessitate more tailored use of corticosteroids according to individual genotyping or phenotyping of the immune-response in the future.

Thalidomide is a drug with immunomodulatory properties through the inhibition of TNFα[119]. In animal studies, TNFα levels in the CSF produced during TBM were shown to correlate with the extent of pathogenesis, although this has been difficult to replicate in humans with TBM[120]. In rabbits infected intracranially with TB, an antituberculosis regimen that included thalidomide, led to reduction of TNFα levels in CSF and prevented death[121]. The same group has used a thalidomide analogue (immunomodulatory drug 3 or IMiD3) for the treatment of experimental animals infected with TBM as an adjunctive to standard antituberculous treatment. IMiD3 has comparable immunomodulatory action to thalidomide, but unlike thalidomide, IMiD3 did not appear teratogenic in rabbits[122]. The additional use of IMiD3 in rabbits resulted in marked improvement in survival, reduced CSF leukocytosis, lower levels of TNF, and attenuated inflammation of the meninges on

histological examination. The beneficial effect on survival and severity of symptoms of IMiD3 was markedly greater than that of thalidomide in this animal model[122].

In children with TBM, a randomized placebo controlled study of adjunctive thalidomide, was stopped early because an increased risk of death and adverse events in the treatment arm[123]. The role of TNFα in tuberculosis is complex and has yet to be fully elucidated. Currently there is no role of TNFα inhibition in the treatment for TBM, however the potential role for IMiD3 in the management of TBM in patients may be a subject of future research.

Anti-retroviral therapy

The mortality in HIV associated TBM is extremely high. Patients generally present with low CD4 counts and are often not on ART. TBM is an AIDS-defining illness and ART should be initiated for HIV co-infected TBM patients. The timing of ART initiation has been under debate. For pulmonary TB, patients with severe immune suppression (CD4 counts<100) benefit from immediate ART upon start of TB treatment [124-127]. However, patients with TBM are severely ill and the development of intracranial IRIS may be detrimental to the patient. TBM-IRIS occurs frequently in ART-naïve patients who commence ART-treatment 2 weeks after initiation of anti-tuberculosis therapy. Patients who present with high CSF neutrophil counts, with high CSF TNFα and low interferon gamma (IFNγ) ,and with *M.tuberculosis* cultured from the CSF, are at increased risk of developing IRIS[128]. In a Vietnamese clinical trial comparing early and delayed initiation (after 2 months of TB treatment) of ART in adult HIV infected patients with TBM, there was no significant benefit of early initiation to delayed initiation[33]. As early ART was associated with increased toxicity and occurrence of IRIS, it is suggested that in patients with TBM is delayed by 8 weeks, in order to reduce the risk of intracranial IRIS, drug-drug interactions, hepatotoxicity and pill-burden. A review of ART and TB drug interactions falls outside the scope of this thesis, but can found in the Centers for Disease Control and Prevention (CDC) guidelines[129].

Managing hydrocephalus

Generally, in TBM hydrocephalus is of the communicating type[130]. Occasionally obstructive hydrocephalus can be diagnosed by imaging, when narrowing of the aquaduct of Sylvius is noted or when a parenchymal mass is demonstrated obstructing the flow of CSF. If hydrocephalus is of the communicating type (in around 80% of cases), furosemide can be used, in combination with acetazolamide to reduce CSF production by the choroid plexus[131]. If medical intervention is insufficient, or hydrocephalus is non-communicating, an external ventricular drain, ventriculoperitoneal shunting, or endoscopic third ventriculostomy may be considered[132]. Outcome depends on the local neurosurgical expertise, correct indication and severity of disease[133]. There is no evidence for the use of osmotic agents, such as mannitol or hypertonic saline, in TBM.

Managing hyponatremia

Hyponatremia in TBM is a risk factor for worse outcome[134]. In resource limited settings, it is difficult to discriminate between the different causes of low sodium, however mostly implicated are the syndrome of inappropriate antidiuretic hormone (ADH) secretion (SIADH) or cerebral salt wasting syndrome (CSW) of which CSW is probably under-diagnosed in TBM. A pragmatic approach is to avoid both hypo-osmolality and hypovolemia. Fluid restriction in CWS can be detrimental and is not recommended. General advice is to correct hyponatremia cautiously with hypertonic saline, if indicated combined with fludrocortisones[132, 135]. Pontine myelinolysis should be prevented by gradual correction.

Stroke prevention

Stroke is associated with poor outcome in TBM. Infiltrative and proliferative vasculitis and necrotising vessel pathologies have been implicated in the pathogenesis. The relative contribution of thrombosis to the development of ischaemic events is unknown. Tuberculous thrombophlebitis has been described in earlier pathological studies[136, 137]. Severe pulmonary TB is characterized by impaired fibrinolysis and a hypercoagulable state[138]. In children with TBM changes were found in procoagulation, anti-thrombotic factors, fibrinolysis, platelet counts and vascular endothelium

functions, all contributing to an increased risk of thrombosis[139]. Aspirin has been subjected to clinical trials in TBM, since it is antithrombotic, and possibly neuro-protective. However, a clinical trial in 146 children with TBM showed no significant effect on either mortality or neurological deficits with low-dose or high-dose aspirin regimens[140]. One open-label randomized study in 118 adults on the role of aspirin showed a beneficial effect on mortality and MRI results[141]. In this study, some patients selectively received corticosteroids, which may have biased the results. Larger appropriately randomized studies are needed to establish the role of aspirin (and dipyridamole) in the management of TBM related ischaemic events. In an intensive neurosurgical care setting in South Africa, cerebral tissue oxygenation was monitored in two children. A decline in oxygenation was reversed by aggressive therapy with oxygen, fluid resuscitation, inotropic support and blood transfusion, which possibly prevented infarction[142]. More profound insights into the pathogenesis of TBM vascular disease are necessary to guide rational therapeutic interventions.

1.3 Conclusion and aims

There is an urgent need for improved early diagnosis of TBM and evaluation of targeted antituberculous treatment regimen for drug susceptible and drug resistant TBM. This thesis addresses these pressing issues. The overarching aims of the studies performed in this thesis are to:

1. Improve diagnosis of TBM by evaluating the performance of a novel molecular diagnostic test; Xpert MTB/RIF (Chapter 2)
2. Improve treatment of TBM by evaluating an intensified antituberculosis treatment regimen. (Chapter 3 and 4)
3. Improve management of drug resistant TBM by exploring factors associated with drug resistance and evaluating response to intensified treatment. (Chapter 5)

CHAPTER 2

2 DIAGNOSIS OF TUBERCULOSIS MENINGITIS USING XPERT MTB/RIF

2.1 Introduction

Early recognition of TBM is pivotal, since prompt initiation of treatment greatly increases chances of survival and reduces disability. However early symptoms are non-specific and diagnostic confirmation has hardly improved since the early 20th century. ZN smear (Figure 2.1) for acid-fast bacilli is central to diagnosis since it gives rapid results, however the reported sensitivity is low. Sensitivity estimates depend upon the criteria used for gold standard and range widely from 1% to 60%. This wide variation is likely to depend on many factors including laboratory performance, workload, technician diligence and experience, time from taking the sample to staining in the laboratory and volume of CSF examined.

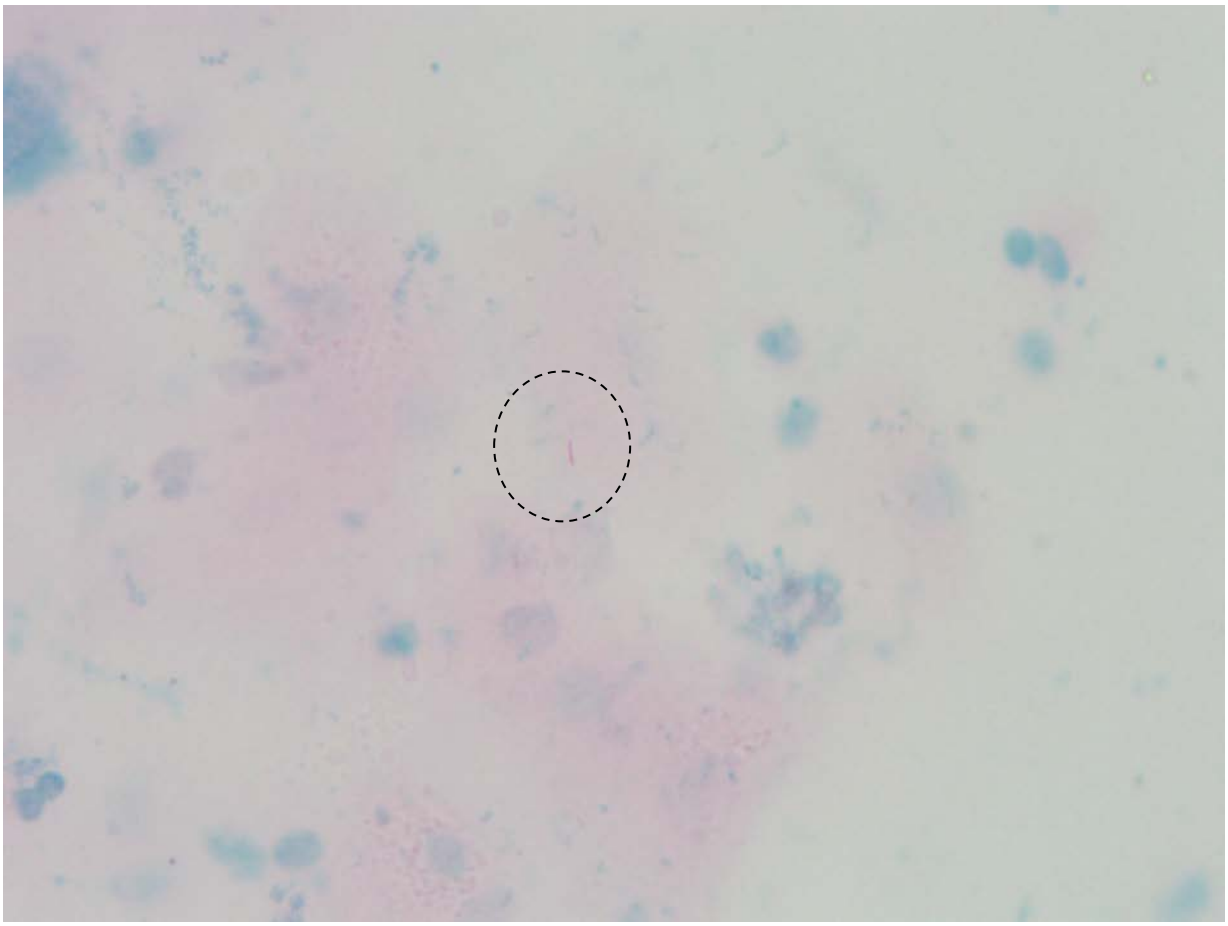


Figure 2.1 Ziehl-Neelsen smear of CSF

CSF= cerebrospinal fluid (image: courtesy of ms. Do Dang Anh Thu)

Liquid culture of *M.tuberculosis* is considered the gold standard for diagnosis, but due to the slow growing nature of mycobacteria, the time to a positive result may range from 2-8 weeks. This renders the test ineffective for clinical decision-making regarding treatment initiation, although a positive result can confirm the decision to continue therapy (although a negative result should not automatically lead to stopping) and provides an isolate for drug susceptibility evaluations. Treatment should be initiated upon clinical suspicion. Different clinical algorithms are published throughout the literature of which those of most use are based on simple clinical and laboratory criteria and can aid clinicians in resource limited settings[143, 144]. There is a desperate need for new rapid, preferably point-of-care diagnostics. The difficulty in evaluating novel diagnostic modalities is the lack of an optimal gold standard.

In order to address the heterogeneity in clinical diagnosis among published research studies of TBM a consensus score-based case definition, based on expert opinion, has been published for use in the research context, with an alternative scoring if imaging is not available. This case definition is not intended to determine treatment decisions, in particular, it should not be used to exclude a diagnosis of TBM[85] (Table 2.1). Rather it is meant to improve comparability of results between clinical studies. In this chapter we set out to evaluate a novel commercially available molecular test, Xpert MTB/RIF on CSF against a clinical diagnosis of TBM using the published case definition.

Table 2.1 Uniform case definition for clinical studies[85]

	DIAGNOSTIC SCORE
CLINICAL CRITERIA	Maximum category score=6
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for more than 2 weeks	2
History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
Altered consciousness	1
CSF CRITERIA	Maximum category score=4
Clear appearance	1
Cells: 10–500 per μl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2.2mmol/L	1
CEREBRAL IMAGING CRITERIA	Maximum category score=6
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
EVIDENCE OF TUBERCULOSIS ELSEWHERE	Maximum category score=4
Chest radiograph suggestive of active tuberculosis: signs of tuberculosis = 2; miliary tuberculosis = 4	2/4
CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
AFB identified or *M.tuberculosis* cultured from another source; i.e., sputum, lymph node, gastric washing, urine, blood culture	4
Positive commercial *M.tuberculosis* NAAT from extra-neural specimen	4
Exclusion of alternative diagnoses An alternative diagnosis must be confirmed microbiologically (by stain, culture, or NAAT when appropriate), serologically (eg, syphilis), or histopathologically (eg, lymphoma). The list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include: pyogenic bacterial meningitis, cryptococcal meningitis, syphilitic meningitis, viral meningo-encephalitis, cerebral malaria, parasitic or eosinophilic meningitis (*Angiostrongylus cantonesis*, *Gnathostoma spinigerum*, toxocariasis, cysticercosis), cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging) and malignancy (eg, lymphoma)	The individual points for each criterion (one, two, or four points) were determined by consensus and by considering their quantified diagnostic value as defined in studies.
Clinical entry criteria • Symptoms and signs of meningitis including one or more of the following: headache,irritability, vomiting, fever, neck stiff ness, convulsions, focal neurological deficits, altered consciousness, or lethargy. **Tuberculous meningitis classification** *Definite tuberculous meningitis* • Patients should fulfill criterion A or B:	

A) Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; *M.tuberculosis* cultured from the CSF; or a CSF positive commercial NAAT.
B) Acid-fast bacilli seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

Probable tuberculous meningitis

• Clinical entry criteria plus a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnoses. At least 2 points should either come from CSF or cerebral imaging criteria.

Possible tuberculous meningitis

• Clinical entry criteria plus a total diagnostic score of 6–9 points (when cerebral imaging is not available) or 6–11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses. Possible tuberculosis cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.

Not tuberculous meningitis

• Alternative diagnosis established, without a definitive diagnosis of tuberculous meningitis or other convincing signs of dual disease.

TST=tuberculin skin test, IGRA=interferon gamma release assay, NAAT=nucleic acid amplification test, CSF=cerebrospinal fluid

2.1.1 MOLECULAR DIAGNOSIS OF TUBERCULOUS MENINGITIS

Examples of commercially available tests have shown heterogenous performance in different field studies in pulmonary TB. The first Food and Drug Administration (FDA) approved commercial NAAT was the Amplified Mycobacterium Tuberculosis Direct Test (MTD) (Gen-Probe Inc., San Diego, CA) which has been approved for both smear positive and smear negative pulmonary TB[145]. The MTD, uses isothermal amplification of 16S ribosomal transcripts[146]. Its interpretation requires the use of a luminometer[147]. Sensitivity in smear positive sputum samples was >95%, and in smear negative samples 75%-90%. The second test was the Amplicor Mycobacterium Tuberculosis Test (Amplicor) (Roche Diagnostic Systems Inc., NJ) which was approved by the FDA, for use on smear positive pulmonary samples[148]. The Amplicor test is a DNA-based test that amplifies a segment of the 16S rRNA gene using genus specific primers, which, after hybridization to oligonucleotide probes, is detected in a colorimetric reaction in a microwell plate format[149]. Sensitivity in smear positive sputum samples was >95% and 60-70% in smear negative respiratory specimens[149].

A systematic review on the use of these commercial NAATs on CSF for diagnosis TBM summarized the performance as having potential to rule-in or confirm diagnosis (specificity 98%), but low sensitivity (56%) precludes the use of these tests to rule-out disease[102].

The most recent major advance in TB diagnosis is the Xpert MTB/RIF (Cepheid, Sunnydale, CA, USA). The Xpert MTB/RIF test is a closed cartridge based system, that is easy to operate by minimally trained staff and will give results in approximately 2 hours[150]. The closed cartridge system reduces the risk of cross contamination and makes it possible for the assay to be used outside the laboratory environment and studies assessing biosafety have suggested that the use of Xpert MTB/RIF carries a smaller biohazard risk than smear microscopy[150].

The principle of the test relies on the amplification of the 81 basepair portion of the rpoB gene containing the rifampicin resistance defining region (RRDR) using a set of probes designed to detect the wild type sequence and mutations associated with rifampicin resistance[151]. Results of the early

studies on the performance of Xpert MTB/RIF calculated a limit of detection of mycobacteria in clinical samples of 131 colony forming units (cfu)/ml sputum (95% CI 106.2-176.4) and Xpert MTB/RIF was able to detect mycobacteria in sputum with bacterial load as low as 10 cfu/ml in 35% of samples[152]. This is comparable to the limit of detection of liquid culture, ranged between 10-100 cfu/ml, contrasting with sputum smear microscopy limit, which is approximately 5,000-10,000 cfu/ml[153]. The buffer contained in the cartridge was shown to reduce viability of mycobacteria, by at least 8 logs, effectively decreasing biohazard[152]. Specificity was evaluated using 20 different NTM species and a panel of 89 flora commonly found in the respiratory tract, which showed no false positive signals[154]. The test was also resilient to contamination with amplicons, since the principle of the test depends of the capture and lysis of whole bacteria[154].

The Xpert MTB/RIF test was approved by WHO in 2010 for the diagnosis of pulmonary TB following extensive evaluation projects in six countries led by the Foundation for Innovative New Diagnostics (FIND)[155][150][152]. In 2013 a Cochrane review was published assessing the performance of Xpert MTB/RIF in clinical settings globally. This meta-analysis included 18 studies, and showed a pooled sensitivity of Xpert MTB/RIF, when used as an initial test replacing conventional smear, of 88% (95%CI 83-92%) and a pooled specificity of 98% (95%CI 97-99%). If Xpert MTB/RIF was used as an additional test, in case of a negative smear result, sensitivity was 67% (95%CI 58%-74%) against culture (either automated liquid media or solid media) as a gold standard[156]. Performance in patients with HIV infection was lower than in uninfected patients: pooled sensitivity respectively 80% (95%CI 67-88%) and 89% (95% CI 81-94%)[156].

Xpert MTB/RIF performed well when compared head to head with two more recently launched commercially available NAAT platforms[157]; the MTBDRplus assay (Hain Lifesciences, GmbH Nehren, Germany) and LightCycler Mycobacterium Detection kit (LCTB) (Roche, Basel, Switzerland). The MTBDRplus is a qualitative line-probe assay, able to identify *M.tuberculosis* and resistance to isoniazid and rifampicin, giving results on a DNA-strip. It can detect the most significant mutations in the rpoB gene for rifampicin resistance and the KatG gene and the promotor region of the inhA gene are examined for isoniazid resistance[158]. The LCTB uses real time PCR, amplifying

the 16s ribosomal RNA gene and hybridisation with fluorogenic probes and can distinguish between *M.tuberculosis*, *M.avium* and *M.kansasii* in human sputum samples[159]. In 177 adult patients with suspected TB, the sensitivity of Xpert MTB/RIF was 86% (95%CI: 76–93%), of MTBDRplus 76% (95%CI: 64–85%) and of LCTB 76% (95%CI: 64–85%) against liquid culture as the gold standard[157].

Prior to this study, several studies have reported successful use of the Xpert MTB/RIF test on extrapulmonary samples, with overall sensitivities reported of over 80% and specificity reaching 100%[160-164]. However, the number of CSF samples in these studies combined was low, including only a total of 62 specimens. Due to the urgency of diagnosis in suspected TBM cases because of a rapid decrease of survival chances with delayed diagnosis together with the rise in drug resistance, a rapid, accurate diagnostic test, which also is able to identify rifampicin resistance, such as the Xpert MTB/RIF could have an impact on survival.

2.2 Materials and methods

2.2.1 Clinical setting

All adult patients (>18 years) presenting to the Hospital for Tropical Diseases (HTD), Ho Chi Minh City (HCMC), Vietnam between 17th April 2011 and 31st December 2012 with suspected TBM who underwent lumbar puncture as part of screening for enrolment in a randomized controlled trial of intensified treatment for TBM were included in the study. The full protocol of this trial has been made publically available (International Standard Randomized Controlled Trial Number ISRCTN61649292)[165]. At HTD clinicians are encouraged to draw at least 8ml of CSF when possible, in order to improve microbiological confirmation rates[83].

2.2.2 DIAGNOSTIC METHODS

CSF preparation

Upon receipt in the laboratory CSF samples were centrifuged at 4,000g for 15 minutes. Supernatant was removed using a sterile pipette, and stored in eppendorfs at -70ºC, to leave 0.5ml deposit. The deposit was vigorously re-suspended in the remaining supernatant, which was then used for ZN smear preparation (100µl), inoculation of MGIT culture (100µl) and Xpert testing (200µl). The remaining deposit was stored at -20°C. All tests were performed by one of three technicians, highly experienced in microbiological tests for TBM diagnosis. Clinical data and results of other microbiological and biochemical investigations were not available to the technicians at the time of the test.

Ziehl-Neelsen smear

ZN smears were prepared using standard methods[166] with two modifications. Firstly, the smear was layered, with two drops of CSF deposit applied. The layered smear was then stained according to standard procedures. Secondly, the ZN smear was meticulously examined for up to thirty minutes under 100x magnification before being recorded as negative. A single AFB observed was recorded as positive[83].

The reagents for the ZN method were prepared as follows.

a. Preparation of the ZN stain:

5g basic fuchsin

25g phenol crystals

50ml ethanol, 95%

500ml distilled water

The fuchsin and phenol were dissolved in the ethanol, and then the water added.

The solution was filtered before use.

b. Preparation of the acidified alcohol decolourising solution

970ml ethanol, 95%

30ml concentrated hydrochloric acid

Mixed well.

c. Preparation of the Counter-stain

2.5g methylene blue

500mls distilled water.

Mixed well.

Two drops of CSF were dried onto a heated clean slide. Once the drops of deposit had dried, the slide was passed through a Bunsen flame twice in quick succession and placed on a staining rack over a sink. Filtered ZN stain was poured over the slide until the deposit was well covered. The slide was then heated from below with a flame until steam rose from the stain. The stain was not allowed to boil, or dry on the slide. After 5 minutes the slide was washed well with running water, and covered with acid-alcohol for 3-5 minutes, or until all the stain had left the deposit. After careful washing with running water the slide was placed on a heating block for 1-2 minutes. Without this step the deposit was frequently washed off the slide when the methylene blue was added. Once the deposit was dry, it was covered with methylene blue for 30-60 seconds, and washed very carefully with running water.

Xpert MTB/RIF

200µl deposit was resuspended in phosphate buffered saline to 500µl volume. 1.5ml sample reagent, supplied with the test, was then added. Prior to August 2011, the mixture was then shaken by hand according to test instructions. From 1st August the mixture was briefly vortexed for 30 seconds to ensure all bacteria were loosened from the tube edges. The sample was left to stand for 15 minutes, as per the manufacturer's instructions, with intermittent manual shaking. The solution was then transferred to the Xpert MTB/RIF cartridge using a Pasteur pipette and the cartridge loaded onto the Xpert MTB/RIF machine for analysis. The maximum valid cycle threshold (Ct) for the five fluorophore labelled molecular beacons is 39.0 Ct for probe A, B, and C and 36.0 for probes D and E. The following results may be reported:

MTB detected: when at least two probes result in Ct values within the valid range and a delta Ct min (the smallest Ct difference between any pair of probes) of less than 2.0.

MTB not detected: when there is only one or no positive probe.

RIF resistance not detected: if the delta Ct max (the Ct difference between the earliest and latest probe) is ≤4.0.

RIF resistance detected: if the delta Ct max is >4.0.

RIF resistance indeterminate: when the following two conditions are met: 1. the Ct value of any probe exceeds the valid maximum Ct (or is zero, i.e. no threshold crossing); and 2. the earliest rpoB Ct value is greater than: [(Valid maximum Ct of probe in condition 1) - (delta Ct max cut-off of 4.0)]

Positive results, i.e. MTB detected, are given a semi quantitative result in four categories; very low, low, medium or high[151].

MGIT culture

100µl deposit was inoculated to a MGIT tube containing PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) antibiotics and OADC supplements (containing oleic acid, albumin, dextrose and catalase). MGIT tubes were incubated in a MGIT 960 machine until automatically detected as positive or for 56 days. All positive cultures were tested for susceptibility to rifampicin, isoniazid, streptomycin and ethambutol using Bactec MGIT SIRE kit (Becton Dickinson, USA) according to manufacturer's instructions [167][161][163].

Line Probe Assay

Cases of rifampicin resistance detected by Xpert were confirmed using the MTBDR*Plus* line probe assay (HAIN Lifesciences, Germany) [162][164] [168] on DNA extracted from a positive MGIT culture isolated from the same CSF sample. DNA was extracted from positive MGIT cultures using CTAB method[169] and the purified DNA then used for MTBDR*plus* test using manufacturers' instructions[166].

Other investigations

All patients underwent routine laboratory investigations for diagnosis of meningitis including CSF biochemistry (protein level, glucose (paired with serum sample), lactate), cell counts (red cell count, white cell count and differential with neutrophil and lymphocyte percentage), India ink stain for fungi, Gram stain, culture and viral PCR (Herpes simplex virus, Varicella zoster virus) and serology (for Japanese encephalitis).

Diagnostic classification

For this study patients were classified as having TBM if no other diagnosis was made and the attending physician made the decision to treat for TBM based on the clinical algorithm. In addition, patients diagnosed with TBM were classified as 'definite', 'probable' or 'possible' TBM using the standardised case definition (Table 2.1) [85]. XpertMTB/RIF results were not included in the case definition because it was the test under evaluation. 'Definite' TBM was defined as a clinical syndrome consistent with TBM, with acid fast bacilli seen on CSF smear or *M.tuberculosis* isolated in CSF MGIT culture. Patients in the 'probable' TBM group had a diagnostic score of 10 or more without cerebral imaging (MRI or CT scan) or 12 or more with cerebral imaging, with at least 2 points from CSF or cerebral imaging criteria. Patients in the probable TBM group had a diagnostic score of between 6 and 9 if without cerebral imaging or between 6-11 if cerebral imaging was performed[85]. All patients who did not meet the criteria, or did not receive treatment for TBM and received an alternative discharge diagnosis were classified as 'not TBM'.

2.2.3 STATISTICAL ANALYSIS

Sensitivity, specificity, positive predictive value and negative predictive value with 95% confidence intervals (CI) were calculated. The proportion of positive results for each test (smear, MGIT culture, Xpert MTB/RIF) was compared using McNemar's test for paired samples. To determine if the introduction of a brief vortexing step after addition of the sample reagent altered senstitivity of the Xpert test, we also analysed sensitivity and specificity in samples processed before and after 1st

August 2011. The proportion of patients with different qualitative results for Xpert MTB/RIF were summarized for the three TBM severity grades and were compared using a univariate proportional odds logistical regression model.

All statistical analyses were done using R version 2.15.1 (The R foundation for Statistical computing, 2012) with packages 'epiR' and 'DTComPair'.

2.3 Results

During the study period, 410 patients presented HTD with suspected TBM. A total of 31 patients were excluded, 19 because no final diagnosis could be made, or clinical information was missing, 7 patients had Xpert 'Error' result, 4 patients had a contaminated culture result and 1 patient had an unknown smear result. 379 eligible patients were included in the analysis. Of these, 151 were finally classified as 'definite TBM', 18 'probable TBM', 13 'possible TBM' and 197 'not TBM' (Figure 2.2).

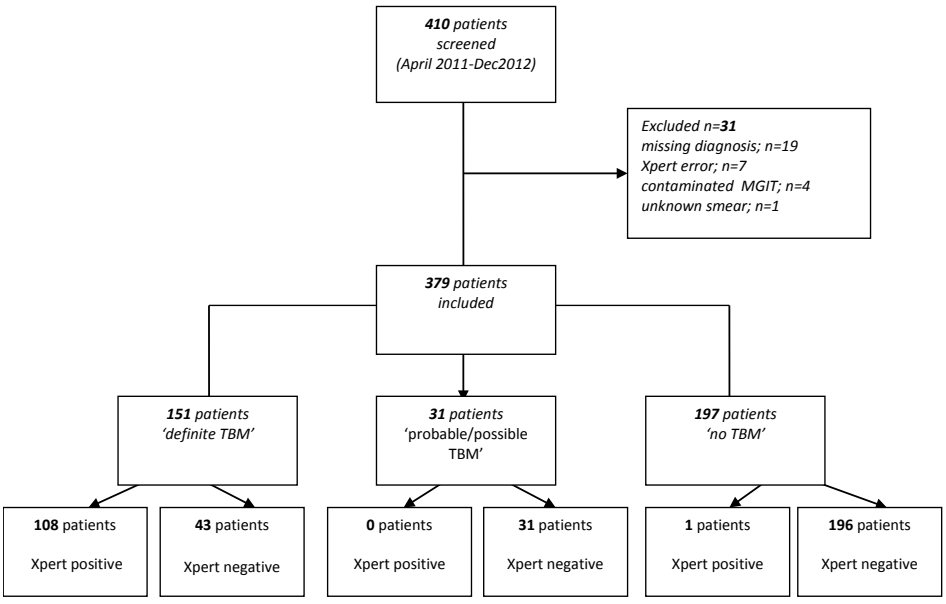


Figure 2.2 Study flow chart

Overall 79/379 (20.8%) patients were HIV infected, 108 (28.5%) HIV uninfected and 192 (50.7%) had an unknown HIV status (declined consent to HIV test or discharged before testing). Of those classified as 'definite', 'probable' or 'possible TBM' (n=182), 66 (36.3%) were HIV infected, 94 (52.6%) HIV uninfected and 22 (12.1%) had an unknown HIV status.

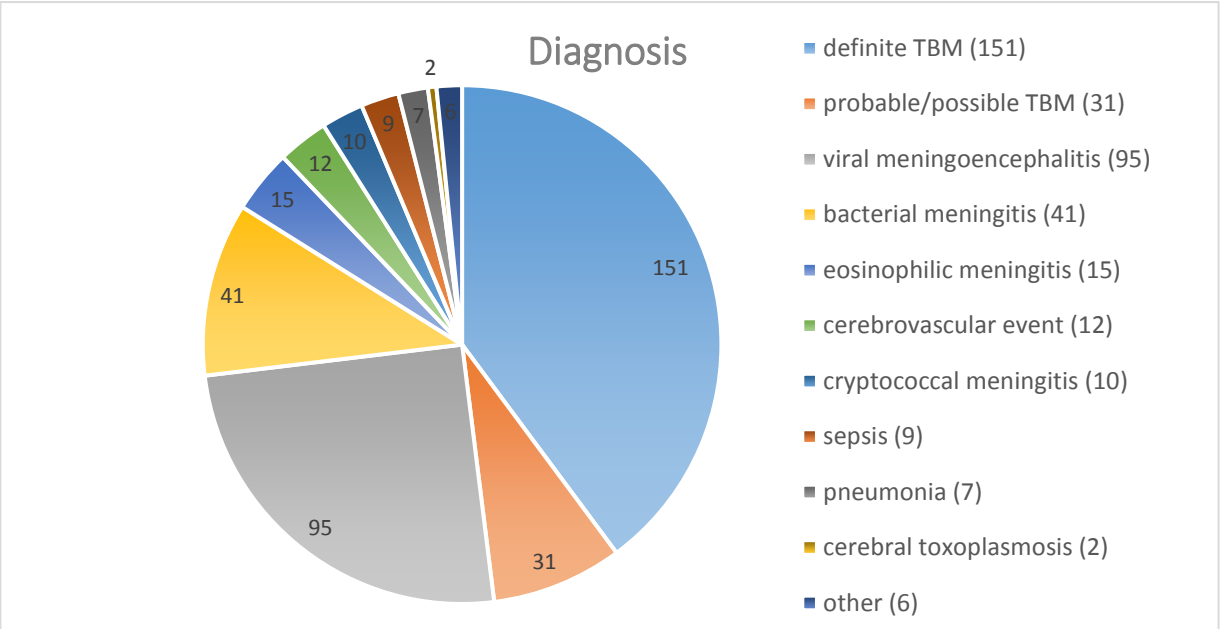


Figure 2.3 Diagnosis of all patients included in the analysis

The six patients in the 'other' category were diagnosed with dengue (n=1), psychiatric disorder (n=1), cerebral tumor (n=1), fever of unknown origin (n=1), cerebral abscess (n=1) and progressive multifocal leukoencephalopathy (n=1).

2.3.1 DIAGNOSTIC ACCURACY

The performances of the different TB diagnostic tests are shown in Table 2.2 below. Overall, the sensitivity of Xpert was 59.3% ([n=108/182 [95% CI: 51.8-66.5]) compared to clinical diagnosis of TBM (definite, probable and possible TBM). Specificity was 99.5% (95% CI: 97.2-100).

The sensitivity of smear against final clinical diagnosis was 78.6% (n=143/182 [95% CI: 71.9-84.3]) and for MGIT culture 66.5% (n=121/182, [95% CI: 59.1-73.3]) (Table 2.2 and Figure 2.4). Since smear and MGIT culture were the reference microbiological tests for diagnosis of TBM, specificity of

these tests could not be determined, however all patients positive by smear/MGIT had a clinical picture consistent with TBM and had no other organisms isolated from the CSF.

Table 2.2 Test results for TB diagnostic test by clinical diagnosis

		TBM n (%)	**Not TBM** n (%)	**Total**
Xpert MTB/RIF	Positive	108(59.3)	1 (0.5)	109
	Negative	74 (40.6)	196 (99.5)	270
	Total	182 (100)	197 (100)	379
ZN smear	Positive	143 (78.6)	0	143
	Negative	39 (21.4)	197 (100)	236
	Total	182(100)	197 (100)	379
MGIT culture	Positive	121(66.5)	0	121
	Negative	61 (33.5)	197 (100)	258
	Total	182 (100)	197 (100)	379

ZN=Ziehl-Neelsen

The sensitivity of Xpert MTB/RIF against clinical diagnosis was significantly lower than the sensitivity of smear to clinical diagnosis (-19.3%, p<0.001) and slightly lower than MGIT culture to clinical diagnosis (-7,2%, p=0.024). The sensitivities of smear and MGIT culture were also significantly different (-12,1%, p<0.001).

The positive and negative predictive value of Xpert MTB/RIF against final clinical diagnosis of TBM were 99.1% (n=108/109, [95% CI: 95.0-100]) and 72.5% (n=196/270, [95% CI: 66.9-77.8]), respectively.

The sensitivity of XpertMTB/RIF against smear was 73.4% (n=105/143[95%CI: 65.4-80.5]) and against MGIT sensitivity was 85.1% (n=103/121[95%CI: 77.5-90.9]).

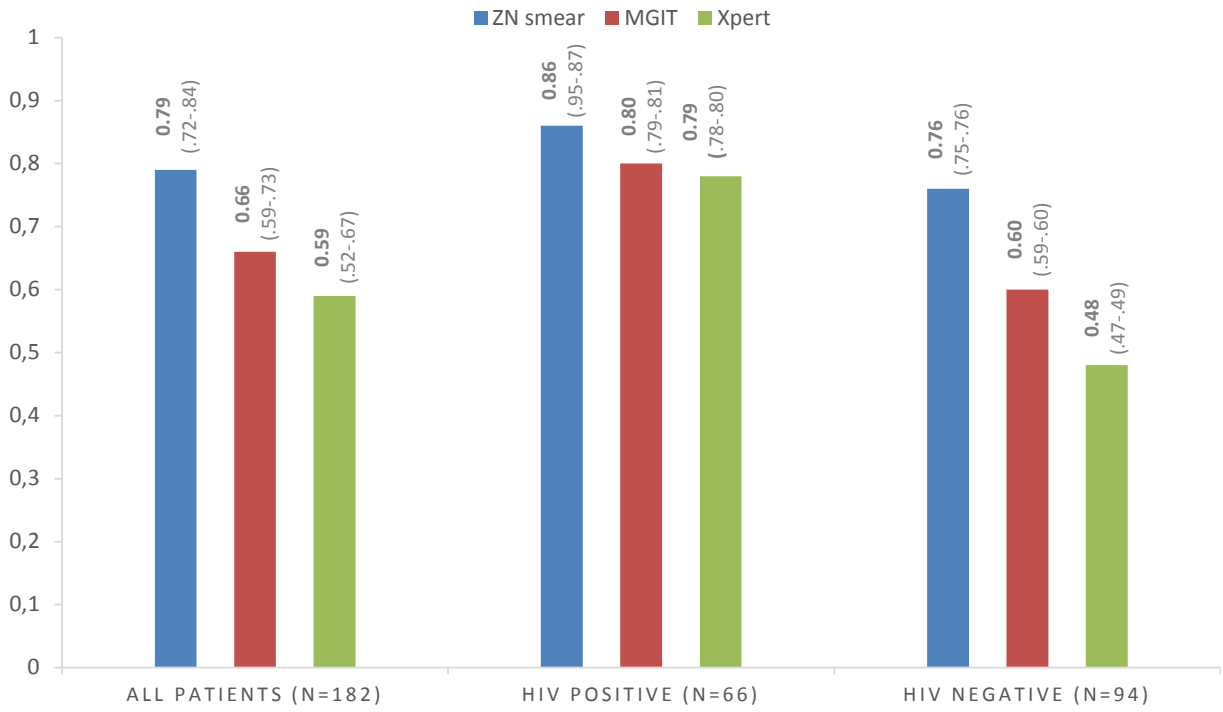


Figure 2.4 Sensitivities of ZN smear, MGIT culture and Xpert MTB/RIF against clinical gold standard for the diagnosis of TB meningitis in all patients and by HIV status

N indicates the number of patients with a clinical diagnosis of TBM. 95% Confidence intervals denoted in brackets. ZN= Ziehl-Neelsen

Figure 2.5 shows in a Venn diagram the number of patients with a clinical diagnosis of TBM with a positive test result for each of the modalities used, ZN smear, MGIT and Xpert MTB/RIF respectively. In total 100 patients had a positive result for all three tests.

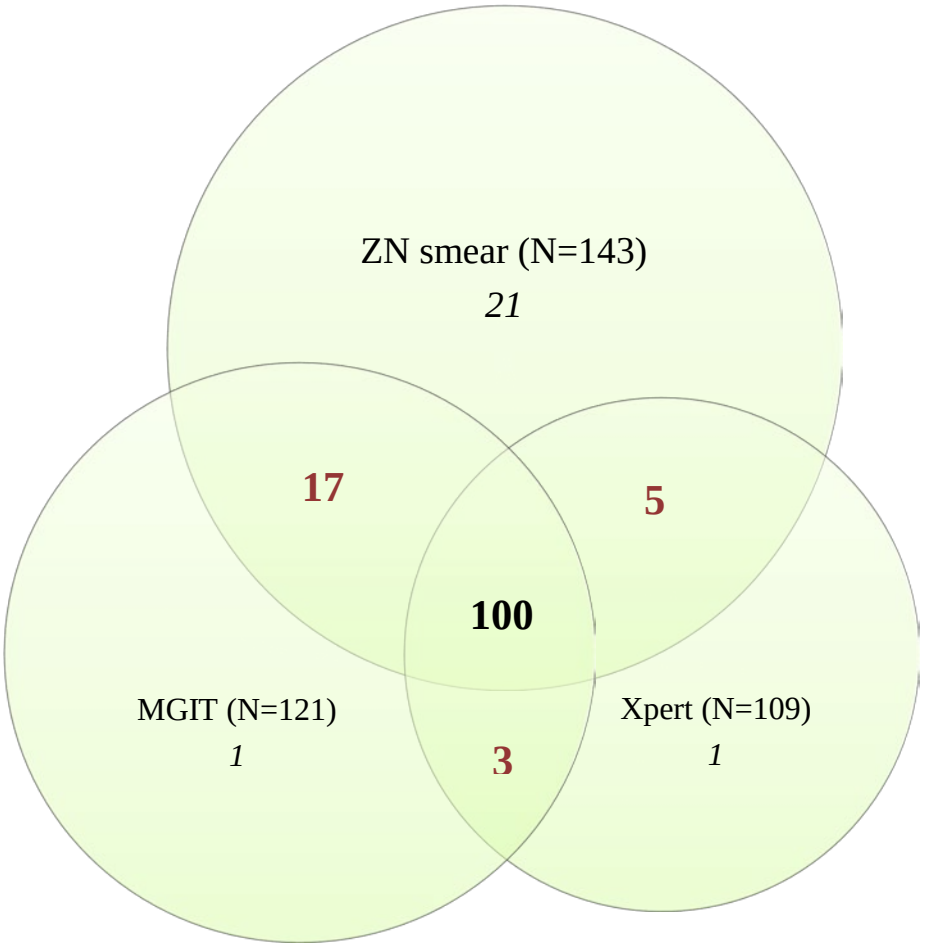


Figure 2.5 Venn diagram of positive test result by diagnostic technique

Number (N) of total positive test results are noted in brackets. Number of shared positive results are shown in bold type. Number of positive test result not shared by other diagnostic techniques are shown in italic type. ZN=Ziehl-Neelsen

Diagnostic accuracy by HIV status

Sensitivity of Xpert MTB/RIF for TBM against clinical diagnosis was significantly higher for HIV infected patients. Among HIV patients, sensitivity was 78.8% (n=52/66, [95% CI: 77.6-79.7]) while it was 47.9% (n=45/94, [95% CI: 47.0- 48.7]) in HIV uninfected patients (P<0.001) (Figure 2.4).

Xpert MTB/RIF positivity by TBM severity grade

There was no difference in the proportion of positive results of Xpert MTB/RIF between the TBM severity categories. The severity grade at baseline was available for 155 patients with a clinical diagnosis of TBM, of which 130/155 (83.9%) had a definite diagnosis of TBM, 15/155 (9.7%) had a probable diagnosis, and 10/155 (6.5%) had a possible diagnosis. Xpert MTB/RIF was positive in 93/155 (60.0%) of patients; in grade 1 32/56 (57.1%), in grade 2 40/69 (58.0%) and in grade 3 21/30 (70.0%) (P=0.46). Similarly, there was no significant difference in proportion of positive results of MGIT culture or ZN smear by TBM severity grade.

Qualitative estimation of bacterial load

The majority of Xpert MTB/RIF results were categorised by Xpert MTB/RIF as 'very low' (n=54/109, 49.5%%) or 'low' (n=46/109, 42.2%) with 9 'medium' (8.3%). No CSF samples reported a 'high' bacterial load by Xpert MTB/RIF.

Qualitative estimation of bacterial load was significantly different between the TBM severity groups. Bacterial load was higher in the higher severity grade groups (Table 2.3).

Table 2.3 Xpert MTB/RIF qualitative load by TBM severity grade

Xpert qualitative load; n (%)	**MRC grade 1 (n=56)**	**MRC grade 2 (n=69)**	**MRC grade 3 (n=30)**	**Comparison* estimate (95%CI); P-value**
no MTB detected	24 (37.5)	29 (24.6)	9 (13.3)	Cumulative OR of a higher
MTB detected; very low	21 (42.9)	17 (42.0)	4 (30.0)	bacterial load
MTB detected; low	10 (17.9)	19 (27.5)	14 (46.7)	Grade 2: 1.94 (1.00-3.74); p=0.048
MTB detected; medium	1 (1.8)	4 (5.8)	3 (10.0)	Grade 3: 4.71 (2.01-11.06); p<0.001

** comparison made using a univariate proportional odds logistical regression model.*
MRC= Medical Research Council, MTB=M.tuberculosis, CI= confidence interval, OR=odds ratio

Diagnostic accuracy of Xpert MTB/RIF by CSF volume.

The volume of CSF received in the TB laboratory was recorded. Of all 379 CSF samples received, 65 (17.2%) were low volume (<2.0 ml), 230 (60.6%) were medium volume (2.1 to 5.0 ml), and 84 (22.2%) samples were high volume (>5 ml). The sensitivities of Xpert MTB/RIF were 51.7% (15/29) (95% CI, 32.5 to 70.6) for low-volume samples, 61.5% (64/104) (95% CI: 44.2-73.0) for medium-volume samples, and 59.2% (29/49) (95% CI:44.2-73.0) for high-volume samples (Figure 2.6). Although the sensitivities for medium- and high-volume samples were greater than those for low-volume samples, this difference did not reach statistical significance (P= 0.34).

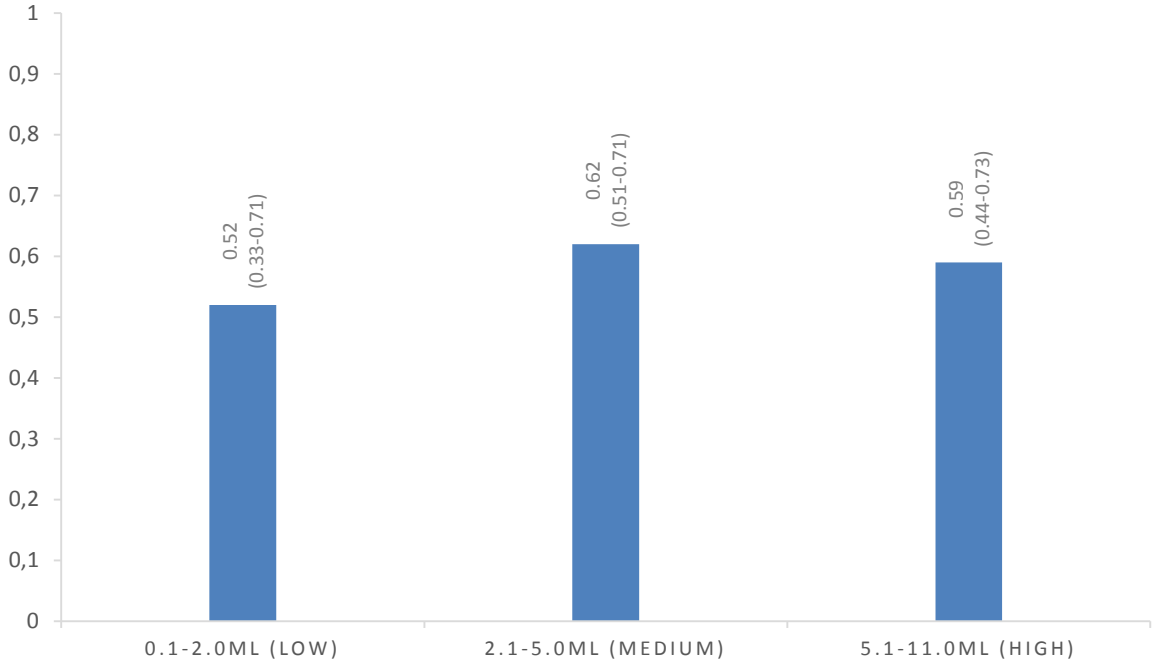


Figure 2.6 Xpert MTB/RIF sensitivity by categorized CSF volume

Low volume samples; n=65 (17.2%), medium volume samples; n=230 (60.6%) and high volume samples; n=84 (22.2%). CSF=cerebrospinal fluid, 95% confidence interval denoted in brackets. Of note: The volume is the total volume received for mycobacterial diagnosis, which means the effective volume used for Xpert testing would have been 40% of the total volume, since the CSF sample was centrifuged and 200µl of the deposit (resuspended in 500µl of supernatant) was used for analysis by Xpert MTB/RIF.

Diagnostic accuracy with addition of vortex step

Prior to 1st August 2011 there were 48 patients included in the study, 26 of whom were finally diagnosed with TBM and 22 as 'not TBM'. The sensitivity of Xpert MTB/RIF in these samples was 50.0% (n=13/26, [95% CI: 29.9-70.1]) and of smear 88.5% (n=23/26, [95% CI: 69.8-97.6]). MGIT culture had a sensitivity of 57.7% (n=15/26, [95% CI: 36.9- 76.6]). The sensitivity of Xpert MTB/RIF to 'definite TBM' was 54.2% (n=13/24, [95% CI: 32.8-74.4])

After the introduction of the vortexing step on 1st August 2011, 331 patients were included in the study. Of these, 156 were finally classified as TBM cases and 175 as 'not TBM'. The sensitivity of Xpert MTB/RIF for these samples was 60.9% (n=95/156, [95% CI: 52.8;-68.6]), the sensitivity of smear and MGIT respectively; 76.9% (n=120/156, [95% CI: 69.5-83.3]) and 67.9% (n=106/156, [95% CI: 60.0-75.2]). The sensitivity of Xpert MTB/RIF to 'definite TBM' was 74.8% (n=95/127, [95%CI: 66.3-82.1]).

The absolute increase of sensitivity of Xpert MTB/RIF to 'definite TBM' before and after the vortex-step was 20.6%, (p=0.04).

2.3.2 DETECTION OF RIFAMPICIN RESISTANCE

Rifampicin resistance was detected in four cases during the study. In three cases the result was confirmed to be MDR TBM by an MTBDR*plus* line probe assay performed on DNA extracted from a positive MGIT culture. One case did not have a positive MGIT culture result. Xpert MTB/RIF testing for rifampicin resistance showed an 'indeterminate result' in two cases. In one case rifampicin resistance was detected using the MGIT SIRE kit. Phenotypic drug resistance testing of all MGIT positive cultures using the MGIT SIRE kit showed 104 RIF susceptible results and 5 RIF resistant cases.

However, it is not possible to draw robust conclusions about the sensitivity of Xpert MTB/RIF for the diagnosis of MDR TBM given the low prevalence of MDR TBM in this study.

2.4 Discussion

We have shown that Xpert MTB/RIF is a rapid, specific test for the diagnosis of TBM. While smear microscopy is a more sensitive test in our laboratory, this exceptional sensitivity depends upon the meticulous examination of individual slides for thirty minutes by a highly skilled and experienced technician. This may be difficult to replicate outside a dedicated research setting[83]. An evaluation of the performance of Xpert MTB/RIF in 235 South African patients with TBM, which was done in the same period as our study, revealed similar sensitivity (62% (95%CI: 48-75%)) and specificity (95% (95% CI: 87-99%)) of the test, while smear microscopy was markedly less sensitive (12%, p=0.001)[170]. Therefore, in high-volume laboratories with low sensitivity for CSF smear microscopy, Xpert MTB/RIF is likely to substantially improve the diagnostic confirmation of TBM since it is less dependent on the skill and time of individual technicians. Xpert MTB/RIF has two significant advantages; the closed cartridge based format and the ability to simultaneously detect *M.tuberculosis* and RIF resistance. The cartridge based format removes the need for manual DNA extraction processing and the closed system dramatically reduces any potential for cross-contamination of samples with PCR amplicons. In our laboratory, the CSF is centrifuged to spin down the cells, after which the deposit is used for the consecutive mycobacterial diagnostic tests. Centrifugation of the neat CSF enhances the detection of bacilli[171]. The addition of a brief vortexing step after addition of the sample reagent improved sensitivity (+ 20%) of Xpert MTB/RIF in these paucibacillary samples and further optimisation of sample processing for extrapulmonary samples may be required to improve detection rates.

The Xpert MTB/RIF test system depends upon capture and lysis of whole bacilli[172] and therefore, as for other microbiological tests for TBM, high volumes (>7mls) of CSF are thought to be crucial to obtaining high sensitivity[83]. However, we could not show a significant difference between three volume categories, in sensitivity of Xpert MTB/RIF (nor in sensitivity of ZN smear or MGIT culture; data not shown) against clinical diagnosis in our study, possibly due to a low number of patients with low volume CSF taken.

Bacterial loads are higher in HIV-infected TBM cases and this is reflected in the higher sensitivity for HIV-associated TBM of all the tests (Figure 2.4), therefore settings with a lower HIV prevalence among TBM patients will have correspondingly lower TBM confirmation rates, and vice versa. This is the inverse of pulmonary TB, where HIV positive individuals with TB are less likely to be smear-positive.

The costs of smear microscopy are substantially lower than of an Xpert MTB/RIF test (consumable and reagent costs approximately 2USD vs 20USD), but the hands-on time required to achieve high sensitivity of smear is greater (approximately 40 minutes for smear vs. 20 minutes for Xpert). Additionally, Xpert MTB/RIF detected four cases of MDR TBM within 2.5 hours. Rapid detection of drug resistance in the paucibacillary CSF has been a major challenge to improving outcome for patients with MDR TBM. Without rapid diagnosis and administration of second line regimens, mortality approaches 100%[64]. However, rare false positive results for rifampicin resistance have been reported with Xpert MTB/RIF[173], and the consequences of mistakenly treating a patient with rifampicin susceptible TBM with weak second line regimens would be grave. It will be extremely difficult to accumulate sufficient data on MDR TBM diagnosis to demonstrate robustly the accuracy of the test for this condition, due to its rarity and accuracy must be inferred from other paucibacillary forms of TB. Therefore, a rifampicin resistant TBM diagnosis on Xpert MTB/RIF should be evaluated in the context of the clinical information, response to treatment and wherever possible be confirmed by a second test such as a line probe assay. An M.*tuberculosis* isolate remains necessary to confirm susceptibility patterns for all drugs including rifampicin since Xpert MTB/RIF detects r*po*B mutations which are present in only 95% of phenotypically rifampicin resistant *M.tuberculosis* isolates [174]. A report from Swaziland informed that 30% (38/125) of MDR strains analysed during a survey in 2009 carried the rpoBI491F mutation, which is not detected by Xpert MTB/RIF[175]. Phenotypic confirmation of resistance would be of particular importance in settings where such strains are circulating. Mutations in other codons outside the field of detection of Xpert MTB/RIF and in rare cases of mixed infection, with both rifampicin sensitive and rifampicin resistant strains, false negative results for rifampicin resistance may also occur. False positive results for rifampicin resistance have

also been reported, due to silent mutations in the RRDR and in some no mutation was found upon rpoB gene sequencing[176]. The cause of the false positive result was unexplained in those cases and only occurred in samples with a very low bacterial load, which should caution in the interpretation of rifampicin resistance results in paucibacillary samples, such as CSF.

Liquid culture methods, where available, have the highest sensitivity for *M.tuberculosis* isolation[177]. However, for patients with rifampicin detected by Xpert MTB/RIF and a clinical suspicion of MDR-TBM, second-line drugs with appropriate CSF penetration should not be withheld until the results from conventional DST become available, since mortality often precedes the microbiological lab-outcome.

One patient in our cohort had a false positive result for *M.tuberculosis* detection on Xpert MTB/RIF. This specificity is consistent with that found for pulmonary TB. The diagnosis in the patient in our case was viral meningoencephalitis. She did not meet the clinical criteria for TBM (she scored 3 points using the published case definition, the minimum score required for diagnostic entry, is 6 points). She was treated with antiviral drugs and antibiotics, but not with antituberculosis treatment. She made a full recovery and was still alive and well when contacted after 10 months of presentation (09/07/13). Without treatment, TBM is invariably fatal.

An updated meta-analysis on the use commercial NAAT for the diagnosis of TBM was published in 2014, unfortunately submitted just prior to the publication of the results of our trial. This review included ten studies, of which half (n=5) evaluated Xpert MTB/RIF, albeit against CSF culture as the gold standard. The summary sensitivity of all included commercial tests (Xpert MTB/RIF, Cobas Amplicor, standard BD ProbeTec ET, modified BD ProbeTec ET, enhanced MTD, Cobas Taqman MTD) was 64% (95% CI: 56-72%), with limited heterogeneity[178]. The investigators analyzed the data of studies only evaluating Xpert MTB/RIF (CSF samples included n=321, of which 133 from a retrospective study) and reported a pooled sensitivity of 70% (95% CI not given), which is lower than the sensitivity found in our study when liquid culture was chosen as the gold standard (85.1% (95%CI: 77.5-90.9%).

WHO published a policy update on the use of XPert MTB/RIF which issued a strong recommendation of Xpert MTB/RIF as the preferred initial test over conventional microscopy and culture in both children and adults[179]. Data from the current study in this chapter was included to form this endorsement. However, this recommendation should be interpreted with some restraint, taking into account the limitations of the test. In particular, the negative predictive value (NPV) of Xpert MTB/RIF, in our study, was only 72.5%, which is too low to exclude the diagnosis, if a patient has clinical considerable a priory chance of having the TBM based on other investigations[180]. A panel of experts emphasized the shortcoming of the recommendation by stating that Xpert MTB/RIF is part of a "package" of TBM diagnostic investigations which should not stop at Xpert MTB/RIF testing[171]. Furthermore, if available, mycobacterial culture remains indispensable for drug resistance confirmation. Conventional smear sensitivity can be significantly improved when large volumes of CSF are examined and slides are meticulously read.

In children, combination of Xpert MTB/RIF and GenoType MTBDRplus (Hain lifeSciences) on CSF improved sensitivity against clinical gold standard from 26% (Xpert alone) and 33% (MTBDRplus alone) to 56% (both NAATs)[181]. This two test approach may not be feasible when resources are limited.

In conclusion, the Xpert MTB/RIF test is able to rapidly confirm a diagnosis of TBM with 59% sensitivity and 99% specificity against a clinical gold standard when using sufficient volumes of CSF, an additional vortex step and the methods outlined in this chapter. This represents a significant advance in the early diagnosis of this devastating condition.

CHAPTER 3

3 INTENSIFIED ANTITUBERCULOSIS TREATMENT FOR TUBERCULOSIS MENINGITIS; TRIAL PROTOCOL

3.1 Background

3.1.1 ANTITUBERCULOSIS TREATMENT OF TUBERCULOUS MENINGITIS

Treatment guidelines for TBM treatment are not uniform. In general, international guidelines recommend 9-12 months of treatment with rifampicin, isoniazid, pyrazinamide and streptomycin (or ethambutol) in the intensive phase, followed by a combination of rifampicin and isoniazid in the continuation phase[114, 182-184]. These treatment regimens are based on the early trials in pulmonary TB involving the introduction of the new first-line antituberculous drugs. The drug dosages and duration of treatment recommended for TBM are derived from pulmonary regimens and are not based on pharmacokinetic principles in the CSF. Regimens for children and adults are similar, however for children often higher dosages are used (Table 3.1).

Table 3.1 Treatment characteristics of first-line antituberculosis agents and recommended treatment dose and duration

First-line agent	Early Bactericidal activity (EBA) *	Minimal Inhibitory Concentration (MIC) †	Estimated CSF:plasma concentration ratio §	Recommended doses (mg/kg) [185]		Treatment duration (months)
				Adults	*Children*	
Isoniazid (H)	0.4-0.8	0.02-0.1	80-90%	5	10	9-12
Rifampicin (R)	0.2-0.6	0.03-0.5	20-30%	10	15	9-12
Pyrazinamide (Z)	0.004 - 0.044‡	0.1	90-100%	25	35	3
Streptomycin (S)	<0.1	0.5-2.0	10-20%	15 (i.m.)	17.5 (i.m.)	3
Ethambutol (E)	<0.1	0.5-2.0	20-30%	15	20	3

** EBA defined as the fall in counts/ml sputum/day during the first two days of treatment (log10/cfu/ml sputum/day) [186]*
† Reported MIC in fully susceptible isolates (µg/ml)
‡ EBA of pyrazinamide is low in the first few days of treatment but at day 4-14 matches that of rifampicin and isoniazid
§ Dependent on degree of blood-CSF barrier disruption
PK=pharmacokinetic, PD=pharmacodynamics, CSF= cerebrospinal fluid, i.m.= intramuscular

In this section we would like to put emphasis on the statement that the CNS "should be regarded as a unique therapeutic compartment"[187] and pharmacokinetic and pharmacodynamic data should be considered in the construction of more effective treatment schedules. The majority of deaths from TBM occur in the first two months of treatment, indicating that effective antimycobacterial killing is most critical in the intensive phase. However, prevention of relapse and the prevention of emerging resistance are additionally important principles of effective multi-drug treatment.

The ability of the different first line anti-tuberculosis drugs to penetrate the CSF is variable and few of the second line drugs are effective in reaching the brain. A summary of antimycobacterial activity and CSF penetration of the first-line drugs used in the intensive phase is appropriate. We will also briefly review some of the second-line agents with favourable CSF levels. Of note little is known about the levels of antimycobacterial drugs in the brain-tissue. Drugs may need to overcome both blood-CSF barrier, consisting of the choroid plexus and the lining epithelial cells and the blood-brain barrier, made up of endothelial tight junctions in capillaries and surrounding glial cells. The level of drugs in the two compartments, CSF and brain tissue may not be equal. A third hurdle may be the penetration and action of drugs in the relatively anerobic conditions within the tuberculoma.

Streptomycin

The initial drug to be introduced for TBM treatment was streptomycin. This aminoglycoside must be given intramuscularly. Streptomycin is a protein synthesis inhibitor. The minimal inhibitory concentration (MIC) in fully susceptible clinical isolates is in the range 0.5-2.0 µg/ml [188]. With the commonly used dosages in adults, the early bactericidal activity [(EBA), generally defined as the fall in counts/mL sputum/day during the first two days of treatment], of streptomycin however is low (<0.1 log10/cfu/ml sputum/day)[186]. With a poor penetration in CSF, the contribution of streptomycin to multi-drug regimens for TBM is probably very limited. In the absence of meningeal inflammation, penetration does not occur. However, in meningitis the penetration can be up to 20% of simultaneous serum levels. In the early course of disease, when blood-brain barrier disruption is prominent, levels above the MIC may be found in CSF (3-16µg/ml). However when clinical improvement is noted, CSF levels barely reach the MIC (0-1.25 to 4µg/ml)[189]. Since TBM may reflect a general state of disseminated tuberculosis, it may be appropriate to maintain streptomycin in treatment schedules in some patients, however at the cost of increased toxicity. For HIV patients, ethambutol should be substituted for streptomycin since injection should be avoided where possible in HIV-infected individuals.

Ethambutol

Ethambutol is bacteriostatic against actively growing TB bacilli, by obstructing the formation of the bacterial cell wall. Ethambutol is slightly more efficient in penetrating the CSF than streptomycin, with levels compared to serum in the range of 0-54%. In healthy adults, despite an oral dose of 50 mg/kg which was twice the usual therapeutic dose, and in the presence of proportionally high blood levels, ethambutol did not appear in the CSF of healthy adults. After oral doses of 18.6-25mg/kg levels, ethambutol did appear in the CSF of patients with active meningitis (0.74-1.98 µg/ml)[189]. Still, with a MIC of 0.5-2.0 µg/ml there may be a limited role for ethambutol in TBM treatment. Both streptomycin and ethambutol have been shown ineffective in sterilizing sputum in pulmonary TB, so their role in TBM may be limited to resistance prevention. It is possible that by increasing the dose of

these drugs therapeutic levels could be achieved in the CSF, but this would come at the cost of increased toxicity.

Isoniazid

After the introduction of isoniazid, a major improvement was seen in the outcome for patients with all grades of TBM. Isoniazid exerts its antimycobacterial activity by inhibiting the synthesis of mycolic acid, required for the mycobacterial cell wall. It is the most bactericidal TB drug and kills approximately 95% of rapidly multiplying organisms in sputum samples within 48 hours[190]. Isoniazid has the highest EBA of the first-line TB drugs ranging from 0.4-0.8log10/cfu/ml sputum/day[186]. MIC in liquid media is low; 0.02-0.04µg/ml [188]. A C_{max} of 3-5µg/ml is needed for optimal action against sensitive *M.tuberculosis* and INH-resistant strains with relatively low MICs[105]. It has good penetration in the CSF in both children and adults. Peak levels are reached at approximately six hours after dose[189]. With an oral dosage of about 9mg/kg isoniazid rapidly diffused into the CSF. By four hours mean CSF isoniazid concentrations measured were 3.2µg/ml, well over the MIC and in the range of optimal C_{max} for sensitive strains[106]. Isoniazid is effective in preventing resistance when used with companion drugs. It is less efficient in eradicating slow growing organisms. Some advocate the administration of higher doses since resistance is increasingly abundant and higher intracerebral C_{max} may lead to killing of strains with low level resistance, which do not carry mutations in the katG gene. Additionally the N-acetyltransferase- 2 genotype of an individual (NAT2) influences the EBA of isoniazid at a given dose and faster isoniazid acetylators consistently have a lower EBA[186]. Conversely slow acetylators may have increased susceptibility to hepatotoxicity[191].

Pyrazinamide

The mechanism of action of pyrazinamide is not completely understood. It is known for its ability to kill semi-dormant *M.tuberculosis* bacilli in low pH milieu, that are not killed by the other TB drugs, possibly by disrupting membrane energetics and inhibiting membrane transport function in *M.tuberculosis*[192]. In pulmonary TB, addition of pyrazinamide to a six-month regimen, significantly reduced relapse rate to less than 5%[185, 190]. Pyrazinamide is very efficient in penetrating the CSF. Generally high levels are found comparable to those in serum[107]. The EBA in the first few days of treatment is low, but at day 4-14 matches that of rifampicin and isoniazid, and is probably also active against extra cellular bacilli[185]. Since rifampicin penetration in CSF is limited and isoniazid resistance is frequent, the role of pyrazinamide in TBM should not be underestimated.

Rifampicin

Rifampicin is considered a key drug in the treatment for TBM, illustrated by the high mortality in MDR-TBM patients compared to isolated isoniazid resistance. In contrast, in a review of the literature, Donald et al. found little effect on mortality in adults after the introduction of rifampicin and pyrazinamide to the TBM treatment schedule, but a significant effect on survival in children[185]. Still, based on the drug-resistance data, rifampicin seems to have a pivotal role in treatment, but it may well be that doses in adults are not sufficient to reach adequate levels in the CSF.

Rifampicin inhibits bacterial RNA synthesis by inhibiting bacterial DNA-dependent RNA-polymerase. Rifampicin has excellent oral bioavailability. It is lipid soluble, and this largely determines its distribution. 80 – 90% of drug is protein bound, which leaves only 10-20% of total drug freely diffusible. It penetrates well into cells, and is active against intra-cellular bacteria, but CSF concentrations are reported to be low[193, 194].

Reported CSF/plasma ratio of maximally 20% are found in early TBM and no drug detectable in CSF in the absence of meningeal inflammation[189]. After an oral dosage of approximately 11mg/kg, serum C_{max} averaging 11.5µg/ml were obtained at two hours. Rifampicin penetrated very slowly into the CSF, and concentrations only slightly in excess of its MIC against *M.tuberculosis* maintained

throughout the period[106]. MICs for *M.tuberculosis* are usually in the range of 0.1 – 1mg/L and in vitro activity is increased in the presence of streptomycin and isoniazid[195].

In general low serum levels of rifampicin are reported, particularly in HIV positive patients, in whom absorption of all TB-drugs may be impaired[196]. It is suggested that rifampicin serum concentrations two hours post dose between 8-24µg/ml are required for optimal treatment of pulmonary tuberculosis[197, 198]. Serum levels below 4µg/ml are defined as very low[198]. In Indonesia 70% of TB-patients had 2-hour plasma concentrations (Cmax) below 4µg/ml[199]. HIV patients have been associated with lower plasma levels of rifampicin [196, 200].

Toxicities

Rifampicin is relatively non-toxic. The most noticeable side effect is red staining of body secretions. Also known as the "red man syndrome"[194]. Other side effects include rash, flushing and gastrointestinal disturbances (usually mild). Drug-induced hepatitis (DIH) is a well-recognized side-effect of TB treatment, with a frequency of between 5 and 33%[201]. The drugs most usually implicated are isoniazid and pyrazinamide. However, transient elevation of transaminases (and less commonly bilirubin) is reported with rifampicin use. DIH usually responds well to treatment interruption. A gradual sequential re-introduction of each drug is usually tolerated without recurrence of hepatitis[201]. Interestingly, in the study of dexamethasone in TB meningitis by Thwaites et al. there was a marked difference in the incidence of DIH between the patients receiving dexamethasone and those receiving placebo – there were no cases of severe hepatitis in the steroid arm but 8 in the placebo arm, suggesting that the reduction in mortality in patients receiving steroids may in part be due to a hepato-protective effect of dexamethasone[32].

3.1.2 HIGH DOSE RIFAMPICIN FOR TUBERCULOUS MENINGITIS

Rifampicin is used throughout the whole of the 9-month treatment period in TBM. The recommended dose is 10mg/kg/day. Formulations of rifampicin usually contain a multiple of 150mg of rifampicin per tablet. Weight based dosing requires dividing tablets but in practice division of tablets is rare. In

Vietnam, the National TB program (NTP) guidelines prescribe rifampicin dosage according to weight categories (Table 3.2). This means that the median dose of rifampicin received by a patient according to weight category is 9.5mg/kg (range 7.7 to 11.3mg/kg). Over 60% of weight categories actually receive less than the recommended 10mg/kg.

Table 3.2 Weight-based dosing schedule of the NTP and additional study treatment

Patient Weight (kg)	NTP Rifampicin dose (mg)	NTP Rifampicin dose (mg/kg)	Study Rifampicin dose (mg)	Study Rifampicin dose (mg/kg)	Dose increase Rifampicin by weight (%)	Additional Rifampicin dose (mg)	Additional Tablets (n)
30	300	10.0	450	15	150.0	150	1.0
31	300	9.7	525	16.9	174.6	225	1.5
32	300	9.4	525	16.4	174.9	225	1.5
33	300	9.1	525	15.9	174.9	225	1.5
34	300	8.8	525	15.4	174.5	225	1.5
35	300	8.6	525	15	175.0	225	1.5
36	300	8.3	600	16.7	200.4	300	2.0
37	300	8.1	600	16.2	199.8	300	2.0
38	300	7.9	600	15.8	200.1	300	2.0
39	300	7.7	600	15.4	200.2	300	2.0
40	450	11.3	600	15	133.3	150	1.0
41	450	11.0	675	16.5	150.3	225	1.5
42	450	10.7	675	16.1	150.3	225	1.5
43	450	10.5	675	15.7	150.0	225	1.5
44	450	10.2	675	15.3	149.6	225	1.5
45	450	10.0	675	15	150.0	225	1.5
46	450	9.8	750	16.3	166.6	300	2.0
47	450	9.6	750	16	167.1	300	2.0
48	450	9.4	750	15.6	166.4	300	2.0
49	450	9.2	750	15.3	166.6	300	2.0
50	450	9.0	750	15	166.7	300	2.0
51	450	8.8	825	16.2	183.6	375	2.5
52	450	8.7	825	15.9	183.7	375	2.5
53	450	8.5	825	15.6	183.7	375	2.5
54	450	8.3	825	15.3	183.6	375	2.5
55	600	10.9	825	15	137.5	225	1.5
56	600	10.7	900	16.1	150.3	300	2.0
57	600	10.5	900	15.8	150.1	300	2.0
58	600	10.3	900	15.5	149.8	300	2.0
59	600	10.2	900	15.3	150.5	300	2.0
60	600	10.0	900	15	150.0	300	2.0
61	600	9.8	975	16	162.7	375	2.5
62	600	9.7	975	15.7	162.2	375	2.5
63	600	9.5	975	15.5	162.8	375	2.5
64	600	9.4	975	15.2	162.1	375	2.5
65	600	9.2	975	15	162.5	375	2.5
66	600	9.1	1050	15.9	175.0	450	3.0
67	600	9.0	1050	15.7	175.0	450	3.0
68	600	8.8	1050	15.4	175.0	450	3.0
69	600	8.7	1050	15.2	175.0	450	3.0
70	600	8.6	1050	15.0	175.0	450	3.0
71	750	10.6	1125	15.8	150.0	375	2.5
72	750	10.4	1125	15.6	150.0	375	2.5
73	750	10.3	1125	15.4	150.0	375	2.5
74	750	10.1	1125	15.2	150.0	375	2.5
75	750	10.0	1125	15.0	150.0	375	2.5

NTP=national TB programme

Studies from Indonesia suggest that an oral dose increase from 10 to 13mg/kg/day is associated with a 65% increase in mean plasma $AUC_{0\text{-}24h}$ and 49% increase in plasma C_{max} without a significant increase in the rate of adverse events[202, 203]. This is likely to be clinically important (particularly in TBM where the therapeutic index is even narrower due to poor drug penetration) because the anti-mycobacterial activity of rifampicin is exposure and concentration dependent[204]. Of note, in the Indonesian study there was a higher rate of grade 1 or 2 transaminitis (indicating rise in liver transaminases in the blood, as measured by aspartate transaminase (AST) and alanine transaminase (ALT), with no apparent symptoms of liver failure) in the high dose arm, but no interruption of treatment was necessary and there was no greater risk of grade 3 or 4 transaminitis. This study was not powered to measure difference in disease outcome, but a study in pulmonary TB found a daily dosage of 1200mg of rifampicin for 3 months to result in significantly improved sputum sterilization [205]. A study comparing 750mg/day with 600mg/day in pulmonary TB found no difference in clinical outcome although both dosages of rifampicin were well-tolerated. However, the situation in pulmonary TB is not analogous to TBM, where the therapeutic index is narrower due to poor drug penetration into CSF.

Rifampicin is not only used to treat tuberculosis, but also in other chronic infections such as brucellosis and chronic staphylococcal disease. In particular, in brucellosis the dosage of rifampicin is higher than that used in TB. The dose most usually trialed has been 15/mg/kg for 6 – 8 weeks, sometimes in combination with ofloxacin[206, 207]. Higher doses of rifampicin appear to be well tolerated in these patients with low rates of transaminitis and treatment interruption. Doses used in staphylococcal disease have been up to 15/mg/kg/day in adults, and 20mg/kg/day in children. Transaminitis and serious adverse events are rarely reported, consistent with the concept that hepatitis in patients on TB treatment is more likely due to isoniazid or pyrazinamide[201, 208].

Based on the data presented in this section we propose an increased dose of rifampicin of 15mg/kg for patients with TB meningitis, to increase serum levels and possibly increase CSF levels of rifampicin. With this strategy, we hope to improve sterilizing power of the antituberculosis regimen in the brain.

3.1.3 LEVOFLOXACIN FOR TUBERCULOUS MENINGITIS

Despite demonstration of *in vitro* activity of various drugs against *M.tuberculosis*, there has been little progress in drug development or assessment of alternative anti-mycobacterial treatment regimes in TB meningitis[72]. Trials in pulmonary TB have demonstrated the safety of prolonged treatment with fluoroquinolones[209, 210]. Initial results with the earlier agents (ciprofloxacin, ofloxacin), where the fluoroquinolone was substituted for one of the standard drugs, were disappointing, but the later generation drugs such as levofloxacin, moxifloxacin and gatifloxacin have improved *in vitro* activity, and there is evidence of good sterilizing activity in sputum in pulmonary TB[111, 211]. Generally, trials of fluoroquinolones in pulmonary TB have been designed to examine the feasibility of substituting other TB drugs with a fluoroquinolone, rather than addition of a new drug to the standard regime[209]. Improved tolerability of the treatment regime has been as much a consideration as improved efficacy. Another approach has been to try to design regimens that enable shortening of the treatment duration. Recent randomized controlled trials investigating the role of fluoroquinolones in shortening treatment for pulmonary TB had disappointing outcome. One trial replaced either isoniazid or ethambutol with moxifloxacin. It showed shorter time to sputum culture conversion, but noninferiority was not shown for the shorter regimens[212]. Another trial involving African patients investigated a short course treatment (4 months) using gatifloxacin for ethambutol, which also did not show noninferiority compared to standard treatment, even though the standard regimen was associated with more drop-outs and treatment failure, more recurrences were seen in the shorter regimen[213].

Since the mortality rate in pulmonary TB is significantly lower than in TB meningitis, the issues facing clinicians are different[53, 214]. In TB meningitis, where the mortality is high, the aim must be to reduce mortality by developing more potent anti-mycobacterial treatment combinations.

Fluoroquinolones are an attractive option for the treatment of TB meningitis because of their demonstrable *in vitro* activity, tolerability, good bioavailability and ease of administration [215-227]. Our center completed a pharmacokinetic study comparing ciprofloxacin, levofloxacin and gatifloxacin in patients with TBM, and examining their pharmacokinetic interaction with rifampicin[110]. We found levofloxacin to have excellent CSF penetration, with a ratio of Area Under the Curve (AUC) in CSF to AUC in plasma of 75%. This compared favorably with gatifloxacin (35%) and ciprofloxacin (14%)[110]. Levofloxacin has the additional advantages of a favorable toxicity profile, affordable cost and available safety data from clinical trials examining its prolonged use in pulmonary TB. We propose to add levofloxacin as a fifth drug in the highly active treatment arm combined with a high dose of rifampicin in this randomized placebo controlled trial.

Microbiological activity

The development of the fluoroquinolones has seen the extension of the spectrum of activity to cover Gram-positive as well as Gram-negative bacteria. All fluoroquinolones inhibit replication and transcription of bacterial DNA by binding to the A-subunit of DNA-gyrase, thus interfering with the resealing of broken DNA strands, frustrating bacterial protein production. This leads to rapid cell death[228, 229].

Levofloxacin has moderate activity against Streptococcus and Staphylococcus species, good activity against the aetiological agents of atypical pneumonia (*L.pneumophila*, mycoplasmas) and enterobacteria, and moderate activity against mycobacterial species in vitro[230]. It is commonly used to treat community acquired pneumonias, sinusitis and enteric fever.

Of the 2nd generation fluoroquinolones, levofloxacin has the greatest anti-tuberculosis activity. MICs for most sensitive isolates are in the order of 0.25 - 1µg/ml[221, 231]. Plasma levels of levofloxacin in Vietnamese patients are comfortably in excess of this, with AUC_{0-12} of 80mg/hr/L[110]. Fluoroquinolone resistance has been identified in strains from Vietnam, but currently is rare and less frequent than rifampicin resistance[232]. In vitro assays do not seem to clearly predict in vivo response to experimental infection and treatment[233].

However, levofloxacin has performed well in human studies using surrogate markers of efficacy such as early bactericidal activity (rate of fall of colony forming units in sputum)[211]. This is probably a reflection of its favourable pharmacokinetic profile resulting in high plasma and intracellular concentrations.

Pharmacokinetics

Effective killing of *M.tuberculosis* is concentration dependent. A recent study comparing the pharmacokinetics of levofloxacin (1g/day) with gatifloxacin (400mg/day) and moxifloxacin (400mg/day) in pulmonary TB patients found levofloxacin to have the most favorable indices, with ratios of free AUC:MIC 1.5 times greater than for gatifloxacin and moxifloxacin[111]. The ratio for levofloxacin was 180 using the MICs needed for the actual study isolates, and 93 using published MIC data. This compares with an established target ratio for AUC:MIC for fluoroquinolones of 40.

Metabolism

Levofloxacin has excellent bioavailability with 99% absorption following oral administration, is only 25% protein-bound, and has excellent CSF penetration, with a ratio of AUC_{csf}/AUC_{blood} of 0.75, which compares favourably with other fluoroquinolones, particularly gatifloxacin[110]. Levofloxacin is predominantly excreted via the renal route with up to 90% of a dose appearing in urine after 48 hours. The plasma half-life is 5 – 7 hours. There is minimal effect on cytochrome p450 enzymes[234].

Levofloxacin in tuberculosis

Prior to the writing of this protocol, a review on the clinical use of fluoroquinolones for TB was published. It included all relevant clinical trials on efficacy and safety of fluoroquinolones in different TB treatment schedules. Overall conclusions were that the use of any of the fluoroquinolones for drug sensitive pulmonary TB was of no benefit to outcome, but the newer fluoroquinolones would be a reasonable option for the treatment of drug resistant TB or for patients who are intolerant to any of the first line TB drugs. An important finding was that the newer fluoroquinolones were well tolerated in all trials[209].

El-Sadr and colleagues investigated in 1998 the effect of adding levofloxacin 500mg daily to the initial phase (first 2 months) of standard anti-tuberculosis quadruple therapy in HIV patients with pulmonary TB. The primary endpoint was sputum culture conversion at 2 months. The investigators found no difference in outcome between treatment arms, but then the overall success rate was high. Sputum clearance rates were 97.3% vs 95.8%[210]. Importantly, there was no difference in the rate of transaminitis or other adverse events between the 2 groups.

Overall the benefit of fluoroquinolones in drug sensitive pulmonary TB may be disappointing, but no clinical trials on the use of fluoroquinolones for TBM have been published yet. Considering the variable penetration in CSF of the first-line anti-tuberculosis drugs and the favourable PK data and optimal CSF penetration of levofloxacin, adding this drug to the regimen might prove of benefit to TBM patients.

Toxicity

By 2001, there had been over 130 million prescriptions of levofloxacin worldwide[235]. Levofloxacin is well tolerated, the commonest side effects being mild gastrointestinal side effects including nausea, vomiting and diarrhoea. Clinical trials also reported a relatively low frequency of CNS reactions such as dizziness, headache, and insomnia. There does not appear to be an increased risk of adverse events as dosage increases[236]. In severe infections levofloxacin is prescribed at a dose of 20mg/kg/day. There is extensive experience with its use in this dosage in enteric fever, when it is prescribed for 1 week, and also in pulmonary TB, when it has been prescribed for 2 months[210].

The overall adverse drug event rate is in the order of 2%, which compares well with other fluoroquinolones[235, 237, 238]. In particular, drug-induced hepatitis, cardiotoxicity and neurotoxicity seem to be less frequent than for the other fluoroquinolones. The incidence of drug-induced hepatitis is approximately 1 per 650 000 prescriptions, and levofloxacin has been used to construct relatively ‘hepato-friendly’ antituberculous treatment regimes in patients who have had this treatment complication[208]. The most notorious of side-effects of the fluoroquinolones in general are

described below in Table 3.3. The risk of toxicities must be considered in the context of the 30% death rate in TB meningitis.

Based on the presented data in this section we propose the use of levofloxacin 20mg/kg as an added fifth drug to the treatment regimen of TB meningitis. Levofloxacin is relatively safe and may be beneficial for these patients, as it has excellent CSF penetration and proven activity against *M.tuberculosis*.

Table 3.3 Infrequent severe toxicities of second generation fluoroquinolones

PROLONGATION OF THE QTC INTERVAL AND HEART ARRHYTHMIAS
Prolongation of the QT interval with possible risk of cardiac arrhythmias has been a recognised side effect of fluoroquinolones for some years. Grepafloxacin was withdrawn from the market place because of prolongation of the corrected QT (QTc) interval resulting arrhythmias and death. Levofloxacin is not generally associated with prolongation of the QT interval[239]. Overall the rate of QT prolongation is estimated at less than 1 per million prescriptions.
TENDONITIS
Tendonitis has been reported following fluoroquinolone therapy, including levofloxacin. However, the overall risk of this is low, estimated at 4 cases per million prescriptions[237]. The risk of tendonitis appears to increase with age[240, 241].
FITS
Generalised convulsions have been described with fluoroquinolone antibiotics. Interestingly, the risk of fits in mice appears to be attenuated with levofloxacin in comparison with ofloxacin[242]. The overall rate of seizures from postmarketing surveillance is estimated at 2 per million prescriptions. In general, convulsions are uncommon in patients with TBM. In the event of prolonged or repeated seizures and the absence of identification of another cause, levofloxacin will be with-held from the patient.
HEPATOTOXICITY
Life threatening hepatotoxicity has been described with some fluoroquinolones (in particular moxifloxacin) but in general is a rare side-effect of their use. There were no episodes of serious hepatic dysfunction noted in cohorts of total 36 000 patients treated with ciprofloxacin, ofloxacin, norfloxacin and enofloxacin[243]. An Italian study of tolerability of levofloxacin in 40 patients with chronic liver disease did not find any episodes of decompensation with a dose of 500mg twice daily[244]. A recently published cohort study showed that both levofloxacin and moxifloxacin caused no additional hepatotoxicity when they were used by patients with hepatitis induced by other first-line TB drugs[245]. Hepatotoxicity is common in TB due to the other drugs used. The drugs most commonly implicated are isoniazid and pyrazinamide.

3.1.4 HYPOTHESIS AND AIMS

We propose enhancing the antimycobacterial efficacy of current treatment for TB meningitis in Vietnam by adding levofloxacin 20mg/kg/day to the intensive phase of treatment and increasing the dose of rifampicin to at least 15mg/kg/day during the intensive phase of treatment for the duration of 2 months.

Hypothesis

Current antimycobacterial regimes are not potent enough to treat TBM effectively, as most of the antimycobacterial drugs have very low CSF penetration. Increasing levels of effective anti-mycobacterial drugs in the CSF and hence at the site of infection will improve treatment outcome.

Aims

The primary aim of this study will be to reduce mortality by intensifying the induction phase of anti-tuberculosis treatment of TBM. Secondary aims are to assess the effect on morbidity and disability of intensifying standard treatment, to assess the safety and tolerability of the intensified treatment.

3.2 Design

This is a randomized, double blind, placebo-controlled trial with two parallel arms, comparing standard antituberculosis treatment for TBM (according to national guidelines) with standard treatment plus an increased dose of rifampicin and additional levofloxacin. We aim to enhance the antimycobacterial efficacy of current treatment for TB meningitis in Vietnam by adding levofloxacin 20 mg/kg/day to the intensive phase of treatment and increasing the dose of rifampicin to 15 mg/kg/day during the intensive phase of treatment for the duration of 2 months (Figure 3.1).

HTD or PNT hospital (stratification)

↓

CONSENT

Inclusion Criteria

Age ≥ 18 years

Clinical diagnosis of TBM

↓

Exclusion criteria

Lack of consent

Creatinin > 3.0 x ULN

Bilirubin > 2.5 x ULN

AST or ALT > 5 x ULN

Positive CSF Gram or India Ink stain

Pregnancy

Diagnosis of multi-drug resistant TBM

↓

HIV test (stratification)

↓

MRC grade (stratification)

Grade 1 = GCS 15 with no focal neurology *or*

Grade II = GCS 11-14 or GCS 15 with focal neurology *or*

Grade III = GCS ≤ 10

↓

Experimental Treatment Randomised to		
Intervention	Dose	Duration
Rifampicin + levofloxacin	5mg/kg/day 20mg/kg/day	2 months
Or Identical Placebos	5 mg/kg/day 20g/kg/day	2 months

↓

Plus Backbone TB treatment

Drug	*Dose*
Isoniazid (H)	5mg/kg od po, max 300mg /day
Rifampicin (R)	10mg/kg od po
Pyrazinamide (Z)	25mg/kg od po, max 2g/day
Ethambutol (E) and/or	15 - 20mg/kg od po, max 1.2g/day
Streptomycin (S)	20mg/kg od im, max 1g/day

After 3 months, stop ZE/S and continue RH for 6 months

↓

Plus Dexamethasone treatment

	Grade 1 TBM	*Grades 2 and 3 TBM*
Week 1	0.3 mg/kg iv	0.4 mg/kg i.v.
Week 2	0.2 mg/kg iv	0.3 mg/kg i.v.
Week 3	0.1 mg/kg iv	0.2 mg/kg i.v.
Week 4	3.0mg total/day po	0.1 mg/kg i.v.
Week 5	2.0mg total/day po	4.0mg total/day p.o.
Week 6	1.0mg total/day po	3.0mg total/day p.o.
Week 7		2.0 mg total/day p.o.
Week 8		1.0 mg total/day p.o.

↓

For HIV-patients: HIV treatment

According to national guidelines. Deferred until 8 weeks, unless already on treatment upon admission

↓

Follow-up

Weekly (0-2 months), Monthly (3-9 months)

Figure 3.1 Trial outline

HTD=Hospital for Tropical Diseases, PNT= Pham Ngoc Thach hospital for tuberculosis and lung diseases. ULN= upper limit of normal, p.o.= oral dose, i.v.= intravenous dose

3.2.1 ENDPOINTS

Primary endpoint

The primary endpoint will be overall survival during a follow-up period of 9 months. For dead patients, their time to death will be analyzed. Survivors will be censored at the date they were last known to be alive (i.e. date of last follow-up visit, or loss to follow-up date, or withdrawal date).

Secondary endpoints

The secondary endpoints are:

Neurological disability at 9 months. This will be assessed using the "simple questions" and Rankin score.

- Time to new neurological event. Neurological events are defined as:
  A. Any of the following adverse events: cerebellar symptoms, coma, hemiplegia, neurological detoriation, paraplegia, seizures, cerebral herniation or cranial nerve palsy
  B. A fall in Glasgow coma score by ≥ 2 points for ≥ 2 days from highest previously recorded Glasgow coma score (including baseline)

Any grade 3 or 4 adverse event (defined in Table 3.7)

- Rate of TB treatment interruption
- The rates of asymptomatic transaminitis and symptomatic hepatitis.
- Time to new or recurrent AIDS defining illness or death (in HIV positive patients only)
- CD4 count at 9 months (in HIV positive patients only)

3.2.2 RANDOMIZATION AND ENROLLMENT

Randomization will be 1:1 and stratified Within strata, we will use block randomization with variable block size. Stratified randomization will ensure that almost equal numbers of patients with equivalent prognosis are included in the two treatment arms.

Eligibility

All adult patients (aged ≥ 18 years) with a clinical diagnosis of TBM (Table 3.4) presenting to the Hospital for Tropical Diseases (HTD), HCMC, or Pham Ngoc Thach Hospital (PNT), HCMC, will be eligible to enter the study.

Table 3.4 Diagnostic clinical algorithm used for trial entry

	Diagnostic criteria
Definite TBM	Clinical meningitis plus acid-fast bacilli seen in the CSF or *M.tuberculosis* cultured from the CSF
Probable TBM	Clinical meningitis plus one of the following criteria: • Radiographic evidence of pulmonary tuberculosis • Acid-fast bacilli seen in sputum or gastric fluid • Evidence of extra-pulmonary tuberculosis • CT or MRI brain scan features consistent with TBM
Possible TBM	Clinical meningitis plus ≥ 2 of the following criteria: • History of previous tuberculosis • Illness duration > 5 days • Glasgow coma score < 15 • Focal neurological signs and ≥ 2 of the following criteria: • Yellow CSF • > 50% lymphocytes in the CSF • CSF glucose < 50% blood glucose

CSF=cerebrospinal fluid

Exclusion criteria are:

- a positive CSF Gram or India Ink stain
- pregnancy, known hypersensitivity/intolerance to fluoroquinolones or rifampicin
- creatinine >3x the upper limit of normal (ULN)
- laboratory contraindications to antituberculous therapy (bilirubin > 2.5 × ULN, AST or ALT > 5 × ULN)
- diagnosis of multi-drug resistant TBM
- lack of informed consent

Randomization

Randomization will be 1:1 and patients will be stratified according to:

- hospital (HTD and PNT),
- HIV status and
- TBM disease severity at presentation (modified MRC grade 1 to 3):
  - Grade 1 = Glasgow coma score (GCS) 15 with no focal neurology
  - Grade 2= GCS 11-14 or GCS 15 with focal neurology
  - Grade 3 = GCS ≤ 10

Within strata, we will use block randomization with variable block size. Stratified randomization will ensure that almost equal numbers of patients with equivalent prognosis are included in the two treatment arms.

Enrolment logs specific to site, HIV positivity and severity of TBM will be used to assign patients to the next available sequential number within the appropriate stratification group. The assigned number will correspond to two pre-packaged bottles which contain a 2-month supply of additional doses of rifampicin and levofloxacin or visually matched placebos of each. Bottles will be prepared centrally by an unblinded study pharmacist and distributed to the sites in batches as required. Only two central study pharmacists who will hold the master randomization list will know the contents of each bottle. This list will be accessed only in the case of emergency unblinding authorized by an investigator as per standard operating procedures.

Blinding

For each group, tablets of intensified treatment or placebo will be placed in bottles in coded sealed packages, which are labeled with the randomization number of the patient. Drug appearance and administration schedules will be identical to maintain blinding amongst the attending physicians and nurses. The admitting physician will be responsible for ensuring the patient satisfies the entry criteria, completes informed consent and starts a study drug treatment package. Clinical details will be recorded in individual patient case record forms (CRFs).

3.2.3 TREATMENTS

Additional treatment for all TBM patients

All patients will receive backbone treatment with standard antituberculous therapy (Table 3.5) and adjunctive dexamethasone (Table 3.6) on study entry, according to PNT Hospital and Vietnamese National TB Programme guidelines. All patients receiving isoniazid will also receive pyridoxine (vitamin B6). Patients, who develop TBM while on treatment for pulmonary TB, will be eligible to enter the study. According to Vietnamese hospital guidelines, these patients will receive TBM-treatment with 5 first line TB-drugs (3SRHZ/E 6RH) which will be the "backbone" or standard TB treatment. If patients consent to take part in the trial, they will be randomized to intensified TB treatment or placebo as described previously. If the patient is comatose, the drugs can be given by nasogastric tube.

Table 3.5 First-line standard antituberculosis treatment

Drug	Dose	Duration
Isoniazid (H)	5mg/kg o.d. p.o., max 300mg/day	9 months
Rifampicin (R)	10mg/kg o.d. p.o. max dose 750mg/day	9 months
Pyrazinamide (Z)	25mg/kg o.d. p.o., max 2g/day	3 months
Ethambutol (E) and/or	20mg/kg o.d. p.o., max 1.2g/day	3 months
Streptomycin (S)	20mg/kg o.d. i.m., max 1g/day	3 months

Table 3.6 Adjunctive dexamethasone treatment

	Grade 1 TBM	Grades 2 and 3 TBM
Week 1	0.3 mg/kg i.v.	0.4 mg/kg i.v.
Week 2	0.2 mg/kg i.v.	0.3 mg/kg i.v.
Week 3	0.1 mg/kg i.v.	0.2 mg/kg i.v.
Week 4	3.0mg total/day p.o.	0.1 mg/kg i.v.
Week 5	2.0mg total/day p.o.	4.0mg total/day p.o.
Week 6	1.0mg total/day p.o.	3.0mg total/day p.o.
Week 7		2.0 mg total/day p.o.
Week 8		1.0 mg total/day p.o.

Second-line antituberculosis therapy

Patients with a definite or clinical diagnosis of multidrug resistant (MDR) TBM will be excluded from the trial and referred to the MDR-TBM department for second-line MDR treatment according to NTP guidelines. Patients who have been randomized and are subsequently diagnosed with MDR-TB will be referred for second-line therapy according to Vietnamese guidelines. They will continue to be followed up in the study and included in the intention to treat (ITT) analysis.

Anti-retroviral therapy

Antiretroviral therapy will be provided for HIV infected patients within the current Vietnamese guidelines. Antiretroviral therapy is available free of charge through the US Government President's Emergency Plan for AIDS Relief (PEPFAR) programme for in-patients with life-threatening opportunistic infections from 2 weeks after admission. HIV positive patients will be referred to the HIV Outpatient Clinic (OPC). To ensure that treatment naïve HIV-positive patients receive ARV treatment at 8 weeks and continue their treatment, patients will be enrolled either at the hospital OPC or through local specialized OPC services, following standard local practice. For ARV-treatment naïve patients, ARV therapy will be initiated after 8 weeks of TB therapy. This is consistent with the results of the recent trial of immediate or deferred antiretroviral therapy in TB meningitis, carried out by our group and consistent with local practice guidelines[33]. There are currently 4 different treatment schedules for first line ARV treatment in Vietnam, all containing two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). Patients already receiving ARVs at the time of diagnosis of TBM will continue ARV therapy. The majority of patients will be on schedules containing nevirapine. According to Vietnamese guidelines nevirapine will be changed to efavirenz for HIV positive patients that require a TB-regimen containing rifampicin. Reports show good clinical outcome for patients on a 600 mg dose of efavirenz who are on TB-regimens containing rifampicin [39]. Accordingly and following National treatment guidelines, the dose of efavirenz will not be increased for patients on TB-regimens containing rifampicin. Second line ARV treatment is rarely prescribed in Vietnam. Very few patients will have a PI in their treatment schedule. Decisions on dose or schedule adjustments for these patients will be

made on an individual basis, following the National guidelines. Liver function tests will be monitored in all patients.

Prophylaxis for opportunistic infections (for HIV positive patients)

Patients will receive prophylaxis for opportunistic infections according to Vietnamese national guidelines. If the CD4 count is less than 200 cells/μL, patients will receive prophylaxis against Pneumocystis jirovecii pneumonia and cerebral toxoplasmosis with cotrimoxazole 960 mg/day.

3.3 Data collection

3.3.1 PATIENT MONITORING

Baseline evaluation

On admission all patients will have a full clinical assessment and examination to determine TBM MRC grade (Table 1.2), and assess any neurological symptoms and signs. The following laboratory tests will be performed at study entry: haematology (full blood count), biochemistry (total protein, albumin, creatinine and liver function tests), cerebrospinal fluid (cell count, protein, glucose, lactate, Gram stain, ZN stain, India Ink stain, cryptococcal antigen, bacterial and mycobacterial culture), HIV test. Additional tests for HIV positive patients will include immunology (CD4 count) and virology (confirmatory HIV test, plasma HIV-1 RNA, HbsAg, HBV DNA, HCV Ab test, HCV RNA). A baseline chest radiograph will be performed for all patients. A CT or MRI brain scan will be performed if there is evidence of raised intracranial pressure or focal neurological abnormalities.

In-patient monitoring

Patients will have daily review until discharge from hospital at 2 months (this period may be adjusted according to clinical findings) for neurological, (drug-related) adverse events (Table 3.7) and new or recurrent AIDS defining illnesses (HIV positive patients only). In-patients will have weekly routine

laboratory monitoring of haematology (full blood count) and biochemistry (creatinine and liver function tests). Cerebrospinal fluid analysis will be done routinely according to local guidelines at 4 and 8 weeks. A subgroup of patients recruited to the pharmacokinetics study will have additional blood and CSF samples taken. Other investigations may be performed if clinically indicated. Uniform management of patients and recording of data will be ensured by the principal investigator who will make a daily round of all study participants. Following discharge, patients will be followed up as part of the National Tuberculosis Programme. Formal outpatient review will occur monthly until the completion of treatment, at 9 months.

Out-patient monitoring

Out-patients will pay monthly visits to the OPC for clinical evaluation and laboratory monitoring until completion of treatment at 9 months. Haematology (full blood count) and biochemistry (creatinine, liver function tests) will be checked monthly. Final cerebrospinal fluid analysis will be at 9 months. HIV positive patients will have additional samples taken for immunology (CD4, CD8) and virology (plasma HIV- 1 RNA) every 3 months until the end of treatment. A subgroup of HIV positive patients who started ARV treatment at week 8 of TBM-treatment will have an additional cerebrospinal fluid analysis at the 3 month OPC-visit.

Imaging

Chest and brain imaging will be performed as clinically indicated - i.e. in the event of pulmonary or neurological deterioration.

Clinical trial specimens

All clinical trial specimens will be labeled with the patient's trial number. Samples will be transferred to the laboratories at the HTD and PNT Hospital for initial processing. Investigation results will be issued to the investigators in a timely manner and a hard copy of the results will be retained in the laboratory for verification. Samples will be stored securely in freezers at the HTD and PNT Hospital prior to transfer to the Oxford University Clinical Research Unit (OUCRU) for further investigations and long term storage.

3.3.2 MANAGEMENT AND REPORTING OF ADVERSE EVENTS

According to the ICH Guidelines for Clinical Safety Data Management: definitions and Standards for Expedited Reporting (1994), a serious adverse event (SAE) is defined as "any untoward medical occurrence that a) results in death, b) is life threatening, c) requires unplanned inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability/incapacity or is a congenital anomaly/ birth defect, e) any other important medical condition, which, although not included in the above, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed." If the patient dies or experiences an adverse event (serious, grade 3 or 4, or one leading to modification of treatment, see Table 3.7) the investigator should inform the principal investigator as soon as possible and complete the specific case report form. When applicable, adverse events will be treated as per the management guidelines in Table 3.8. All SAEs will be recorded on the SAE form and reported to the principal investigator, the Oxford Tropical Research Ethics Board and the Ethical Committee of the Ministry of Health Vietnam within 72 hours of the event. Unblinded adverse event and mortality summaries will be reviewed by the trial's independent Data and Safety Monitoring Committee (DSMC) at regular time points (see section "ethical issues" for details.) If there is a protocol violation for any reason this will be fully recorded. Protocol violations which affect patient safety will be reported to the Oxford Tropical Research Ethics Board and the Ethical Committee of the Ministry of Health Vietnam.

Table 3.7 Table of common toxicity criteria

Haematological	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haemoglobin	8.0 - 9.4 g/dl	7.0 - 7.9 g/dl	6.5 –6.9g/dl	<6.5 g/dl
White cell count	3.0 - 3.9 x 10^3 cells/μl	2.0 - 2.9 x 10^3 cells/μl	1.0 - 1.9 x 10^3 cells/μl	<1.0 x 10^3 cells/μl
Neutrophils	1.0 – 1.5 x 10^3 cells/μl	0.75 – 0.99 x 10^3 cells/μl	0.5 – 0.74 x 10^3 cells/μl	<0.5 x 10^3 cells/μl
Platelets	75 - 99 x 10^3 cells/μl	50 - 74 x 10^3 cells/μl	20 - 49 x 10^3 cells/μl	<20 x 10^3 cells/μl
Prothrombin time	>1.0 – 1.25 x ULN	>1.25 – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 x ULN
Biochemical	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Hyponatraemia	130 – 135 mmol/l	123-129 mmol/l	116-122 mmol/l	<116 mmol/l

Hypernatraemia	146 – 150 mmol/l	151 – 157 mmol/l	158 – 165 mmol/l	>165 mmol/l
Hypokalaemia	3.0 – 3.4 mmol/l	2.5 – 2.9 mmol/l	2.0 – 2.4 mmol/l	<2.0 mmol/l
Hyperkalaemia	5.6 – 6.0 mmol/l	6.1 – 6.5 mmol/l	6.6 – 7.0 mmol/l	>7.0 mmol/l
Hypoglycaemia	3.1 – 3.6 mmol/l 55 – 64 mg/dl	2.2 – 3.0 mmol/l 40-54 mg/dl	1.7 – 2.1 mmol/l 30 – 39 mg/dl	<1.7 mmol/l <30 mg/dl
Hyperglycaemia (fasting)	6.5 – 9.0 mmol/l 118 – 164 mg/dl	9.1 – 14.0 mmol/l 165 – 255 mg/dl	14.1 – 28.0 mmol/l 256 – 509 mg/dl	>28.0 mmol/l, >509 mg/dl or ketoacidosis
Urea	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
Creatinine	>1.0 – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 6.0 x ULN	>6.0 x ULN
Bilirubin	>1.0 – 1.5 x ULN	>1.5 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 x ULN
AST or ALT or GGT	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
Alkaline phosphatase	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN	>10.0 x ULN
Amylase	>1.0 – 1.5 x ULN	>1.5 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 x ULN
Urinalysis	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Haematuria	Microscopic	Gross, no clots	Gross and clots	Obstruction or requiring transfusion
Proteinuria	1+ or <0.3% or <3g/l or 200mg-1g loss/day	2-3+ or 0.3 - 1.0% or 3-10g/l or 1-2g loss/day	4+ or >1.0% or >10g/l or 2-3.5g loss/day	Nephrotic syndrome or >3.5g loss/day
Gastrointestinal	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Stomatitis/mouth ulcers	Mild discomfort, no limits on activity	Some limits on eating or talking	Eating/talking very limited	Requiring IV fluids
Nausea	Mild or transient discomfort, maintains reasonable intake	Moderate discomfort or significantly decreased intake for > 3 days	Severe discomfort or minimal intake for ≥ 3 days	Hospitalization required
Vomiting	Mild or transient, 2-3 episodes per day or mild vomiting lasting < 1 week	Moderate or persistent, 4-5 episodes/day or vomiting lasting ≥ 1 week	Severe vomiting of all foods/fluids in 24 hours or orthostatic hypotension or IV fluids required	Hypotensive shock or hospitalization required for IV fluids
Diarrhoea	Mild or transient, 3-4 loose stools/day or mild diarrhoea lasting < 1 week	Moderate or persistent, 5-7 loose stools per day or diarrhoea lasting ≥ 1 week or nocturnal loose stools	Bloody diarrhoea or orthostatic hypotension or ≥ 7 loose stools per day or requiring IV fluids	Hypotensive shock or hospitalization required for IV fluids

Clinical pancreatitis	Mild abdominal pain, amylase < 2.5 x ULN, other causes excluded	Moderate abdominal pain, amylase < 2.5 x ULN, other causes excluded	Severe abdominal pain, amylase > 2.5 x ULN, hospitalization required.	Severe abdominal pain, shock/hypovolaemia, amylase > 5 x ULN, hospitalization required
Neurological	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Headache	Mild, no treatment	Moderate or requires non-narcotic analgesia	Severe or responds to first narcotic	Intractable or requiring repeated narcotics
Consciousness	Difficulty in concentration or memory	Mild confusion or lethargy <50% waking hours	Disorientation or stupor >50% of waking hours	Coma or seizures
Mood	Mild anxiety or depression	Treatment required for anxiety or depression	Treatment and assistance required, severe depression, mania or anxiety	Acute psychosis or hospitalization
Psychosis	Mild agitation or confusion	Some limitation in activities of daily living and minimal treatment required	Treatment and assistance required, severe agitation or confusion	Toxic psychosis or hospitalization
Cerebellar	Slight incoordination or dysdiadichokinesia	Intention tremor or dysmetria or slurred speech or nystagmus	Ataxia requiring assistance to walk or arm incoordination interfering with activities of daily living	Unable to stand
Neurological	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Motor	Mild weakness in feet but able to walk or mild increase or decrease in reflexes	Moderate weakness in feet (unable to walk on heels or toes), mild weakness in hands but still able to do most hand tasks, or loss of previously present reflex or development of hyperreflexia or unable to do deep knee bends due to weakness	Marked distal weakness (unable to dorsiflex toes or foot drop) and moderate proximal weakness (e.g. in hands interfering with activities of daily living or requiring assistance to walk or unable to rise from chair unassisted)	Confined to bed or wheelchair because of muscle weakness

Clinical myopathy	Minimal findings	Moderate myalgia or difficulty climbing stairs or rising from sitting position, able to walk, may need NSAID	Moderate to severe myalgia needing NSAID, assistance required for walking or general activities	Severe myalgia unrelated to exercise requiring narcotics, unable to walk or necrosis or oedema
Sensory	Mild impairment (decreased sensation e.g. vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution	Moderate impairment (moderately decreased sensation e.g. vibratory, pinprick, hot/cold to ankles) or joint position or mild impairment that is not symmetrical	Severe impairment (decrease or loss of sensation to knees or wrists) or loss of sensation of moderate degree in multiple different body areas (e.g. upper and lower extremities)	Sensory loss involves limbs and trunk
Parasthaesia	Mild discomfort, no treatment	Moderate discomfort, requiring non-narcotic analgesia	Severe discomfort or symptoms respond to narcotic analgesia	Incapacitating or not responsive to narcotics
Peripheral neuropathy	Mild paraesthesia, numbness, pain or weakness, not treated	Moderate paraesthesia, numbness or pain, objective weakness, requires analgesic	Severe, narcotic required, interferes with normal activity	Intolerable, incapacitating, unable to walk despite narcotics, paralysis
Respiratory	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Bronchospasm	Transient, no treatment, 70-80% peak flow or FEV1	Requires treatment, normalizes with bronchodilator, 50-69% peak flow or FEV1	No normalization with bronchodilator, 25-49% peak flow or FEV1, retractions	Cyanosis, intubated or <25% peak flow or FEV1
Cardiovascular	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Cardiac arrhythmia		**A**symptomatic, transient dysrhythmia, no treatment	Recurrent or persistent dysrhythmia, symptomatic**,** treatment required	Unstable dysrhythmia, hospitalization and treatment required
Hypertension	Transient, increase >20mm/Hg, no treatment	Recurrent, chronic increase >20mm/Hg, requires treatment	Acute treatment required, outpatient, hospitalization possible	Hospitalization required

Hypotension	Transient, orthostatic hypotension, no treatment	Symptoms correctable with oral fluid treatment	IV fluid required, no hospitalization required	Hospitalization required
Pericarditis	Minimal effusion	Mild/moderate asymptomatic effusion, no treatment	Symptomatic effusion, pain, ECG changes	Tamponade or pericardiocentesis or surgery required
Haemorrhage	Microscopic or occult	Mild, no transfusion	If Hb <6.6 g/l or if Hct <20% transfuse packed red cells or whole blood based on clinical care Gross blood loss or transfused 1-2 units	Massive blood loss or transfused >2 units
Other	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Fever, oral, > 12 hours	37.7-38.5°C	38.6-39.5°C	39.6-40.5°C	>40.5°C
Fatigue	Normal activity reduced by <25%	25-50% decrease in normal activity	>50% decrease in activity,	Cannot work, unable to care for self
Hypersensitivity	Pruritus without rash	Localized urticarial	Generalised urticaria or angioedema	Anaphylaxis
Rash	Rash, erythema or pruritus	Diffuse maculopapular rash or dry desquamation	Vesiculation or moist desquamation or ulceration	Exfoliative dermatitis or mucous membrane involve-ment or suspected Stevens-Johnson or erythema multiforme or necrosis requiring surgery
General	General mild, transient, easily tolerated, no treatment	Moderate, discomfort, interrupts usual activity, may require minor treatment	Severe, considerable interference with usual activity, requires treatment or intervention	Incapacitating or lifethreatening, requires treatment and/or hospitalization

ULN = upper limit of normal local reference range

Table 3.8 Guide to management of toxicities

Grade 1 clinical or laboratory toxicities Continue study drugs
Grade 2 clinical or laboratory toxicities Continue study drugs If relevant, monitor more closely and consider more frequent laboratory assessments Investigate to exclude other causes
Grade 3 clinical or laboratory toxicities Monitor more closely Perform more frequent laboratory assessments Investigate to exclude other causes For AST or ALT > 5 x ULN stop all study drugs until toxicity resolves and consider reintroduction of antituberculous drugs sequentially (Appendix 7.5). For other grade 3 toxicities the clinician may immediately stop study drugs if confirmatory test cannot be performed within 72 hours or if the clinician determines that continuation of study drugs is unsafe while awaiting test results *Fill in an adverse event form and inform the DSMC*
Grade 4 clinical or laboratory toxicities Monitor more closely Perform more frequent laboratory assessments Investigate to exclude other causes For all grade 4 toxicities that are attributable to antituberculous drugs, stop all drugs until toxicity resolves and restart antituberculous drugs sequentially (Appendix 7.5) For all grade 4 toxicities that are clearly attributable to antiretroviral drugs, stop relevant drugs until toxicity resolves and consider switching to alternative drugs as indicated in Appendix 7.6 For other grade 4 toxicities the clinician may immediately stop study drugs if confirmatory test cannot be performed within 72 hours or if the clinician determines that continuation of study drugs is unsafe while awaiting test results If any doubt about management discuss with the principal investigator *Fill in an adverse event form and inform the DSMC*

3.4 Statistical considerations

3.4.1 SAMPLE SIZE AND POWER CALCULATIONS

The trial is powered for the primary endpoint, i.e. overall survival during the 9-month follow-up period. Based on previous publications from our research group, the 9-month mortality in the control arm is expected to be 60-65% in HIV-positive and around 25% in HIV-negative TBM patients [2]. Approximately 50% of TBM patients in the participating hospitals are HIV-positive; we therefore expect an overall 9-month mortality rate of around 40% in the control arm of our trial. An absolute risk reduction of 10% in 9-month mortality from 40% to 30% due to intensified treatment was judged as both realistic and clinically relevant. Assuming proportional hazards, these mortality estimates translate into a HR of 0.7 [= log(1-0.3)/ log(1-0.4)], i.e. a 30% risk reduction due to intensified treatment on the HR scale. Based on Schoenfeld's formula, a total of 247 deaths are required to detect a HR of 0.7 based on a two-sided test at the 5% significance level with 80% power; assuming an overall mortality rate of 35% in the trial, this translates into 706 required patients. In order to account for potential deviations from our assumptions and losses to follow-up, a safety margin of 6% was added to this number leading to a total sample size of 750 patients (375 per treatment group). HIV-positive TBM patients with a very high mortality are a particularly important subgroup of our study population and we aimed to have sufficient power to also detect a benefit in this subgroup of patients alone. If intensified treatment reduces 9-month mortality by 15% in HIV-positive patients (from 65% to 50%), corresponding to a HR of 0.67, a total of 196 deaths in HIV-positive patients are required to detect this difference with 80% power; approximately 350 HIV-positive patients are necessary to observe 196 deaths during follow-up. To guarantee both sufficient power in the subgroup of HIV-positive TBM patients and a sufficiently high event rate in the total population, the trial will continue recruitment until both a total of 750 patients and a minimum of 350 HIV-positive patients have been recruited.

3.4.2 PRIMARY AND SECONDARY ENDPOINT ANALYSIS

The primary endpoint of this trial is overall survival, i.e. time from randomization to death, during the entire follow- up period of 9 months. Overall survival will be analyzed with a log-rank test stratified by HIV status (positive/negative) and TBM disease severity at presentation (modified MRC grade I, II or III). Kaplan-Meier plots and explicit survival estimates at 3, 6 and 9 months of follow-up will also be calculated for the full populations and in the subgroups defined by HIV status and TBM disease severity separately. In a second stage, overall survival will be modeled using the Cox proportional hazards regression model and the following covariates (in addition to the treatment group): TBM disease severity (grade I, II, or III), HIV status (positive/negative), participating hospital (PNT/HTD), previous TB treatment (yes/no), drug resistance (drug sensitive/MDR-TB/Isoniazid resistant non-MDR). A separate analysis for HIV positive patients only will be performed which will include prior antiretroviral therapy (yes/no), CD4 cell count and log10-HIV viral load at baseline as additional covariates. The homogeneity of the treatment effect on overall survival in the subgroups defined by MRC grade (1, 2, or 3), HIV status (positive/negative), prior TBM treatment (yes/no), drug resistance (drug sensitive/MDR-TB/ isoniazid resistant non-MDR) respectively, will be examined and tested using tests of interaction between treatment and the grouping variable. For the secondary endpoints concerning neurological disability, the disability score at month 3, 6, and 9 of follow- up is defined as the higher (worse) of the “simple question” and the Rankin score assessed at that time point as previously described [2]. Disability score will be defined as 4 (worst outcome) if the patient died prior to the respective time point. The score of primary interest is the month 9 score which will be compared between the two arms with the generalized Cochran-Mantel-Haenzsel test as described in Mantel’s generalized statistics [40] taking into account that the disability score is ordinal. The test will be stratified by HIV status and TBM disease severity at presentation. Patients lost to follow up will be analyzed according to their last recorded disability status. If the rate of patients lost to follow-up exceeds 10%, we will also perform an alternative analysis based on multiple imputation of missing values. Time-to-event endpoints, i.e. time to new neurological event or death and time to new or recurrent AIDS defining illness or death (in HIV positive patients only), will be analyzed with a log-

rank test, Kaplan-Meier curves and Cox regression models as described for the primary endpoint above. All reported serious and grade 3&4 adverse reactions will be listed; their overall frequencies and the rate of treatment interruptions due to adverse events will be compared between the two treatment groups using a generalized Cochran-Mantel-Haenszel test stratified by HIV status and TBM disease severity at presentation.

Analysis populations

All patients will be analyzed in the primary analysis according to their randomization arm (intention-to treat, ITT). The primary endpoint, overall survival, will in addition be analyzed on the per-protocol (PP) population which excluded the following patients: patients with a final diagnosis other than TBM, major protocol violations and those receiving less than 2 months of administration of the randomized study drug for reasons other than death.

A full description of the analysis, including some minor changes to the statistical methods proposed in this section is provided in the statistical analysis, which is an appendix to this thesis.

3.5 Ethical considerations

3.5.1 Ethical approval

This protocol, the patient information sheet, the patient consent form have been reviewed and approved by the Oxford Tropical Research Ethics Committee (OxTREC) and the Institutional Review Boards of the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital. The study and study materials have also been approved by the Ethical Committee of the Ministry of Health Vietnam.

3.5.2 INFORMED CONSENT

A patient cannot enter the trial without informed consent. Written informed consent will be sought for all patients entering the trial. When written consent is not possible verbal consent will be considered acceptable in the presence of a witness who can attest to the accurate reading of the informed consent form and the agreement of the patient. The doctor entering the patient into the trial is responsible for obtaining informed consent. If the patient is unconscious, the consent of the relatives or family members is acceptable. If there are no relatives, the consent of two independent physicians will be considered acceptable. In this case consent from the patient will be sought as soon as the patient regains the ability to give or refuse consent.

Withdrawal from the trial

Patients may voluntarily withdraw from the trial for any reason. If this occurs, the trial researchers are under no obligation to provide treatment. The withdrawal of the patient from the trial will not affect their access to the best standard of care within the national health system. Clinical and laboratory assessment should be performed and recorded at the time of withdrawal.

Confidentiality

A unique trial number will be assigned to each patient entering the trial and will be used to identify all laboratory specimens and the case record forms. All records will be stored securely on the wards or in the OUCRU. Clinical information will not be released without written permission of the patient.

3.5.3 INTERIM SAFETY ANALYSIS

An independent DSMC will oversee the trial. Interim analyses are planned after 20 deaths have been observed, after 6 and 12 months of recruitment and yearly thereafter until the completion of the trial. The DSMC will be provided with unblinded summary tables of grade 3&4 and serious adverse events and an analysis of overall survival. These analyses will be performed by an independent statistician

not otherwise involved with the trial. Based on these data, the committee will make one of the following recommendations:

- Continue the trial without modification
- Continue the trial with modification
- Stop the trial due to safety concerns

Unless the benefit of intensified treatment is shown “beyond reasonable doubt” at an interim analysis, no formal stopping for efficacy is foreseen. The Haybittle-Peto boundary, requiring $p<0.001$ at interim analysis to consider stopping for efficacy, should be used as a guidance. However, the DSMC recommendation should not be based purely on statistical tables but also requires clinical judgment. As the dissemination of preliminary summary data could influence the further conduct of the trial and introduce bias, access to interim data and results will be confidential and strictly limited to the involved independent statistician and the monitoring board and results (except for the recommendation) will not be communicated to the outside and/or clinical investigators involved in the trial.

3.6 Discussion

Currently very few options are available for the treatment of TBM. There are 5 “first-line drugs” and a small number of “second-line drugs”. With the exception of fluoroquinolones, the second-line drugs are relatively toxic and apart from ethionamide, cycloserine and some of the fluoroquinolones, penetration into the CSF is poor. Several new agents are now in the early stages of clinical evaluation, but will not be evaluated in treating TBM in the immediate future. The amplification of MDR-TB strains globally and the exceptionally high mortality among MDR-TBM patients are worrying signs of insufficient TB and TBM treatment globally. This trial is based on the hypothesis that current anti-mycobacterial treatment schedules for TBM are not potent enough and that outcomes will be improved by increasing the CSF penetrating power of this regimen by optimizing dosage and using

additional drugs with better CSF penetration. We acknowledge the fact that this trial is testing this hypothesis by essentially including two interventions in one arm of the trial. From a clinical point of view, the main interest of this research is improving treatment, however scientifically it would be satisfying to know, if positive results are observed, to which drug they can be attributed or which modifications are strictly necessary in this treatment regimen. In order to assess the effect of either intervention alone and the combined effect it is necessary to either perform a series of trials or do a 2 × 2 factorial trial. In a companion paper published alongside to the present study protocol[246] we show that an adequately powered 2 × 2 factorial design would require an eight-fold increase in sample size that would transform our study protocol from what will be the largest trial ever conducted in TBM to an impossible study. Currently 40% of all adult patients with TBM die from the disease. In view of this high mortality we argue for a pragmatic approach. Subsequent trials to further refine the optimal treatment can be initiated if the present working hypothesis proves successful.

CHAPTER 4.

4 INTENSIFIED ANTI-TUBERCULOSIS CHEMOTHERAPY FOR THE TREATMENT OF TUBERCULOUS MENINGITIS

4.1 Background

The rationale and methods of the study are discussed in the previous chapter (chapter 3). In this introduction, we will discuss any amendments to the original protocol, issues concerning recruitment and data analysis.

4.1.1 TRIAL DURATION

The full duration from the formation of the trial hypothesis to final publication of the trial results is given in Figure 4.1. This chart summarizes the course of events from the initial trial idea, which was formed in early 2009, to protocol content review, grant obtainment, ethical approval and from then patient recruitment and follow up. It serves to illustrate the regularly lengthy process of ethical approval and the relatively rapid recruitment of the required sample size.

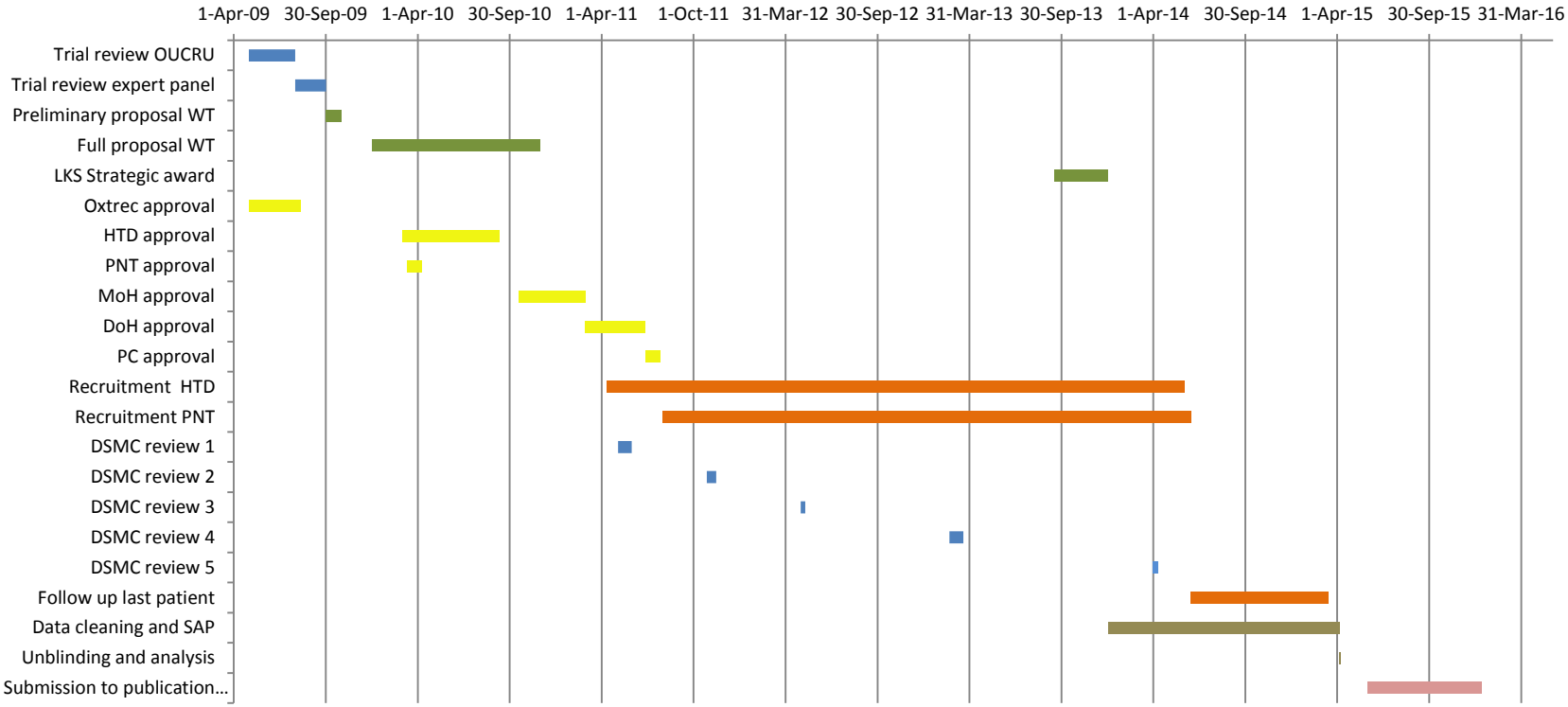


Figure 4.1 Timeline from trial conception to results

The initial version of the protocol was written in May 2009. The trial was reviewed internally at the Oxford University Clinical Research Unit (OUCRU) and externally by a panel of TBM experts. The original protocol was published in February 2011, prior to recruitment (not shown no graph). The trial was funded by a Project Grant from the Wellcome Trust (WT). A second grant, a strategic award, was obtained from the LiKaShing (LKS) foundation. The protocol was ethically reviewed by The Oxford Tropical Research Ethics Committee (OxTREC), the Institutional review board (IRB) of the Hospital for Tropical Diseases (HTD), Pham Ngoc Thach hospital (PNT) IRB, the Vietnamese Ministry of Health (MoH), The Department of Health (DoH) and the Ho Chi Minh City People's Committee (PC). Total recruitment, including last patient follow up lasted 47 months from April 2011 until March 2015. During the trial, the results were reviewed by the Data Safety Monitoring Committee (DSMC) of the trial, while the study team remained blinded. After the last patient, the study team was unblinded and results were published in the New England Journal of Medicine (NEJM) in March 2016. SAP=statistical analysis plan.

4.2 Changes to the trial protocol

4.2.1 XPERT MTB/RIF TESTING

After the trial initiated recruitment, a few minor changes were made to the protocol. In July 2011, Xpert MTB/RIF testing for CSF was added to the array of tests performed at baseline. It was also stated that patients who tested positive for *M.tuberculosis* with rifampicin resistance detected on Xpert MTB/RIF, will not be recruited, if the results were available prior to enrollment. Patients with rifampicin resistance detected on Xpert MTB/RIF were transferred to the MDR TB programme of the NTP, based at PNT hospital in HCMC.

4.2.2 PATIENT RECRUITMENT

The trial was powered to detect a mortality reduction from 40% to 30% in the overall population (corresponding to a target HR of 0.7) and a reduction in mortality from 65% to 50% in the subgroup of HIV-infected patients with 80% power at the two-sided 5% significance level leading to a target sample size of at least 750 subjects and a minimum of 350 HIV-infected patients, assuming 50% of recruited patients are HIV infected. By February 2013, when more than 500 patients had been recruited, it became apparent that the rate of recruitment of HIV infected patients was slower (only 39% of all recruited patients) than expected. After careful consideration of the study team and the DSMC, in order to retain power of observing a treatment effect in this important population it was decided to continue recruitment of HIV infected patients, after the total sample size of 750 patients was obtained, to ensure 350 HIV positive patient included in the dataset. Strictly, this was no change to the original protocol, however it involved careful thought and thorough power calculations of different recruitment scenarios in order to make this decision.

4.2.3 DSMC REVIEWS, STATISTICAL ANALYSIS AND SAP

During the trial there were no concerns from the DSMC, and in the conclusion after each interim analysis was to continue the trial without modification. Statistical analysis followed the protocol and the statistical analysis plan (SAP) (appendix to this thesis).

4.3 Results

4.3.1 PATIENT ENROLLMENT

Between 18 April 2011 and 18 June 2014, 817 adult patients were randomly assigned to receive either standard anti-tuberculosis treatment with placebo (409 patients) or with additional rifampicin and levofloxacin (intensified treatment) (408 patients). Fifty-three patients did not complete follow-up for reasons other than death (28 in the standard arm, 25 in the intensified arm).

One hundred and twenty-one patients were excluded from the per-protocol population (59 in the placebo arm, 62 in the intensified treatment arm). Fourteen patients had an alternative diagnosis and 8 patients were deemed unlikely to have tuberculous meningitis (3 in the placebo arm, 5 in the intensified treatment arm). One hundred and three patients received less than 50 days of study drugs for other reasons than death and 15 patients were diagnosed with MDR tuberculous meningitis (Figure 4.2). We assessed compliance with the 8-week intervention and 4.0% (33/817) were judged as non-compliant (19 in the standard arm, 14 in the intensified arm), i.e. <100% medication doses received for reasons other than death, confirmed other diagnosis, MDR, adverse events leading to treatment interruption, or loss to follow-up.

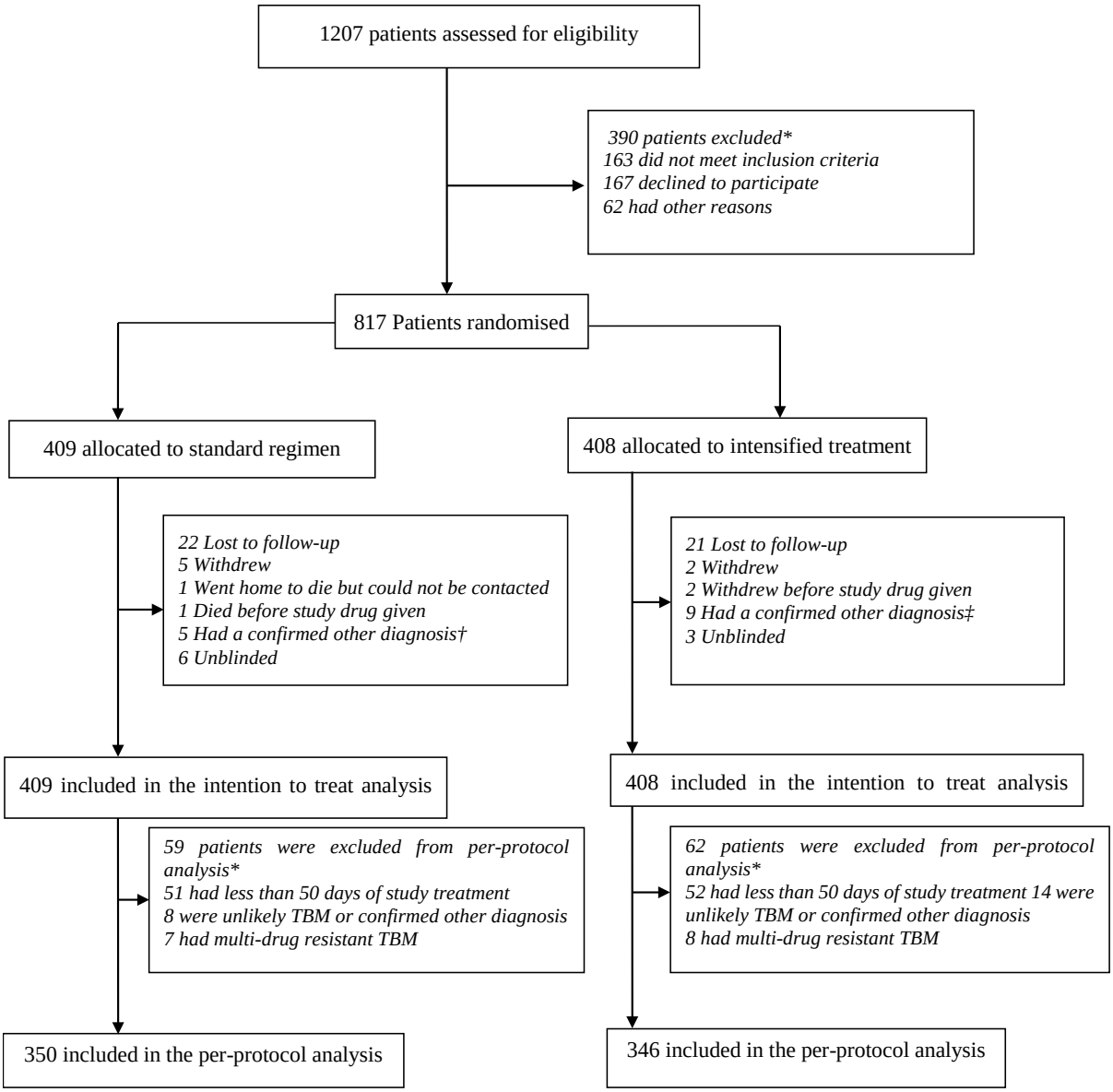


Figure 4.2 Trial flow diagram

**One patient may have more than a single reason to be excluded. † Four patients had cryptococcal meningitis, one patient had an intracranial malignancy. ‡ Seven patients had cryptococcal meningitis, one patient had encephalitis caused by Herpes simplex, one patient had eosinophilic meningitis*

4.3.2 BASELINE CHARACTERISTICS

Baseline characteristics were balanced between the two treatment groups (Table 4.1). The predominant sex was male (68.5%), the median age and duration of illness was 35 years and 15 days respectively. The majority of patients had mild to moderate illness severity; only 17.4% were grade 3 at enrollment. 42.7% of patients were HIV-infected. The published diagnostic criteria[85] defined 49.8% of patients with definite tuberculous meningitis, 26.2% with probable and 21.3% with possible tuberculous meningitis. Amongst patients with culture-confirmed disease, 26.7% had isoniazid resistant infection and 4.7% had MDR tuberculous meningitis. The baseline clinical characteristics of patients in the three severity grades are provided in Table 4.2.

Table 4.1 Baseline characteristics by treatment arm

Characteristic	**Standard regimen (N=409)**	**Intensified regimen (N=408)**	**All patients (N=817)**
Male sex - no.(%)	278 (68.0%)	282 (69.1%)	560 (68.5%)
Age [years] – median (IQR)	35 (30,47)	35 (29,45)	35 (29, 46)
Weight [kg] – median (IQR)	50 (44,55)	47 (43,54)	48 (44, 55)
History of previous tuberculosis – no.(%)	62 (15.2%)	84 (20.6%)	146 (17.9%)
Duration of illness [days] – median (IQR)†	15 (10,30)	15 (10,30)	15 (10,30)
Cranial nerve palsies – no.(%)	95 (23.2%)	106 (26.0%)	201 (24.6%)
Hemiplegia – no.(%)‡	62 (15.2%)	68 (16.7%)	130 (15.9%)
Paraplegia – no.(%) ‡	45 (11.0%)	33 (8.1%)	78 (9.5%)
Quadriplegia – no.(%)‡	2 (0.5%)	8 (2.0%)	10 (1.2%)
MRC grade – no.(%) §			
- 1	160 (39.1%)	158 (38.7%)	318 (38.9%)
- 2	178 (43.5%)	179 (43.9%)	357 (43.7%)
- 3	71 (17.4%)	71 (17.4%)	142 (17.4%)
Chest X-ray result – no.(%) ¶			
- consistent with tuberculosis	214 (53.0%)	186 (45.9%)	400 (49%)
- miliary tuberculosis	45 (11.1%)	64 (15.8%)	109 (13.3%)
- other abnormalities	51 (12.6%)	54 (13.3%)	105 (12.9%)
- normal	94 (23.3%)	101 (24.9%)	195 (23.9%)
HIV–infected – no.(%)	174 (42.5%)	175 (42.9%)	349 (42.7%)
ART-naive HIV-infected patients– no.(%)	115/174(66.1%)	114/175 (65.1%)	229/349 (65.6%)
HIV-infected patients on ART prior to enrolment– no.(%)	59/174 (33.9%)	61/175 (34.9%)	120/349 (34.4%)
CD4 cell count [cells/mm^3]– median (IQR) ‖	38 (15,82)	38 (14,113)	38(14, 101)
Sodium [mmol/l]– median (IQR)**	129 (124,133)	127 (123,132)	128(123,132)
Cerebrospinal fluid analysis††			
Clear appearance– no.(%)	284 (74.7%)	283 (75.3%)	567 (75.0%)
White cell count [cells/mm^3]– median (IQR)	92 (27,263)	135 (45,303)	115 (34,279)
Lymphocyte percentage [%]– median (IQR)	95 (75,100)	90 (65,100)	90 (69,100)
Protein [g/l] – median (IQR)	1.2 (0.6-1.4)	1.2 (0.6,2.0)	1.2 (0.6, 2.0)
Glucose [mmol/l] – median (IQR)	1.9 (1.3,2.8)	1.9 (1.3,2.6)	1.9 (1.3, 2.7)
Lactate [mmol/l] – median (IQR)	4.9 (3.3,6.6)	4.8 (3.7, 6.4)	4.8 (3.5, 6.5)
Diagnostic category – no.(%) ‡‡			
- definite tuberculous meningitis	201 (49.1%)	206 (50.5%)	407 (49.8%)
- probable tuberculous meningitis	109 (26.7%)	105 (25.7%)	214 (26.2%)
- possible tuberculous meningitis	91 (22.3%)	83 (20.3%)	174 (21.3%)
- unlikely tuberculous meningitis	3 (0.7%)	5 (1.2%)	8 (1.0%)
- confirmed other diagnosis	5 (1.2%)	9 (2.2%)	14(1.7%)
Resistance category – no.(%)			
DST available– no. §§	156	166	322
- No isoniazid or rifampicin resistance – no.(%)	107 (68.6%)	113 (68.1%)	220 (68.3)
- Isoniazid mono-resistance – no.(%)	41 (26.3%)	45 (27.1%)	86 (26.7%)
- Rifampicin mono-resistance – no.(%)	1 (0.6%)	0 (0%)	1 (0.3%)
- MDR – no.(%)	7 (4.5%)	8 (4.8%)	15 (4.7%)

IQR= inter-quartile range
†Duration of illness not known for 1 patient in the standard arm and 3 patients in the intensified treatment arm
‡Data on were not available for 1 patient in the intensified treatment arm
§MRC denotes modified British Medical Research Council criteria. Grade 1 indicates a Glasgow coma score of 15 with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less.
¶Chest X-ray results were missing for 5 patients in the standard arm and 3 patients in the intensified treatment arm.
‖CD4 cell counts were only performed in HIV-infected patients. Data were missing in 30 patients in the standard arm and 25 patients in the intensified treatment arm.
***Sodium levels at baseline were performed for 383 patients in the standard arm and 362 patients in the intensified treatment arm.*
††Results for cerebrospinal fluid results at baseline were available for the following number of patients in the standard arm and intensified arm respectively; appearance: 380 vs. 376, total white cell counts: 394 vs. 392, lymphocyte percentage: 374 vs. 377, protein: 379 vs. 380, glucose: 382 vs. 382, lactate: 348 vs. 354
‡‡Diagnostic categories were assigned according to the consensus case definition[85]. Patients with an unlikely diagnosis of tuberculous meningitis had a score of <6. Confirmed other diagnosis was only made based on microbiological evidence.
§§DST= drug susceptibility test.
¶¶Isoniazid mono-resistance is defined as resistance to isoniazid, but not to rifampicin. Rifampicin mono-resistance is defined as resistance to rifampicin, but not to isoniazid. MDR (multidrug-resistance) is defined as resistance to at least both isoniazid and rifampicin. In all categories, other resistance may be present.

Table 4.2 Baseline characteristics by severity grade

Characteristic	**MRC grade 1* (N=318)**	**MRC grade 2 (N=357)**	**MRC grade 3 (N=142)**	**Comparison † (P-value)**
Male sex-no.(%)	219(68.9%)	235(65.8%)	106(74.6%)	0.16
Age [years] – median (IQR)	35(28,42)	39(31,52)	33(28,43)	<0.001
Weight [kg] – median (IQR)	49(44,55)	48(44,53)	48(44,53)	0.76
History of previous tuberculosis – no.(%)	55(17.3%)	63(17.6%)	28(19.7%)	0.80
Duration of illness [days] – median (IQR) ‡	15(10,30)	15(10,30)	15(8,21)	0.55
Cranial nerve palsies – no.(%)	7(2.2%)	138(38.7%)	56(39.4%)	<0.001
Hemiplegia– no.(%) §	3(0.9%)	74(20.7%)	53(37.6%)	<0.001
Paraplegia– no.(%) §	4(1.3%)	64(17.9%)	10(7.1%)	<0.001
Quadriplegia– no.(%)§	0(0%)	4(1.1%)	6(4.3%)	<0.001
Glasgow coma score	15(15,15)	14(13,15)	8(7,10)	<0.001
Sodium [mmol/l]- median (IQR)‖	129(124,132)	128(123,132)	127(121,133)	0.41
Chest X-ray result¶				0.07
- abnormal consistent with TB	134(42.5%)	184(52.3%)	82(57.8%)	
- abnormal miliary TB	52(16.5%)	42(11.9%)	15(10.6%)	
- abnormal other	44(14.0%)	46(13.1%)	15(10.6%)	
- normal	85(27.0%)	80(22.7%)	30(21.1%)	
Cerebrospinal fluid analysis**				
White cell count [cells/mm^3]– median (IQR)	131(39,295)	114(28,274)	102(45,264)	0.37
Lymphocyte percentage [%]– median (IQR)	90(72,100)	91(71,100)	85(60,100)	0.03
Protein [g/l] – median (IQR)	1.0(0.5,1.7)	1.3(0.7,2.0)	1.5(0.9,2.4)	<0.001
Glucose [mmol/l] – median (IQR)	2.0((1.4,2.8)	1.9(1.2,2.7)	1.7(2.7)	0.07
Lactate [mmol/l] – median (IQR)	4.3(3.0,5.7)	4.9(3.7,6.8)	6.0(3.9,7.9)	<0.001
HIV-infected- no (%)	149(46.9%)	135(37.8%)	65(45.8%)	0.04
CD4 cell count [cells/mm^3] - median (IQR) ††	43(19,112)	34(10,97)	39(13,74)	0.21
ART-naive HIV-infected patients‡‡	89(59.73%)	93(68.89%)	47(72.31%)	0.12
HIV-infected patients on ARTprior to enrollment	60(40.27%)	42(31.11%)	18(27.69%)	
Diagnostic category- no (%)**§§**				0.07
- definite tuberculous meningitis	148(46.54%)	171(47.9%)	88(61.97%)	
- possible tuberculous meningitis	79(24.84%)	75(21.01%)	20(14.08%)	
- probable tuberculous meningitis	80(25.16%)	102(28.57%)	32(22.54%)	
- unlikely tuberculous meningitis	4(1.26%)	4(1.12%)	0(0%)	
- confirmed other diagnosis	7(2.2%)	5(1.4%)	2(1.41%)	

**MRC denotes modified British Medical Research Council criteria. Grade 1indicates a Glasgow coma score of 15 with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less. Of note, the MRC grade at study enrolment is reported here whereas for neurological signs, the value at study screening is reported. 14 patients in the MRC grade 1 group had mild neurological signs upon presentation. However these symptoms had resolved before enrolment to the study.*

†Summary statistics are frequency (percentage) for categorical and median (IQR) for continuous variables. P-values are based on Fisher's exact test (categorical data) or the Kruskal-Wallis test (continuous data).

‡Duration of illness not known for 4patients.

§Data on were not available for 1 patient.

¶Chest X-ray results were missing for 8 patients.

‖Sodium levels at baseline were missing for 72 patients.

***Results for cerebrospinal fluid results at baseline were available for the following number of patients in MRC Grade groups 1, 2 and 3 respectively; total white cell counts: 308, 341 and 137, lymphocyte percentage:300, 321 and 130, protein:295, 333 and 131, glucose:295, 337 and 132, lactate:269, 311 and 122.*

††CD4 cell counts were only performed in HIV-infected patients. Data were missing in 55patients.

‡‡ART; anti-retroviral therapy

§§ Diagnostic categories were assigned according to the consensus case definition[85]. Patients with an unlikely diagnosis of tuberculous meningitis had a score of <6. Confirmed other diagnosis was only made based on microbiological evidence.

4.3.3 PRIMARY OUTCOMES

During 9 months of follow-up, 113 and 114 patients died in the intensified treatment and placebo arm respectively (HR 0.94; 95%CI 0.73 to 1.22, P=0.66). Kaplan Meier survival curves of all patients included in the intention to treat population are shown in Figure 4.3. Survival by HIV status is shown in Figure 4.4 and Figure 4.5.

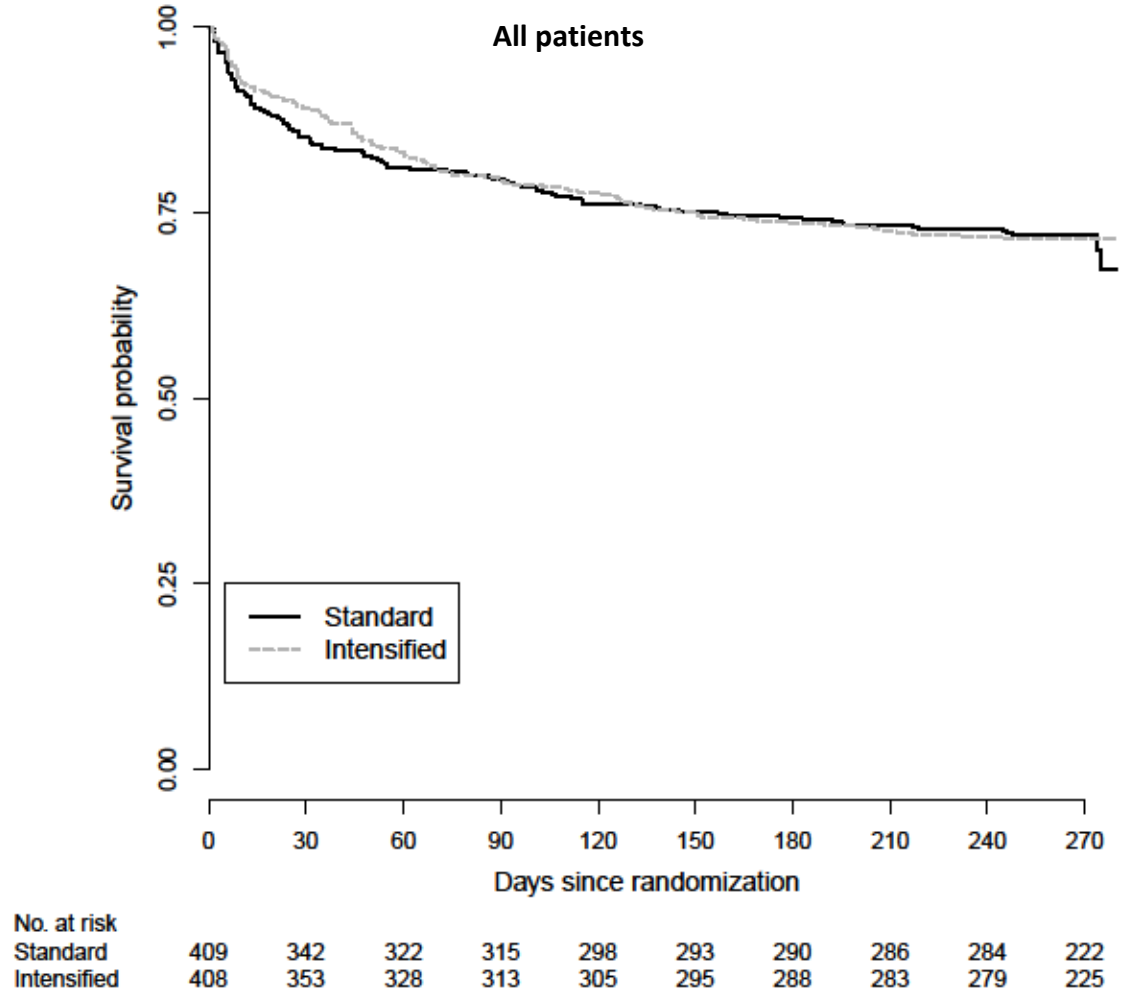


Figure 4.3 Kaplan Meier survival estimates of all patients

*including all 817 patients in the intention to treat population. * The statistical analysis plan pre-defined to include all deaths on the database in the final analysis and this included two deaths on days 274 and 275. As the 9 month follow-up visit was on days270-272 for most subjects, the number of patients at risk on days 274 and 275 was low explaining the drop in the Kaplan-Meier curves at the end.*

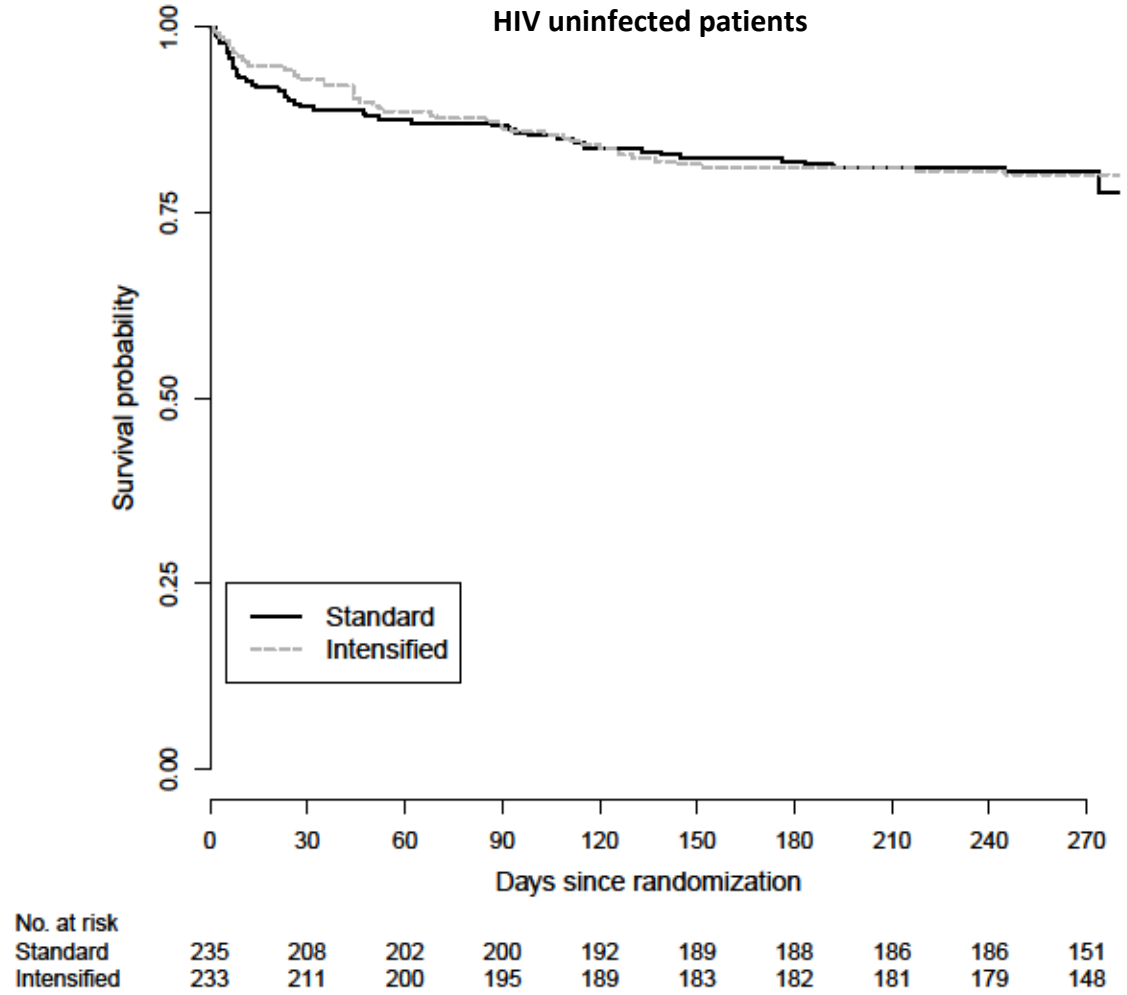


Figure 4.4 Kaplan Meier survival estimates of all HIV uninfected patients

Including all 468 HIV uninfected patients in the intention to treat analysis.

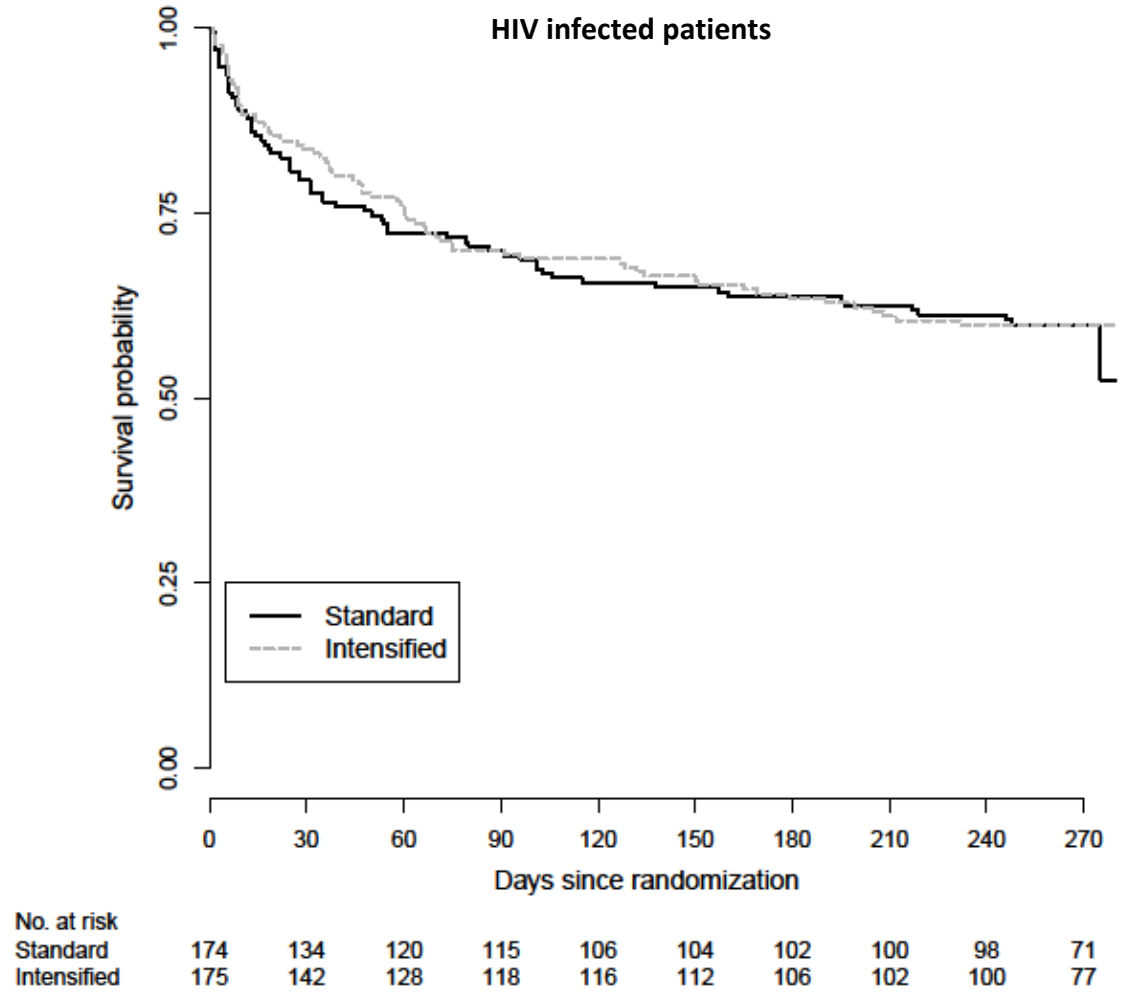


Figure 4.5 Kaplan Meier survival estimates of all HIV infected patients

Including all 349 HIV infected patients in the intention to treat analysis.

There was no evidence of a treatment effect in the overall population or any of the pre-defined subgroups, except for a suggestion of benefit of intensified treatment in subjects with isoniazid resistance (P=0.06) (Table 4.3 Primary endpoint: overall result and predefined subgroup analysis).

Table 4.3 Primary endpoint: overall result and predefined subgroup analysis

Subgroup	Standard regimen (n=409)	Intensified regimen (n=408)	Comparison	Test for heterogeneity*
	deaths/n	deaths/n	Hazard Ratio (95% CI); P-value	(P-value)
ITT	114/409	113/408	0.94(0.73-1.22); p=0.66	
Per protocol population	99/355	98/351	0.91(0.69-1.21); p=0.53	
MRC grade				0.69
- 1	25/160	21/158	0.82(0.46-1.46); p=0.49	
- 2	50/178	52/179	1.07(0.72-1.57); p=0.74	
- 3	39/71	40/71	0.87(0.56-1.36); p=0.55	
HIV status				0.74
- uninfected	46/235	45/233	1.00(0.66-1.51); p=1.00	
- infected†	68/174	68/175	0.91(0.65-1.27); p=0.57	
Previous tuberculosis				0.82
- no	89/347	78/324	0.91(0.67-1.23); p=0.53	
- yes	25/62	35/84	0.99(0.59-1.67); p=0.97	
On anti-tuberculosis treatment at enrolment‡				0.02
- no	36/150	49/149	1.38(0.89-2.12); p=0.15	
- yes	78/259	64/259	0.74(0.53-1.03); p=0.07	
Diagnostic score§				0.93
- definite TBM	56/201	55/206	0.90(0.62-1.31); p=0.57	
- probable TBM	30/109	33/105	1.10(0.66-1.83); p=0.71	
- possible TBM	28/91	25/83	0.94(0.54-1.63); p=0.82	
Resistance category				0.04
- No or other resistance	22/107	30/113	1.54(0.88-2.71); p=0.13	
- Isoniazid resistant	16/41	11/45	0.45(0.20-1.02); p=0.06	
- Rifampicin resistant/MDR	6/8	5/8 (62.50)	0.63(0.15-2.69); p=0.53	

CI= confidence interval

**Heterogeneity was tested with a likelihood ratio test for an interaction term between the sub-grouping variable and the randomized treatment group. As pre-defined, the Cox regression was stratified by MRC grade and HIV status.*

† Amongst patients not on ARV at enrolment, of 49/114 (43%) subjects died in the intensified arm (30 of them in the first 8 weeks) and 44/115 (38%) died on placebo (29 of them in the first 8 weeks). Amongst patients on ARV at enrolment, of 19/61 (31%) subjects died in the intensified arm (9 of them in the first 8 weeks) and 24/59 (41%) died on placebo (18 of them in the first 8 weeks).

‡ Patients were not eligible to enter the trial if they had received > 7days of anti-tuberculosis treatment prior to enrollment. The median duration of anti-tuberculosis treatment was 4 days (interquartile range2-5).

§ 22 subjects with unlikely tuberculous meningitis or confirmed other diagnosis not included.

Overall survival by treatment arm in the per protocol population and MRC-grade groups are shown in Figure 4.6 to Figure 4.9.

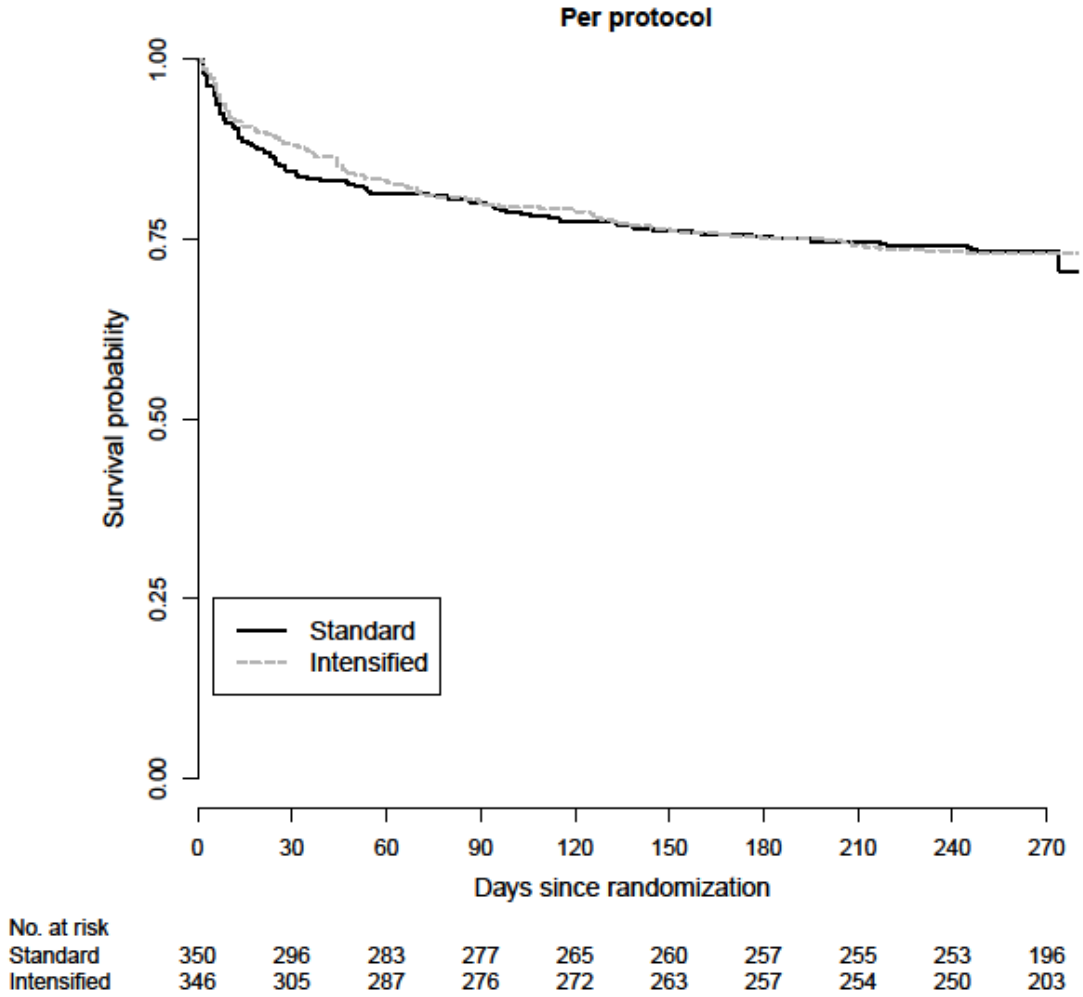


Figure 4.6 Kaplan-Meier survival estimates of the per protocol population

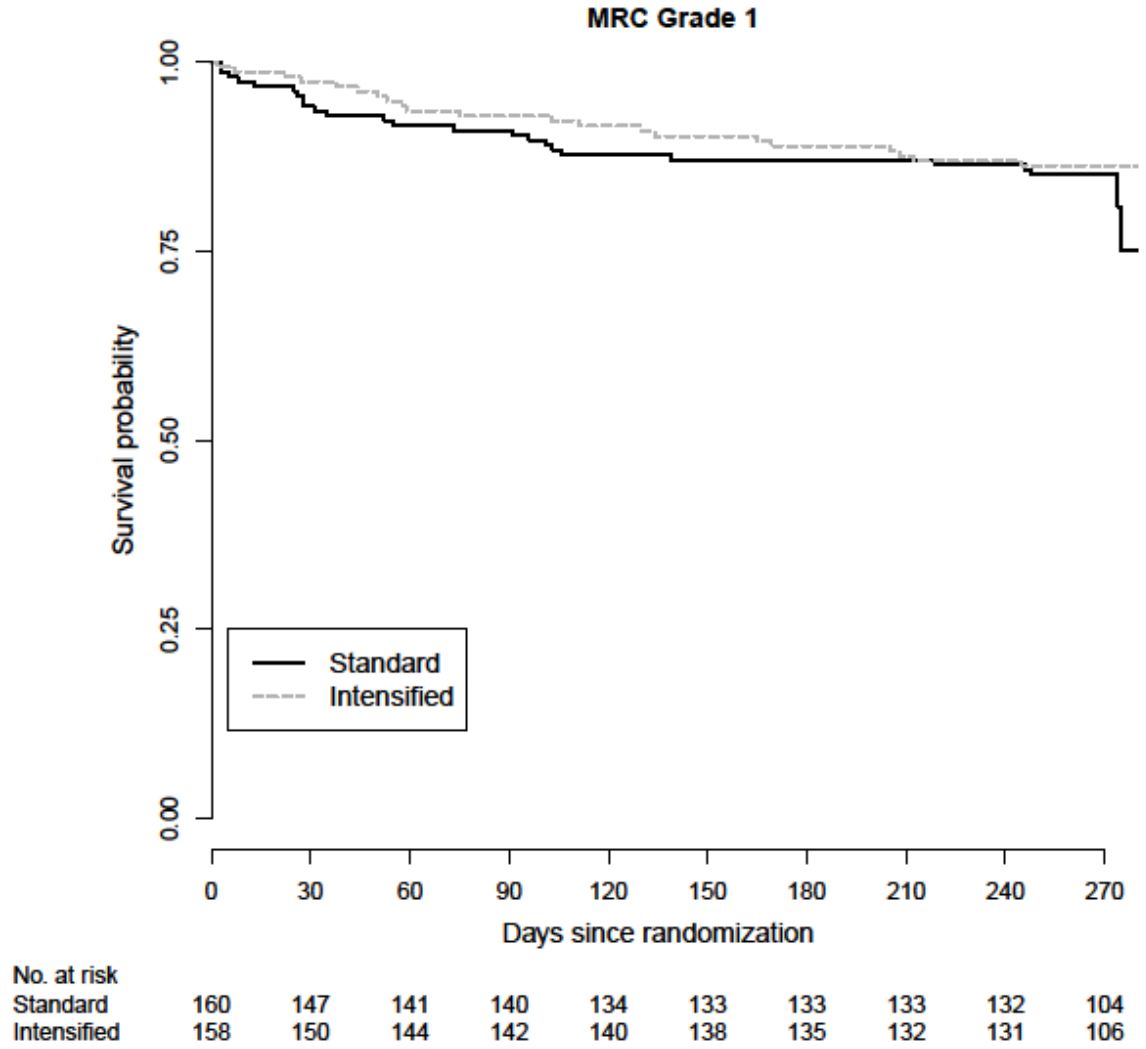


Figure 4.7 Kaplan-Meier survival estimates for patients with MRC severity grade 1

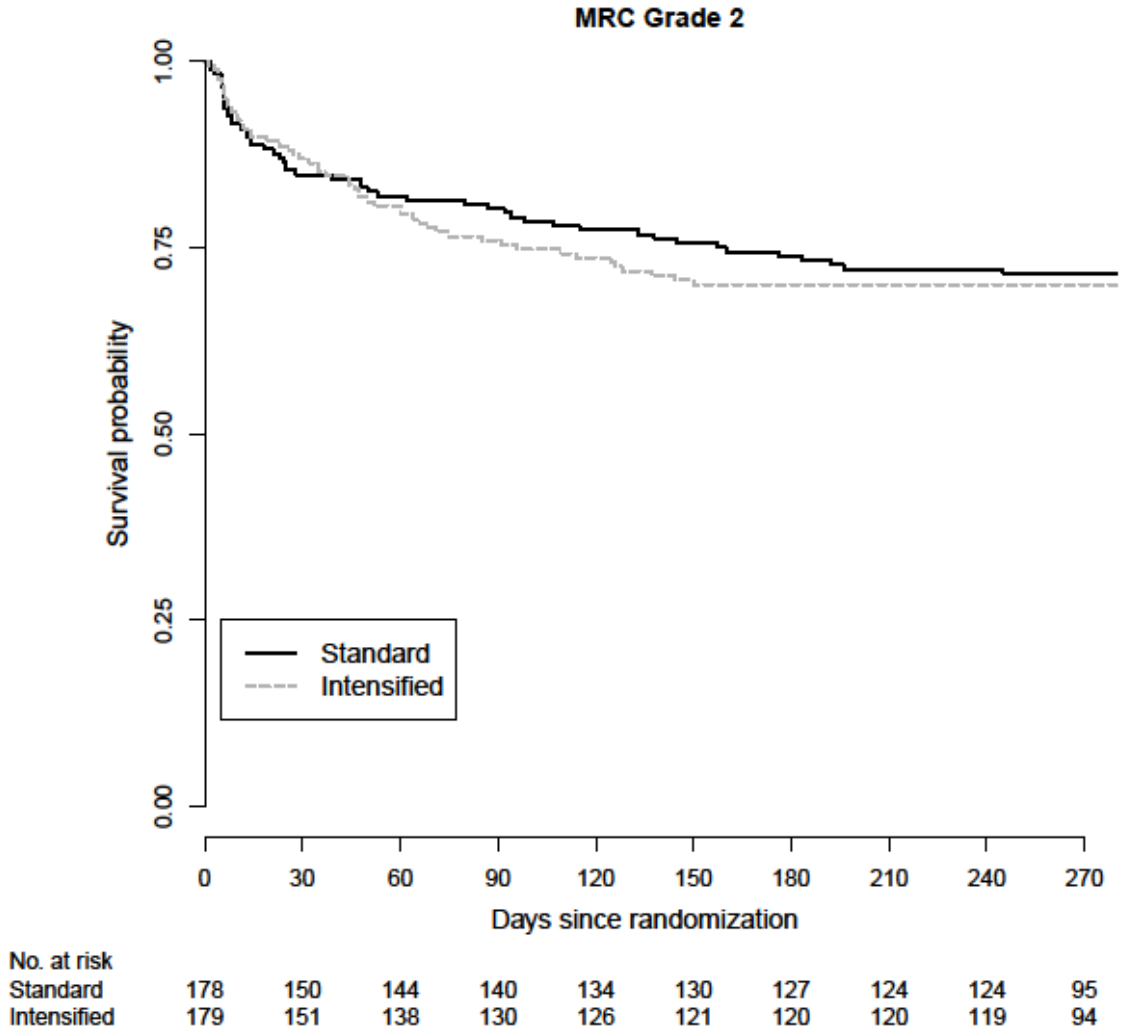


Figure 4.8 Kaplan-Meier survival estimates for patients with MRC severity grade 2

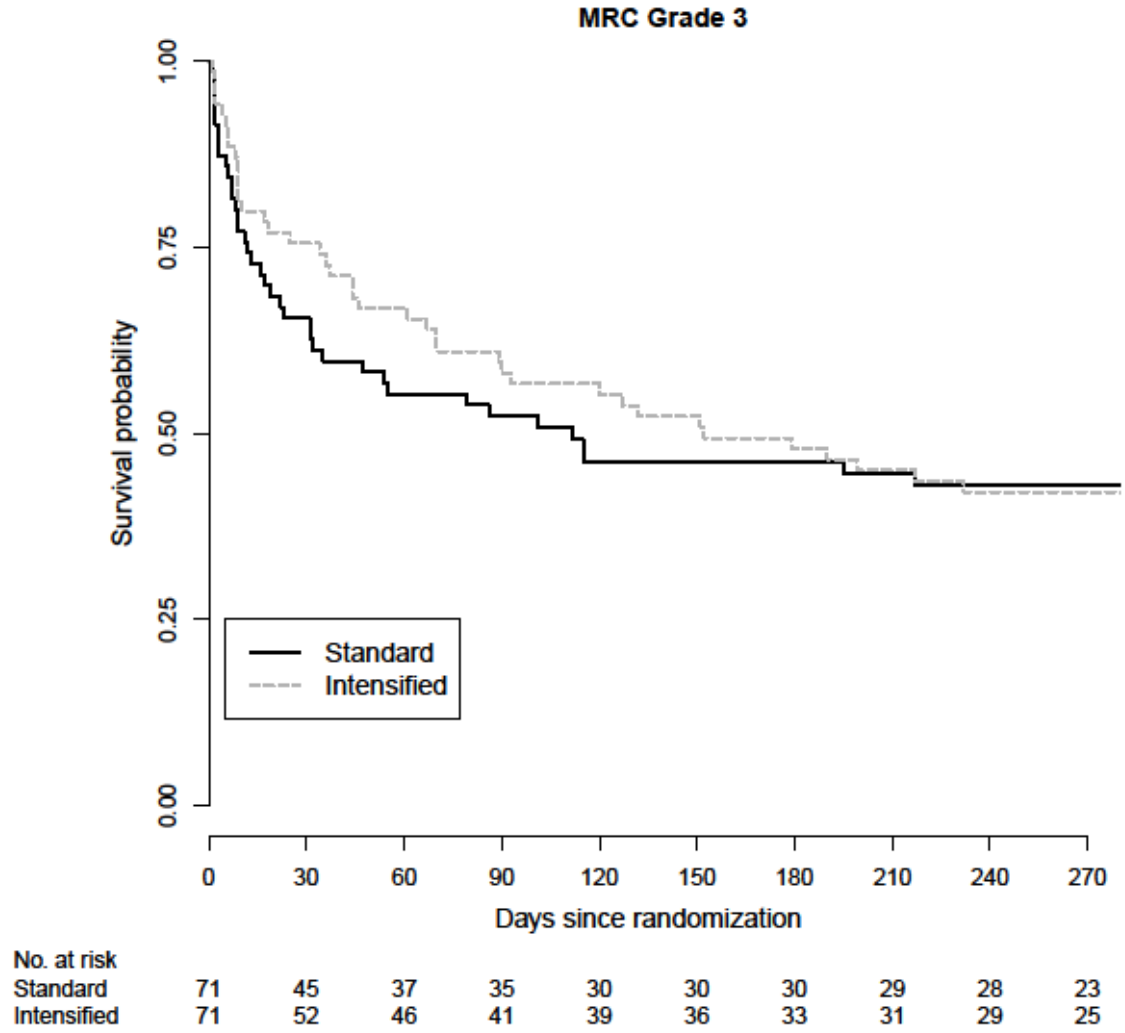


Figure 4.9 Kaplan-Meier survival estimates for patients with MRC severity grade 3

Cox regression analyses (Table 4.4) identified the following factors as predictors for poor survival: more severe neurological compromise upon treatment initiation, indicated by higher MRC grade (HR, 2.41; 95% CI 1.70 to 3.42; and 6.31; 95% CI 4.36 to 9.12 for grade 2 and 3 vs. grade 1); HIV infection (HR, 2.53; 95% CI 1.90 to 3.36), and MDR/rifampicin resistance (HR, 4.72; 95% CI 2.41 to 9.24) or unknown resistance (HR, 1.76; 95% CI 1.27 to 2.45, compared to no isoniazid or rifampicin resistance). In HIV-infected patients, a higher CD4 cell count was associated with reduced mortality (HR, 0.62; 95% CI 0.44 to 0.87 per +100cells/mm^3).

Table 4.4 Cox regression of pre-defined variables

A. All subjects

Covariate	Hazard ratio (95% confidence interval); P-value
Intensified treatment arm	0.92 (0.70-1.19); p=0.51
MRC grade*	
1	1 (reference category)
2	2.41 (1.70-3.42); p<0.001
3	6.31 (4.36-9.12); p<0.001
HIV positive	2.53 (1.90-3.36); p<0.001
Previous TB episode†	1.27 (0.93-1.74); p=0.14
Resistance category	
No isoniazid or rifampicin resistance	1 (reference category)
Isoniazid resistance‡	1.21 (0.76-1.95); p=0.42
MDR/rifampicin resistance§	4.72 (2.41-9.24); p<0.001
Unknown resistance¶	1.76 (1.27-2.45); p=0.001

B. HIV positives

Covariate	Hazard ratio (95% confidence interval); P-value
Intensified treatment arm	0.96 (0.68-1.36); p=0.84
MRC grade*	
1	1 (reference category)
2	1.86 (1.20-2.87); p=0.005
3	4.97 (3.16-7.81); p<0.001
Previous TB episode †	1.11 (0.76-1.63); p=0.58
Resistance category	
No isoniazid or rifampicin resistance	1 (reference category)
Isoniazid resistance‡	1.38 (0.79-2.41); p=0.27
MDR/rifampicin resistance§	5.66 (2.71-11.81); p<0.001
Unknown resistance¶	2.07 (1.37-3.14); p=0.001
Baseline CD4 cell count (per +100 cells/mm^3)	0.62 (0.44-0.87); p=0.006
ART on enrollment**	0.87 (0.59-1.29); p=0.49

** MRC denotes modified British Medical Research Council criteria. Grade1 indicates a Glasgow coma score of 15 with no neurologic signs (baseline), grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less.*

† Patients who reported having a previous episode of disease.

‡ Resistance to isoniazid (INH), with or without other resistance, but not rifampicin resistance

§ MDR; multi-drug resistance to at least rifampicin and isoniazid, or isolate d rifampicin resistance from culture results.

¶ Patients for whom no drug resistance results were available.

*** ART; anti-retroviral therapy*

4.3.4 SECONDARY OUTCOMES AND ADVERSE EVENTS

There was no evidence of an effect of treatment on any of the pre-defined secondary outcomes (Table 4.5).

Table 4.5 Summary of neurological disability at 9 months and time to new neurological events or death

Outcome	**Standard treatment (n=409)**	**Intensified treatment (n=408)**	**Comparison Estimate (95% confidence interval); P-value**
Disability at 9 months – ITT*			Cumulative odds ratio of a worse
- Good	163 (41%)	175 (45%)	disability status – OR†
- Intermediate	80 (20%)	65 (17%)	
- Severe disability	42 (11%)	39 (10%)	ITT: 0.93 (0.71-1.22);p=0.61‡
- Death	114 (29%)	113 (29%)	HIV negatives: 0.99 (0.70-1.41); p=0.97
- No with a disability assessment	399	392	HIV positives: 0.86 (0.57-1.30); p=0.47
Time to first new neurologic event§or death during the 9 month follow-up	144	148	Hazard ratio (combined endpoint) ITT: 0.99 (0.80-1.23); p=0.94
- Number of new neurologic events	24	27	HIV negatives: 1.30 (0.95-1.77); p=0.10
- Number of deaths without prior to new neurological event			HIV positives: 0.78 (0.58-1.04); p=0.09

**In case of missing disability assessment at 9 months, the last reported disability assessment from month 2 or 6 was used instead for 20 subjects in each arm.An analysis which included only subjects with a valid 9-month disability assessment gave a cumulative OR=0.92 (95% CI 0.70-1.22), p=0.56.ITT; intention to treat population.*

† OR<1 represent more favourable outcomes for the intensified treatment group.

‡ An alternative analysis based on multiple imputation of missing disability outcomes for all subjects (as detailed in the statistical analysis plan) gave a cumulative OR=0.91 (95% CI 0.70-1.19), p=0.51.

§Neurological events were pre-defined as any of the following events: cerebellar symptoms, coma/consciousness deterioration, mono-, hemi-, para-, or tetraplegia, neurological detoriation requiring ventilation, seizures, cranial nerve palsy, or a fall in Glasgow coma score (GCS) by ≥ 2 points for ≥2 days from the highest previously recorded GCS.

Overall there was no significant difference between the treatment arms in clinical adverse events, apart from an increased frequency of seizures (11 vs. 23, P=0.04) and vision impairment (4 vs. 14, P=0.02) in the intensified arm (Table 4.6). Signs of drug allergy were more frequent in the intensified arm, however this did not reach statistical significance (17 vs. 30 patients, P=0.052).

Table 4.6 Summary of clinical grade 3/4 adverse events

	Standard regimen (n=409)	**Intensified regimen (n=408)**	**Comparison (P-value) †**
Total number of patients with at least one event – no.(%) *	229 (56.0%)	240 (58.8%)	0.44
Total number of adverse events – no.	446	534	0.09
Selected adverse events specified - no.(%) *:			
Neurological event	155 (37.9%)	173 (42.4%)	0.20
- deterioration of consciousness	89 (21.8%)	90 (22.1%)	0.93
- headache	30 (7.3%)	36 (8.8%)	0.44
- hemiplegia	21 (5.1%)	31 (7.6%)	0.16
- paraplegia	9 (2.2%)	10 (2.5%)	0.82
- urinary retention	12 (2.9%)	10 (2.5%)	0.83
- cranial nerve palsies	11 (2.7%)	13 (3.2%)	0.69
- seizures	11 (2.7%)	23 (5.6%)	0.04
- vision impairment	4 (1.0%)	14 (3.4%)	0.02
Hepatotoxicity	28 (6.9%)	17 (4.2%)	0.12
Jaundice	17 (4.2%)	29 (7.1%)	0.07
Respiratory event	18 (4.4%)	18 (4.4%)	1.00
Event required patient to be ventilated	10 (2.4%)	14 (3.4%)	0.42
Signs of drug hypersensitivity ‡	17 (4.2%)	30 (7.4%)	0.052
Cardiological events	14 (3.4%)	13 (3.2%)	1.00
Diarrhea	7 (1.7%)	5 (1.2%)	0.77
Vomiting	13 (3.2%)	18 (4.4%)	0.37
Severe abdominal pain	12 (2.9%)	5 (1.2%)	0.14
Fever	8 (2.0%)	9 (2.2%)	0.81
Hemorrhage / anemia	16 (3.9%)	17 (4.2%)	0.86
Other haematological event	4 (1.0%)	3 (0.7%)	1.00
New AIDS defining illness	6 (1.5%)	10 (2.5%)	0.33
Gastrointestinal bleed	3 (0.7%)	1 (0.3%)	0.62
Other gastrointestinal symptoms	3 (0.7%)	9 (2.2%)	0.09
Renal event	2 (0.5%)	3 (0.7%)	0.69
Urinary symptoms	0 (0%)	1 (0.3%)	0.50
Dermatological symptoms	5 (1.2%)	6 (1.5%)	0.77
Peripheral oedema	0 (0%)	4 (1.0%)	0.06
Musculoskeletal symptoms	3 (0.7%)	3 (0.7%)	1.00
Exhaustion	2 (0.5%)	4 (1.0%)	0.45
Other	14 (3.4%)	12 (2.9%)	0.84

**Numbers refers to the number of patients with at least one adverse event of the respective type.*
†P-values are based on Fisher's exact test for all comparisons except for the comparison of the total number of adverse events which is based on a comparison of the number of events per patients between the two arms with the Wilcoxon rank sum test.
‡ Includes patients with hypersensitivity reactions ranging from mild (skin rash, itch) to severe (Steven's-Johnson syndrome)

The difference in adverse events leading to anti-tuberculosis drug interruptions between arms did not reach statistical significance (64 vs. 95 events, P=0.08;Table 4.7). There were more interruptions in the intensified arm due to jaundice (7 vs. 19 patients, P=0.02).

Table 4.7 Summary of clinical grade 3/4 adverse events leading to interruption of anti-tuberculosis treatment

	Standard treatment (n=409)	**Intensified treatment (n=408)**	**Comparison (P-value) †**
Total number of patients with at least one event - no.(%) *	58 (14.2%)	75 (18.4%)	0.11
Total number of adverse events - no.	64	95	0.08
Selected adverse events specified - no.(%) *:			
Neurological event	13 (3.2%)	15 (3.7%)	0.71
Signs of drug allergy	13 (3.2%)	22 (5.4%)	0.12
Jaundice	7 (1.7%)	19 (4.7%)	0.02
Transaminitis	17 (4.2%)	9 (2.2%)	0.16
Vomiting	1 (0.2%)	3 (0.7%)	0.37
Severe abdominal pain	2 (0.5%)	3 (0.7%)	0.69
Other gastrointestinal symptoms	2 (0.5%)	2 (0.5%)	1.00
Cardiological event	0 (0%)	1 (0.3%)	0.50
Hemorrhage / anemia	3 (0.7%)	2 (0.5%)	1.00
New aids defining illness	0 (0%)	2 (0.5%)	0.25
Respiratory event	1 (0.2%)	1 (0.3%)	1.00
Haematological event	1 (0.2%)	1 (0.3%)	1.00
Renal event	0 (0%)	2 (0.5%)	0.25
Musculoskeletal symptoms	0 (0%)	1 (0.3%)	0.50
Peripheral oedema	0 (0%)	1 (0.3%)	0.50
Dermatological symptoms	1 (0.2%)	2 (0.5%)	0.62
Other	2 (0.5%)	5 (1.2%)	0.29

**Numbers refers to the number of patients with at least one adverse event of the respective type.*
† P-values are based on Fisher's exact test for all comparisons except for the comparison of the total number of adverse events which is based on a comparison of the number of events per patients between the two arms with the Wilcoxon rank sum test.

Additional laboratory abnormalities are given in Table 4.8. There were significantly more grade 3 and 4 raises in bilirubin (31 vs. 49, P=0.04) and hyponatremia (81 vs. 112, P=0.01) in the intensified arm.

Table 4.8 Summary of new grade 3/4 laboratory abnormalities.

	Standard treatment (n=409)	**Intensified treatment (n=408)**	**Comparison (P-value) †**
Total number of patients with at least one new laboratory abnormality – no.(%) *	161 (39.36%)	185 (45.34%)	0.09
Total number of new laboratory abnormalities – no.	422	533	0.06
Specific lab abnormalities – no.(%) *:			
Hyponatremia	81 (19.8%)	112 (27.5%)	0.01
Hypernatremia	0 (0%)	2 (0.5%)	0.25
Hypokalemia	35 (8.6%)	48 (11.8%)	0.13
Hyperkalemia	1 (0.2%)	2 (0.5%)	0.62
Raised bilirubin	31 (7.6%)	49 (12.0%)	0.04
Raised ALT	29 (7.1%)	18 (4.4%)	0.13
Rraised AST	27 (6.6%)	24 (5.9%)	0.77
Anemia	21 (5.1%)	29 (7.1%)	0.25
Leukopenia	17 (4.2%)	22 (5.4%)	0.42
Neutropenia	11 (2.7%)	7 (1.7%)	0.48
Thrombocytopenia	17 (4.2%)	14 (3.4%)	0.72
Hyperglycemia	17 (4.2%)	13 (3.2%)	0.58
Hypoglycemia	1 (0.2%)	2 (0.5%)	0.62
Raised creatinine	1 (0.2%)	0 (0%)	1.00

** New laboratory abnormalities are defined as any worsening of a lab value to grade 3 or 4 (including changes from grade 3 to 4) compared to the subject's previous lab value. In addition, to be conservative, if a subject's baseline lab value was missing, the first post-enrolment lab value was also considered to be a new lab abnormality if it was of grade 3 or 4. A grading table for laboratory abnormalities is provided in the appendix of the statistical analysis plan (Supplementary material).*
Numbers refers to the number of patients with at least one new lab abnormality of the respective type.
†P-values are based on Fisher's exact test for all comparisons except for the comparison of the total number of new laboratory abnormalities which is based on a comparison of the number of abnormalities per patients between the two arms with the Wilcoxon rank sum test.

The median duration of the initial hospitalization was 31 days in the intensified arm and 30 days in the placebo group. There were 11 patients who had QTc prolongation above the critical threshold of 500ms (calculated using Framingham's formula) at any time point between baseline and 4 weeks of treatment (4 and 7 in the standard and intensified arm, respectively).

4.4 Discussion

In this pragmatic, randomized, double-blind, placebo-controlled trial intensified anti-tuberculosis treatment did not improve survival in adults with tuberculous meningitis. The results contradict the findings of previous studies which suggested an increase in rifampicin dose[109] and the addition of a fluoroquinolone to the standard regimen[110] may improve outcome from tuberculous meningitis.

A limitation of our study was that we tested a regimen rather than the contribution of individual drugs. A factorial design may have enabled the latter but would have led to a prohibitively large sample size[246]. However, our negative findings suggest neither higher dose rifampicin nor levofloxacin improve tuberculous meningitis treatment. There are a number of possible explanations for our results. It is possible that the oral rifampicin dose used in our study (15mg/kg/day) did not increase intracerebral drug concentrations sufficiently to enhance bacterial killing. Recent data suggests much higher doses of rifampicin (up to 35mg/kg/day) may be both tolerated and necessary to significantly increase *M.tuberculosis* killing in pulmonary tuberculosis[247]. Furthermore, oral administration probably results in substantially lower plasma rifampicin concentrations than equivalent doses given intravenously[248]. Some reports suggest that the relative benefit of rifampicin on outcome of tuberculous meningitis may be modest in the presence of optimal mycobacterial killing by isoniazid[185]. Rifampicin's main role in pulmonary tuberculosis treatment is probably to shorten treatment, rather than enhance early mycobacterial killing[190, 249]. By contrast, fluoroquinolones have enhanced the early sterilization of sputum but have not allowed therapy to be shortened due to unacceptable increases in disease relapses[212, 213, 250]. Previous studies have reported fluoroquinolones to have either no impact on tuberculous meningitis outcome[109] or a possible benefit in those with mild disease[110]. A planned pharmacokinetic and pharmacodynamic analysis of patients recruited to our trial will help address these possibilities.

An intensified anti-tuberculosis regimen may, however, benefit patients infected with isoniazid resistant *M.tuberculosis*. How this finding should influence clinical practice is uncertain given that isoniazid resistance detection usually requires bacterial culture and often takes many weeks. The

development of rapid molecular tests that can reliably detect isoniazid resistance in CSF may aid early diagnosis and treatment adjustments. But empirical regimen intensification may be warranted in patients at high risk of isoniazid resistance or in settings with high isoniazid resistant bacteria prevalence.

The overall mortality in our population was lower than anticipated from previous reports. This may be due to a combination of earlier diagnosis (38.9% of patients had grade 1 disease at randomization), increased availability of second-line drugs for drug resistant infections and improved HIV management. Although the results of our study do not support a change to the currently recommended treatment regimens for tuberculous meningitis, enhanced anti-tuberculosis treatment with higher doses of first-line anti-tuberculosis drugs, including intravenous rifampicin, or the newer anti-tuberculosis drugs bedaquiline and delamanid, still require investigation. In the meantime, the key determinants of survival from this dangerous infection are earlier diagnosis and treatment.

CHAPTER 5

5 INTENSIFIED ANTI-TUBERCULOUS CHEMOTHERAPY FOR THE TREATMENT OF DRUG RESISTANT TUBERCULOUS MENINGITIS

5.1 Background

5.1.1 DRUG RESISTANT TB

Although TB incidence is declining globally, mycobacterial drug resistance is increasing. This rise jeopardizes progress made in global TB control. Multidrug resistance (MDR) resistance, defined as resistance to at least rifampicin and isoniazid, was reported in 3.3% of new patients and in 20% of retreatment cases globally in 2014[4]. Only half of people with MDR TB are treated successfully. In 2014 there were an estimated 190,000 deaths from MDR TB. Isoniazid resistance without rifampicin resistance, is more prevalent; reported in 8.1 % of new cases in 2014[4]. In pulmonary TB, patients with isoniazid resistant infection are more likely to fail treatment and are at risk of developing MDR TB[251]. Current treatment regimens need to be adjusted to target this growing population of patients with isoniazid resistance. A recent retrospective study showed more favorable treatment outcomes in patients receiving a fluoroquinolone versus standard treatment in pulmonary TB patients with pretreatment isoniazid resistance(97.3% vs 84.6%, P=0.007)[252]. The World Health Organization (WHO) currently only recommends adding ethambutol to the continuation phase of treatment to protect rifampicin in pulmonary TB; however the evidence is weak and the recommendation is based on expert opinion.

5.1.2 DRUG RESISTANT TBM

Prior data on drug resistant TBM is sparse and of a descriptive nature. A total of 16 papers were published reporting on prevalence of drug resistant TBM in the last 15 years. These included 12 different cohorts, including 3,338 adults and children from 4 different regions (Europe, South Africa, India, South East Asia and the United States). Reports on drug resistance in the context of TBM are limited due to the difficulty of isolating mycobacteria from the CSF. Reported prevalence of isoniazid resistant TBM varies from 6% in the United States to 64% in a Chinese cohort, but are broadly consistent with the prevailing background isoniazid resistance rates in *M.tuberculosis* for a region [64, 70, 253, 254] [53, 64, 66, 68-70, 164, 253-260].

Drug resistance in the context of TBM is more critical than in pulmonary TB. Isoniazid and rifampicin are the principal drugs in treatment of TBM and are given throughout the regimen. Isoniazid penetrates readily through the blood-brain barrier and has the highest early bactericidal activity (EBA) of all first line drugs, killing approximately 95% of mycobacteria in the first two days of treatment[190]. Despite low penetration into the CSF, rifampicin, is thought to be highly active in the days thereafter and against both dividing and persistent mycobacteria. The complementary role of isoniazid and rifampicin is illustrated by the high mortality of patients with confirmed MDR TBM, which approaches 100% when treated with standard first line regimens and exceeds 40% when second line regimens are used[64, 256]. Isoniazid resistance, without rifampicin resistance has also been associated with increased mortality in TBM. In an American cohort, patients with isoniazid resistant TBM were found more likely to die (OR 2.07, 95% CI 1.30-3.29) independent of HIV status[255]. In Vietnam, isoniazid resistance in HIV associated TBM, was associated with higher mortality (adjusted HR 1.78, 95% CI 1.18-2.66), but this was not shown in Vietnamese HIV negative TBM patients. However, a trend was seen towards a greater proportion of bacterial culture positivity of CSF after treatment initiation, suggesting slower bacterial clearance[64, 261]. This study may have been underpowered to show an effect on clinical outcomes.

To date there have been no clinical trials exploring antituberculosis treatment for drug resistant TBM. The WHO's recommendation of adding ethambutol to the continuation phase in isoniazid resistant pulmonary TB is unlikely to benefit patients with TBM, as the majority of deaths occur in the first two months of treatment. Moreover, ethambutol is a bacteriostatic drug and penetration of the blood-cerebrospinal fluid barrier is extremely poor. In healthy volunteers with uncompromised blood-CSF barrier, ethambutol did not penetrate the CSF even at doses of 50mg/kg. In TBM patients levels in CSF reported rarely exceed the MIC for ethambutol (0.5-2.0 µg/ml)[105]. During the continuation phase in TBM treatment, blood-CSF barrier disruptions may have been restored.

Fluoroquinolones are an attractive candidate for treatment of TBM; they are well tolerated, resistance rates in *M.tuberculosis* are low, have high bactericidal activity comparable to isoniazid, have good penetration in the cerebrospinal fluid and are inexpensive[105, 110, 111, 211, 262]. Prior to our trial, three trials were published examining the addition of a fluoroquinolone to the antituberculosis regimen for TBM [109, 110, 263]. These trials were primarily pharmacokinetic safety trials, not adequately powered for clinical outcomes, and not double-blinded. There was no substantial increase in toxicity in fluoroquinolone containing regimens, and a weak suggestion that fluoroquinolones may be of benefit to outcome[110]. These trials included patients with TBM, but were not targeted to examine the effect in drug resistant TBM. In drug resistant TBM, when bactericidal activity of key drugs, such as isoniazid and rifampicin is lost, the addition of a fluoroquinolone to the regimen may result in a more pronounced benefit to outcome.

Our randomized trial addressed the question whether intensified treatment would benefit patients with TBM, rooted on the hypothesis that excess death in TBM is due to limited intracranial bactericidal activity of current antituberculosis treatment regimens. Intensified treatment consisted of standard treatment with a higher dose of rifampicin (15mg/kg) and additional levofloxacin (20mg/kg) during the intensive phase of the treatment[165]. Although the trial did not show a benefit of intensified treatment in the overall population, the results suggest reduced mortality in isoniazid resistant infection (HR 0.45, 95%CI 0.20-1.02, p=0.06)[264]. The addition of a (second generation)

fluoroquinolone may have an impact on the early bactericidal activity of the regimen and subsequent mortality, in the absence of the optimal early bacterial killing of isoniazid. Higher doses of rifampicin are also associated with increased early bactericidal activity[265] and are being trialed for the treatment of susceptible tuberculosis[266]. The main objectives of using higher doses of rifampicin for the treatment of susceptible pulmonary TB are treatment shortening and relapse prevention[250, 266, 267]. No studies have evaluated the use of high dose rifampicin in isoniazid resistant TB. Theoretically, higher doses of rifampicin might contribute to improved early killing of isoniazid resistant mycobacteria. In the following analysis we describe the drug resistant population in this trial and the effect of intensified treatment on clinical response and outcome in this group of patients.

5.2 Objectives

The main objective of this analysis is to describe the presentation and treatment response of patients with a known drug susceptibility profile. I aim to identify baseline clinical features associated with drug resistance, in particular isoniazid resistance, that may raise awareness of drug resistance with treating clinicians. Being alerted to the possibility of a patient being infected with a drug resistant strain, may prompt additional investigations or empirically adjusted treatment regimens if appropriate. Secondly I aim to evaluate which patients may benefit from adjusted intensified treatment regimens. I will address the following questions;

1) Is drug resistance in TBM associated with presenting clinical or laboratory features?
2) Are there any clinical or laboratory factors after treatment initiation associated with drug resistance in TBM?
3) Is drug resistance in TBM associated with mortality and neurological disability?
4) What is the effect of intensified treatment on neurological outcome and survival in drug resistant TBM?

5.3 Methods

5.3.1 STUDY DESIGN AND PARTICIPANTS

Patients were recruited from two tertiary referral hospitals in HCMC, Vietnam. The full protocol and primary results from the trial are presented in chapter 3 and 4 of this thesis respectively. In short, adult patients with a clinical diagnosis of TBM were eligible to enter the trial. Exclusion criteria were a positive CSF Gram or India ink stain, known or suspected pregnancy; laboratory contraindications to antituberculosis therapy; MDR TBM diagnosed prior to enrolment; lack of consent. Written informed consent to participate in the study was obtained from all patients or their relatives if the patient could not provide consent.

5.3.2 TREATMENT

Patients were randomized to receive TBM standard treatment or intensified treatment, as described in the previous chapters. All patients received adjunctive dexamethasone and HIV infected patients received antiretroviral treatment according to Vietnamese guidelines. For patients who were found to be infected with *M.tuberculosis* resistant to isoniazid on drug susceptibility testing (DST), the treating clinician made the decision to adjust treatment. In general, an active fluoroquinolone and in some cases a second-line injectable aminoglycoside (kanamycin 20mg/kg/day for up to 3 months) were added to the regimen. However, in PNT hospital it was not standard practice to change treatment for patients with isoniazid resistant TBM, hence not all patients had treatment adjustment with a fluoroquinolone. Consequently, a subset of patients in the standard treatment arm also received additional treatment with a fluoroquinolone. Patients with known or suspected infection with resistance to rifampicin were transferred to the MDR treatment unit, after consultation with the MDR specialist. Second-line treatment was initiated if available and appropriate.

5.3.3 ASSESSMENT OF OUTCOME

Patients were followed up for 9 months. In-patients were assessed daily, out-patients were assessed at a monthly clinic visit. The primary outcome was death during the 9-month follow-up period. Patients were monitored closely for drug toxicity, neurological deterioration, and other clinical parameters. Neurological events are defined as any new event with onset after enrollment, including cerebellar symptoms, coma/consciousness deterioration or a fall in Glasgow coma score (GCS) by ≥ 2 points for ≥ 2 days from the highest previously recorded, cranial nerve palsy, hemiplegia, paraplegia, tetraplegia or monoplegia, neurological deterioration requiring ventilation, seizures or cerebral herniation.

Disability was assessed based on the two simple questions and the modified Rankin score and classified as good outcome, intermediate outcome, severe disability, or death, as previously described in chapter 3 and 4 of this thesis[33, 51, 59].

5.3.4 INVESTIGATIONS

Patients underwent lumbar puncture at baseline, 1, 2 and 9 months of treatment. Upon receipt in the TB laboratory, CSF samples were centrifuged at 4,000 *g* for 15 min. Supernatant was removed to leave a 0.5ml deposit, which was then used for ZN smear preparation (100µl), Xpert MTB/RIF (Cepheid, USA) testing (200µl) [268] and inoculation of Bactec MGIT culture (Becton Dickinson, USA)(100µl). TB diagnostic methods are described more elaborately in section 2.2.2 of this thesis.

A 100µl portion of the deposit was used to inoculate a MGIT tube containing 0.8ml MGIT supplement (PANTA and growth supplements). MGIT tubes were incubated in a MGIT960 machine until they were automatically identified as positive or for 56 days. All positive cultures were tested for drug susceptibility (DST) to rifampin, isoniazid, streptomycin, and ethambutol using a Bactec MGIT SIRE kit (Becton, Dickinson) according to the manufacturer's instructions.

5.3.5 RESISTANCE CATEGORIES

Drug resistance was categorized based on the results of MGIT DST performed against rifampicin, isoniazid, ethambutol and streptomycin on baseline samples (defined as sample taken anytime in between 7 days prior or 3 days after enrollment) of either CSF, sputum, gastric fluid or blood. Drug resistance categories are defined as follows:

Multidrug resistant (MDR): resistance against both isoniazid and rifampicin. **Rifampicin resistant (RIF-r)**: resistance against rifampicin but susceptible to isoniazid. **Isoniazid resistant (INH-r)**: resistance against isoniazid but susceptible to rifampicin. **No isoniazid or rifampicin resistance (INH-s+RIF-s)**: no resistance to isoniazid or rifampicin documented. In all categories resistance to streptomycin and/or ethambuthol may be present.

5.3.6 PCR AND DNA SEQUENCING

Isoniazid resistant *M.tuberculosis* isolates were sequenced using multiplex allele specific PCR to detect mutations in KatG315 and inhA-15. The first primer pair targeted the inhA promoter, with the reverse primer designed to detect mutation C-15T in this region. Another primer pair is located inside the katG gene where the forward primer will yield a PCR product if the isolate is wild type. PCR products yield three bands after electrophoresis on agarose 2% for 40min at 140V. The largest band of 656bp represents for Hsp65 of *M.tuberculosis*. The second band of 329bp represents isolate with genetic wild type at katG315, thus the isolates with absence of this band have a katG315 mutation, or isoniazid resistance. The lowest band of 174bp represents the isolates carrying mutation -15 C→T in the inhA promoter. The primers were designed using Primer Express version 2.0 software (Applied Biosystems Inc. Foster City, CA, USA). KatG315 mutations are associated with high level resistance (>2µg/ml) [269]. InhA-15 mutations are associated with intermediate level resistance (0.1-0.4µg/ml) [270].

5.3.7 STATISTICAL ANALYSIS

1) Baseline characteristics for each of the resistance categories are summarized as median (IQR) for continuous data and number of patient (%) for categorical data. Clinical variables associated with specified resistance categories were assessed by univariable analysis. The Kruskall-Wallis test was used to compare continuous parameters and Chi-square or Fisher's exact test for comparisons between categorical parameters.

2) Time series plots were drawn for serum sodium levels during treatment. Association of longitudinal sodium measurements with drug resistance was estimated by multiple regression using a generalized estimating equations (GEE) approach, corrected for HIV status and TBM severity grade. CSF parameters at baseline and after one and two month of treatment are summarized as median (IQR) for continuous data and number of patients (%) for categorical data, and compared between resistance categories. The Kruskall-Wallis test was used to compare continuous parameters and Chi-square or Fisher's exact test for comparisons between categorical parameters, where appropriate.

3 and 4) Kaplan-Meier estimates were used to display survival of patients in the defined resistance categories, by randomized treatment arm for INH-r group and MDR/RIF-r resistance group and by isoniazid resistance mutations (inhA and KatG). The HR of death within the resistance categories was calculated by Cox regression analysis on the population with a known resistance profile, including the following predictors of outcome as co-variates; trial treatment arm, TBM severity grade, HIV status and resistance category. Similar analyses were done for the combined endpoint of time to new neurological event or death. For the survival analysis of the isoniazid resistance mutation categories (KatG and inhA), in case of dual mutations in both regions (inhA and KatG) the isolate was classified as a KatG mutation, while isolates with no mutation in the two regions were grouped with inhA mutations. Calculation of HR of death between the mutation groups by Cox regression, was corrected for TBM severity grade and HIV status.

Additionally, time dependent covariate cox model for fluoroquinolone usage, adjusted for TBM severity and HIV status at baseline was performed on the population of isoniazid resistant patients.

The proportion of patients in the different disability categories were summarized for the three resistance categories and were compared using a proportional odds logistical regression model depending on HIV status and TBM severity grade.

Results with a p-value of 0.05 or less will be considered statistically significant.

All statistical analyses are performed using the statistical software R v3.0.2.

5.4 Results

5.4.1 Management of drug resistance and baseline characteristics

A total of 817 patients were randomized from April 2011 to June 2014. Of those patients, 322 had a known drug resistance profile; 283 (87.9%) were from CSF culture, 39 (12.1%) from extraneural culture (sputum (n=36), gastric fluid (n=2), blood (n=1)). Overall, from these 86/322 (26.7%) were classified as INH-r, 15/322 (4.7%) patients as MDR, one (0.3%) patient as RIF-r, 220/322 (86.3%) patients as INH-s (+RIF-s).

Overall only 176 of 322 patients (54.7%) of patients had fully susceptible infection with no resistance to any of the first-line antituberculosis drugs detected (Figure 5.1).

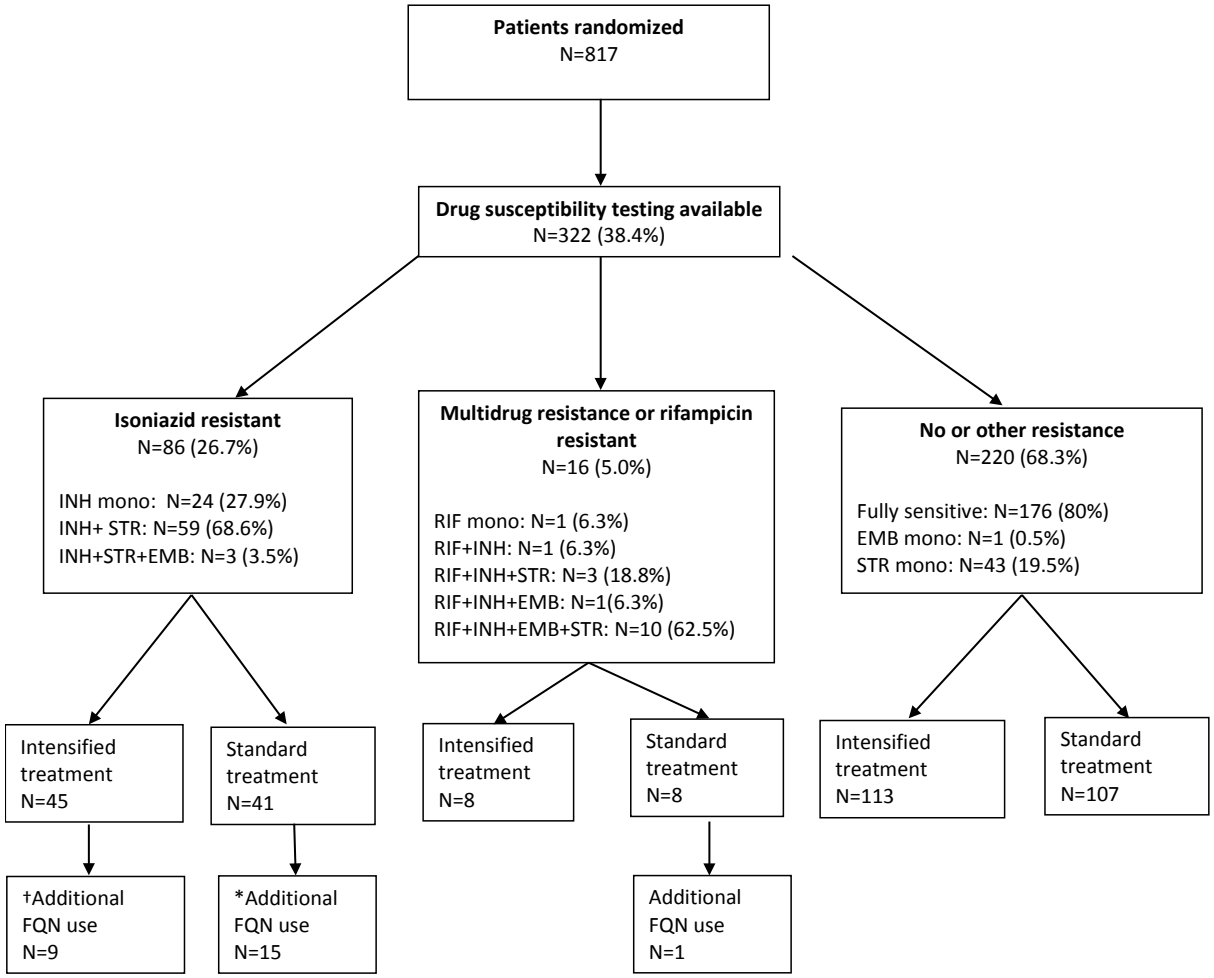


Figure 5.1 Study flow diagram

*Treatment allocation in the defined resistance categories and additional use of fluoroquinolone (FQN) as part of adjustment of regimen after detection of drug resistance is given: † Nine patient in the INH-r category who were randomised to intensified treatment received open label fluoroquinolones after isoniazid resistance detection either after the study intervention, or study intervention was stopped *15 patients in the INH-r category, who were allocated to standard treatment received a fluoroquinolone as part of treatment for isoniazid resistant infection.*
INH= isoniazid, STR= streptomycin, EMB= ethambutol, RIF= rifampicin, mono= mono-resistance

5.4.2 TIME TO DIAGNOSIS OF ISONIAZID RESISTANCE AND REGIMEN ADJUSTMENT

The median time from randomization to diagnosis of resistance to isoniazid was 70 days (IQR 48-83). In the INH-r group, 45 patients were allocated to levofloxacin and high dose rifampicin through randomization in the trial. The remaining 41 patients were allocated to placebo and only received standard first line antituberculosis drugs initially. However, during the treatment period, 15 patients in the standard treatment arm received a fluoroquinolone for confirmed isoniazid resistance (levofloxacin 750 mg (n=14) or moxifloxacin 400mg (n=1)) despite their allocation, with a median time to initiation of fluoroquinolone treatment of 76 days (IQR 61-92). In the intensified treatment arm, 9 patients with INH-r received additional fluoroquinolone treatment for confirmed isoniazid resistance (levofloxacin 750mg), despite their allocation, with a median time to treatment initiation of the added fluoroquinolone of 105 days (IQR 66-113).

5.4.3 BASELINE CHARACTERISTICS

Of the patients with a known DST, 185 (57.5%) were co-infected with HIV. Patients with HIV associated TBM are more likely to have *M.tuberculosis* isolated from the CSF[268], which accounts for the higher proportion of HIV infected patients in this subset than in the overall population (349/817, 42.7%)[264]. Patients in the different resistance categories had a similar clinical presentation, although patients infected with a strain resistant to isoniazid or MDR were more likely to report a previous episode of TB treatment ($p<0.001$) (Table 5.1). We also found paraplegia upon presentation to be statistically significantly associated with resistance category, with a higher proportion in the INH-r group with paraplegia (10.5%, $p=0.03$). However, due to the rarity of the symptom (16/322 (5.0%) patients), this finding is clinically less relevant. Other presenting factors were similar across groups. In particular, disease grade on presentation was similar between groups, there were no differences in findings on chest X-ray and CSF results. Although not statistically significant, a higher proportion of patients with MDR infection were HIV infected (56.8% vs. 81.3%, P=0.13). A higher proportion of non-rifampicin resistant patients had a positive reading on baseline Xpert MTB/RIF (82.2% vs 50.0%, P=0.02).

In 66 patients with a previous treated episode of TB, 49 (74.2%) were HIV infected. In this group with reported previous anti-TB treatment, resistance to streptomycin was detected in 31 (47.0%), ethambutol resistance in 8 (12.1%), isoniazid resistance in 32 (48.5%) and rifampicin resistance in 8 (12.1%).

Table 5.1 Baseline characteristics by resistance category

	No or other resistance (n=220)		**INH resistant (n=86)**		**RIF resistant or MDR (n=16)**		**All patients (n=322)**		**P-value***
Number of patients (%)	N	220/322 (68.3)	n	86/322 (26.7)	n	16/322 (5.0)	n	322/322 (100)	NA
Age (years) (median (IQR))	220	34 (29,41)	86	34 (29,41)	16	34 (31,41)	322	34 (29,41)	0.84
Male sex n(%)	220	156 (70.9)	86	61 (70.9)	16	14 (87.5)	322	231 (71.74)	0.42
Weight (kg) (median (IQR))	220	47.7 (43.0,52.0)	86	48.0 (42.1,50.0)	16	50.0 (42.8,52.0)	322	48.0 (43.0,51.0)	0.54
Duration of illness days (median (IQR))	220	15.0 (10.0,30.0)	86	15.0 (10.0,29.5)	16	25.0 (10.3,30.0)	322	15.0 (10.0,30.0)	0.68
Previous episode of TB n (%)	220	33 (15.0)	86	25 (29.1)	16	8 (50.0)	322	66(20.5)	<0.001
HIV infected n (%)	220	125 (56.8)	86	47 (54.7)	16	13 (81.3)	322	185 (57.5)	0.13
Cranial nerve palsy n (%)	220	52 (23.6)	86	23 (26.7)	16	2 (12.5)	322	77 (23.9)	0.51
Hemiplegia n (%)	220	31 (14.1)	86	19 (22.1)	16	3 (18.8)	322	53 (16.5)	0.23
Paraplegia n (%)	220	7 (3.2)	86	9 (10.5)	16	0 (0)	322	16 (5.0)	0.03
Quadriplegia n (%)	220	4 (1.8)	86	1 (1.2)	16	0 (0)	322	5 (1.6)	1.00
GCS (median (IQR))	220	14 (11,15)	86	15 (11,15)	16	15 (13,15)	322	14 (11,15)	0.40
MRC grade† n (%)	220		86		16		322		0.41
Grade 1		87 (39.5)		29 (33.7)		8 (50.0)			
Grade 2		83 (37.7)		39 (45.3)		7 (43.8)			
Grade 3		50 (22.7)		18 (20.9)		1 (6.2)			
Serum sodium level mmol/l (median (IQR))	205	126.0 (122.0,131.0)	81	125.0 (121.0,130.0)	16	125.5 (121.0,128.0)	302	126.0 (122.0,131)	0.16
Chest Xray n (%)	220		85		16		321		0.75
Consistent with TB		124 (56.4)		45 (52.9)		9 (56.3)		178 (55.5)	
Miliary TB		51 (23.2)		16 (18.8)		3 (18.8)		70 (21.8)	
Abnormal other		21 (9.6)		12 (14.1)		1 (6.3)		34 (10.6)	
Normal		24 (10.9)		12 (14.1)		3 (18.8)		39 (12.2)	
CSF results									
White cell count cells/mm^3 (median (IQR))	216	199.5 (72.8,386)	86	108.5 (32.3,316)	16	253.5 (64.3,340.0)	318	180.0 (58.0,382)	0.06
Lymphocyte% (median (IQR))	210	74.5 (40.0,90.0)	84	86.0 (53.5,100.0)	15	80.0 (34.0,95.0)	309	78.0 (44.0,95.0)	0.04
Protein g/l (median (IQR))	211	1.48 (1.02,2.38)	82	1.24 (0.81,1.87)	16	1.28 (0.74,2.38)	309	1.41 (0.90,2.30)	0.15
Lactate mmol/l (median (IQR))	199	5.93 (4.50,7.69)	78	5.20 (4.40,6.84)	15	5.50 (4.81,7.30)	292	5.80 (4.49,7.40)	0.15
Glucose mmol/l (median (IQR))	212	1.49 (0.98,2.00)	81	1.58 (1.04,2.11)	16	1.60 (0.95,2.28)	309	1.51 (1.00,2.10)	0.52

CSF/blood glucose ratio (median (IQR))	177	0.25 (0.16,0.33)	72	0.25 (0.19,0.34)	15	0.26 (0.18,0.36)	264	0.25 (0.16,0.33)	0.69
ZN smear positive n (%)	210	135 (64.3)	83	45 (54.2)	15	7 (46.7)	308	187 (60.7)	0.14
Xpert result positiven (%)	202	166 (82.2)	79	62 (78.5)	14	7 (50.0)	295	235 (79.7)	0.02
Duration of initial admission (days) (median (IQR))	219	30 (23,38)	86	31 (26,37)	16	30 (12,32)	321	30 (24,37)	0.46

**Summary statistics are frequency (percentage) for categorical and median (IQR) for continuous variables. P-values are based on Fisher's exact test (categorical data) or the Kruskal-Wallis test (continuous data).*

†MRC denotes modified British Medical Research Council criteria. Grade1 indicates a Glasgow coma score of 15 with no neurologic signs (baseline), grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less.

INH=isoniazid, RIF=rifampicin, MDR=multidrug resistant, CSF=cerebrospinal fluid, ZN=Ziehl-eelsen

5.4.4 TREATMENT RESPONSE AS AN INDICATOR OF DRUG RESISTANCE

In the absence of early microbiological confirmation, the use of surrogate clinical indicators may be of importance in clinical awareness of the possibility of drug resistance. In order to evaluate the significance of the results of follow up CSF analysis, we explored differences in clinical, biochemical and CSF parameters in the drug resistance categories, with particular interest in differences in the INH-r and the INH-s group. Evolution of sodium levels in the first two months of treatment in the different resistance categories in all patients and in those who survived the nine month follow up and those who died are shown in Figure 5.2.

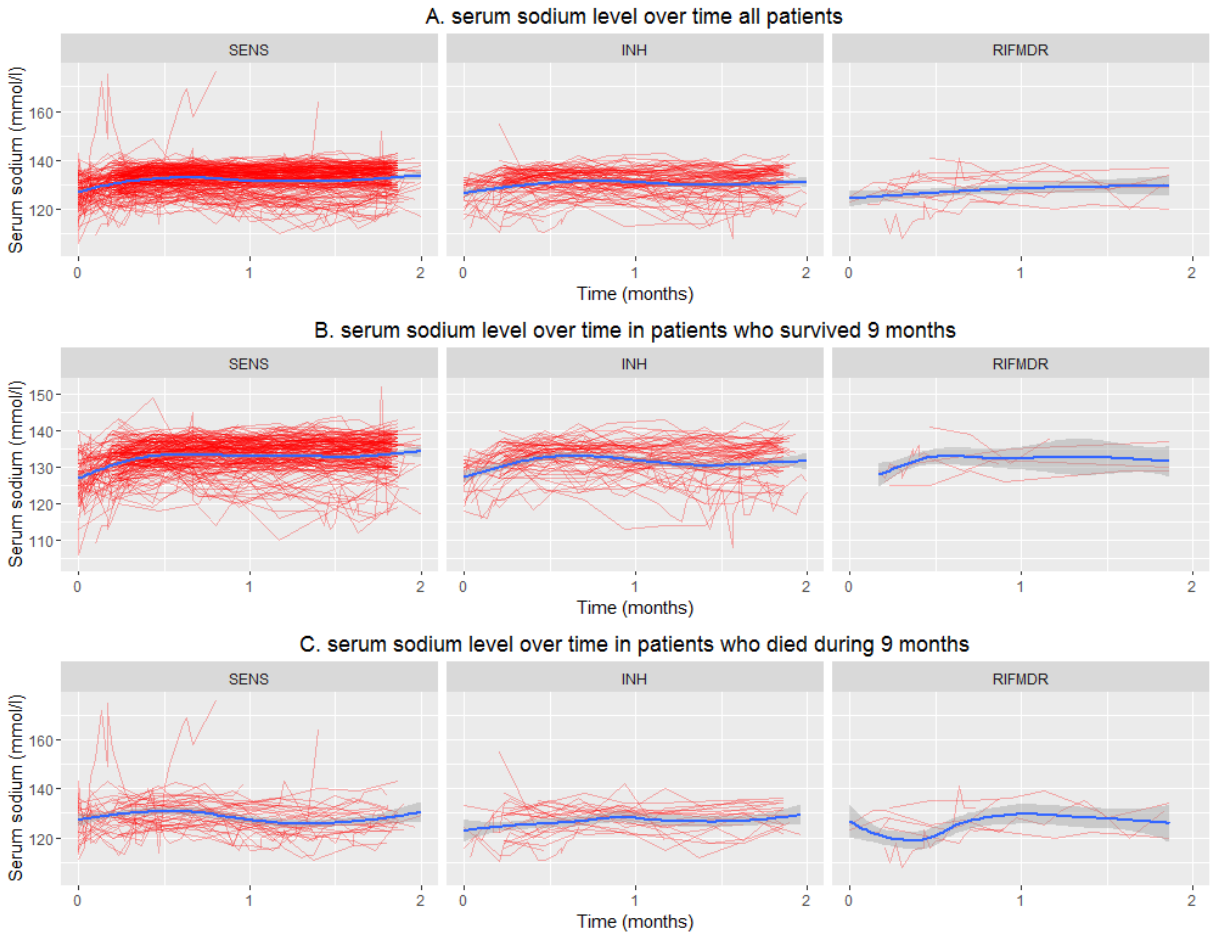


Figure 5.2 Serum sodium levels over time during first two months by resistance category

A.All patients included, 220 in INH-s+RIF-s group, 86 in INH-r group, 16 in RIFMDR group. B. Including only patients who survived nine months or were censored for other reasons than death; 168 in the INH-s+RIF-s group, 59 in INH-r group, 5 in RIFMDR group Three patients in the INH-s+RIF-s group were lost to follow up prior to 2 months. C. including patients who died prior to nine months (and prior to 2 months) of follow-up; 52 (19) in INH-s+RIF-s group, 27 (7) in INH-r group, 11 (7) in RIFMDR group.
SENS=no isoniazid or rifampicin resistance, INH=isoniazid resistance, RIFMDR=rifampicin monoresistance or multidrug resistance

Overall, lower sodium levels were associated with worse outcome; patients who survived had statistically significantly higher baseline sodium levels than patients who died during nine month follow up (126 mmol/l (95%CI 122.8-131.0) vs. 124 mmol/l (95% CI 120.0-130.0), P=0.03). Analysis of longititudinal measurement of sodium up to 61 days, using a GEE linear model corrected for HIV status and TBM severity grade, showed association of lower sodium levels with RIFMDR resistance (-4.09mmol/l (SE 1.05), P<0.001) and HIV status (-1.72 mmol/l (SE 0.49), P<0.001), but not INH-r (-1.16 mmol/l (SE 0.62), P=0.06). There was no significant difference in the slope considering the model is linear; i.e. no significance difference in change in sodium over time.

No clear difference was seen in CSF cell count or biochemical results between the resistance categories after one month of antituberculosis treatment, although protein levels were slightly higher in patients without resistance to isoniazid or rifampicin (Figure 5.3 and Table 5.2). Overall, median CSF protein level after one month was 1.19 g/l (IQR 0.66-2.09 g/l), median CSF glucose was 2.5 mmol/l (IQR 2.1-3.0 mmol/l), CSF lactate 3.00 mmol/l (IQR 2.29-4.19 mmol/l), median CSF white cell count was 44.5 cell/mm^3 (IQR 12.0-101.0 cells/mm^3). After two months of treatment, in all resistance categories, median protein levels were still raised (1.31g/l (IQR 0.64-2.29 g/l)).

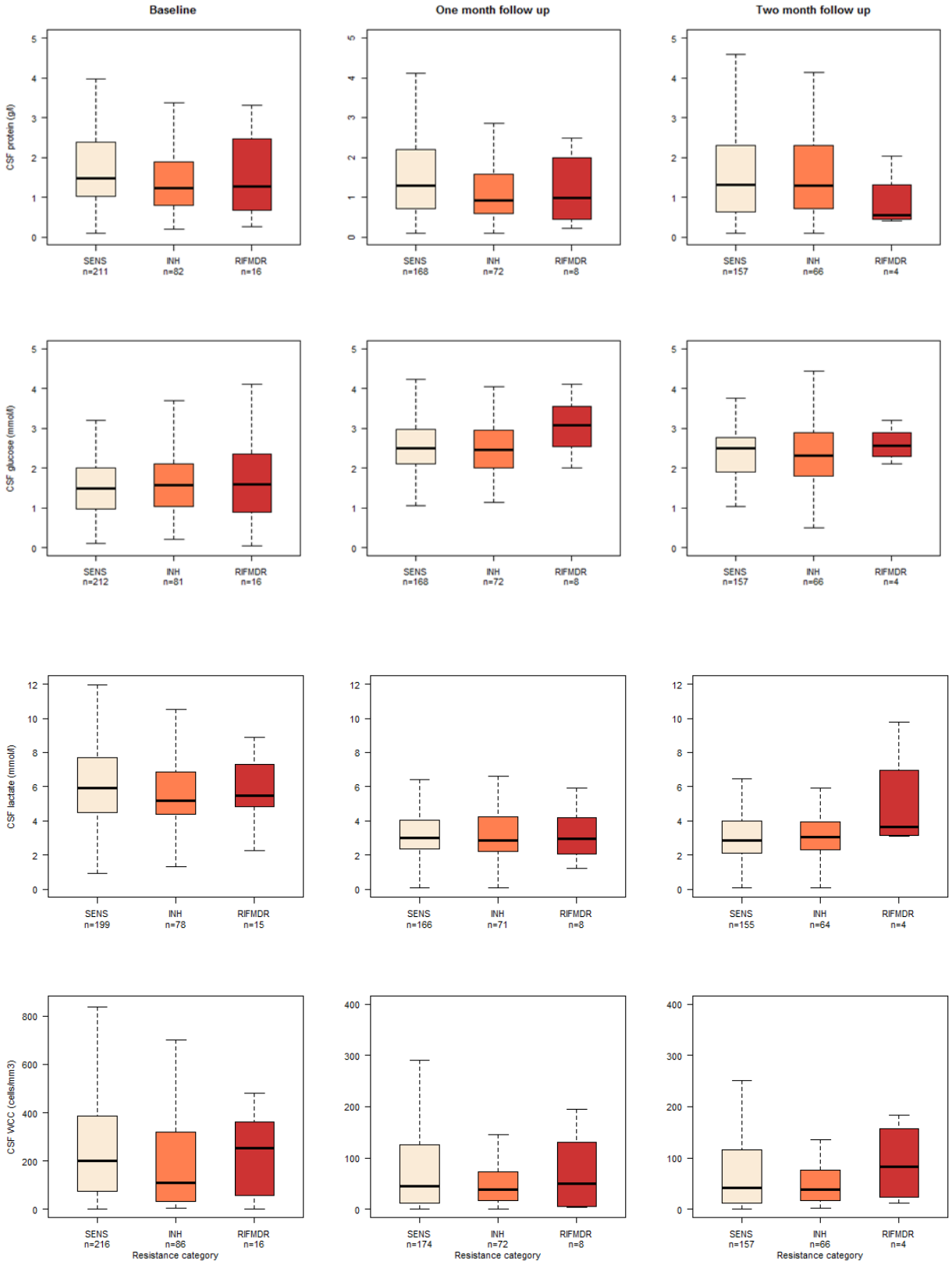


Figure 5.3 CSF parameters at baseline, one and two months of antituberculosis treatment

At baseline, CSF=cerebrospinal fluid, WCC= white cell count, SENS=no isoniazid or rifampicin resistance, INH=isoniazid resistance, RIFMDR=rifampicin monoresistance or multidrug resistance

Table 5.2 Comparison of follow up CSF parameters by resistance category

	All patients (N=322)		**INH-r (N=86)**		**INH-s+RIF-s (N=220)**		**RIFMDR (N=16)**		**Comparison**
Characteristic	n	Summary statistic median (IQR)	n	Summary statistic median (IQR)	n	Summary statistic median (IQR)	n	Summary statistic median (IQR)	(P-value)
Baseline									
White cells (/mm^3)	318	180.0 (58.0,381.8)	86	108.5 (32.3,316.0)	216	199.5 (72.8,386.0)	16	253.5 (64.3,340.0)	0.06
Glucose (mmol/l)	309	1.51 (1.00,2.10)	81	1.58 (1.04,2.11)	212	1.49 (0.98,2.00)	16	1.60 (0.95,2.28)	0.52
Lactate (mmol/l)	292	5.80 (4.49,7.40)	78	5.20 (4.40,6.84)	199	5.93 (4.50,7.69)	15	5.50 (4.81,7.30)	0.15
Protein (g/l)	309	1.41 (0.90,2.30)	82	1.24 (0.81,1.87)	211	1.48 (1.02,2.38)	16	1.28 (0.74,2.38)	0.15
One month									
White cells (/mm^3)	254	44.5 (12.0,101.0)	72	39.0 (16.5,70.5)	174	45.5 (12.0,123.8)	8	49.5 (6.5,121.0)	0.80
Glucose (mmol/l)	248	2.50 (2.10,3.00)	72	2.46 (2.00,2.92)	168	2.50 (2.10,2.96)	8	3.07 (2.67,3.52)	0.13
Lactate (mmol/l)	245	3.00 (2.29,4.19)	71	2.86 (2.20,4.22)	166	3.00 (2.37,4.04)	8	2.95 (2.12,4.15)	0.93
Protein (g/l)	248	1.19 (0.66,2.09)	72	0.93 (0.60,1.58)	168	1.30 (0.72,2.20)	8	0.98 (0.51,1.75)	0.04
Two months									
White cells (/mm^3)	227	41.0 (14.0,98.0)	66	37.5 (16.5,75.5)	157	42.0 (12.0,115.0)	4	83.5 (30.0,144.0)	0.69
Glucose (mmol/l)	227	2.44 (1.89,2.80)	66	2.32 (1.80,2.90)	157	2.50 (1.90,2.76)	4	2.55 (2.40,2.75)	0.70
Lactate (mmol/l)	223	2.94 (2.20,4.00)	64	3.04 (2.30,3.89)	155	2.85 (2.12,4.00)	4	3.65 (3.18,5.53)	0.22
Protein (g/l)	227	1.31 (0.64,2.29)	66	1.30 (0.71,2.30)	157	1.32 (0.64,2.30)	4	0.55 (0.48,0.96)	0.37

Diagnostic testing for mycobacteria on CSF is notoriously insensitive. CSF is rapidly sterilised after treatment initiation, reflecting limitation of the tests probably rather than eradication of mycobacteria.

Figure 5.4 shows the percentage of positive results by diagnostic test used in all patients (i.e. in this dataset, with culture confirmed diagnosis).

For the exploration of mycobacterial test results by resistance category, Xpert testing was considered to be most representative and applicable to clinical guidance, since ZN stain is a very insensitive diagnostic test in most settings, and MGIT culture results take too long to guide clinicians to adjust treatment. Results of baseline and follow Xpert results after one and two months of treatment by resistance category are shown in Figure 5.5.

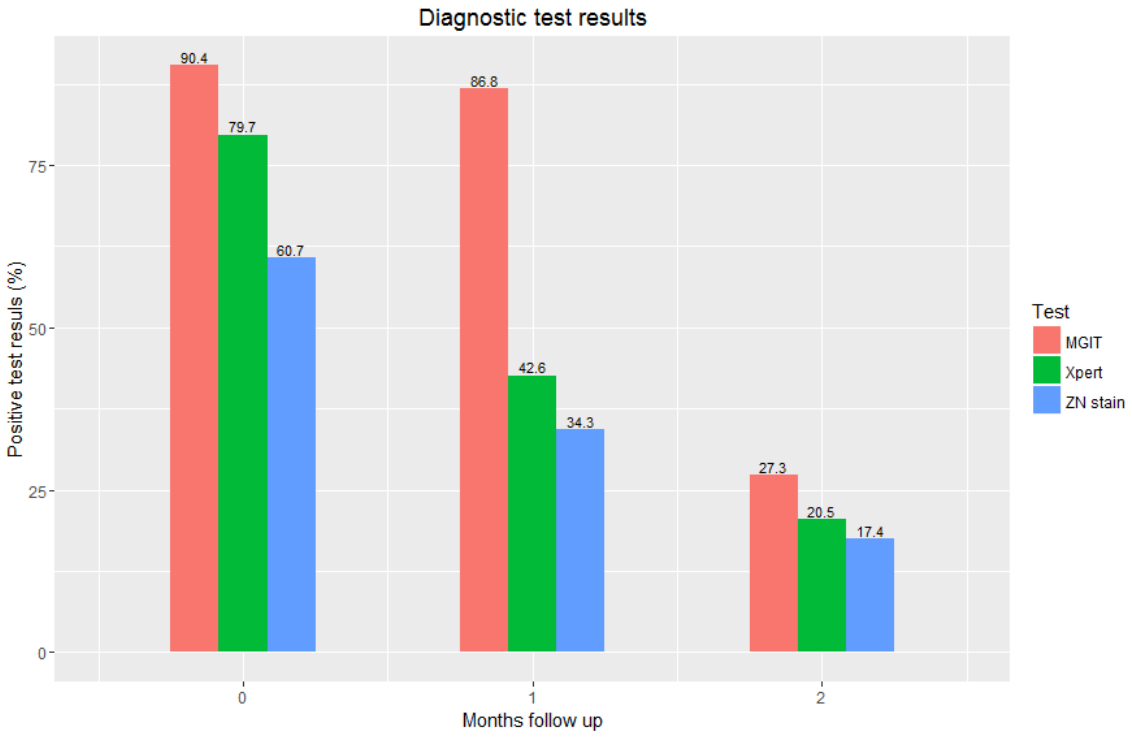


Figure 5.4 Mycobacterial test result of CSF by month of follow up

Percentages are based on number of positive/number tested, i.e. excluding missing patients. Baseline results for MGIT culture, Xpert and ZN stain were missing for 9, 27 and 14 patients respectively. After one month follow up, 286 patients were still at risk (i.e. 36 patients were censored); the number of patients tested for MGIT culture, Xpert and ZN stain were 53, 155 and 181 respectively. After two months of follow up, 271 patients were at risk (51 patients were censored); the number of patients tested for MGIT culture, Xpert and ZN stain were 11, 132 and 144 respectively.
MGIT= MGIT culture, ZN=Ziehl-Neelsen

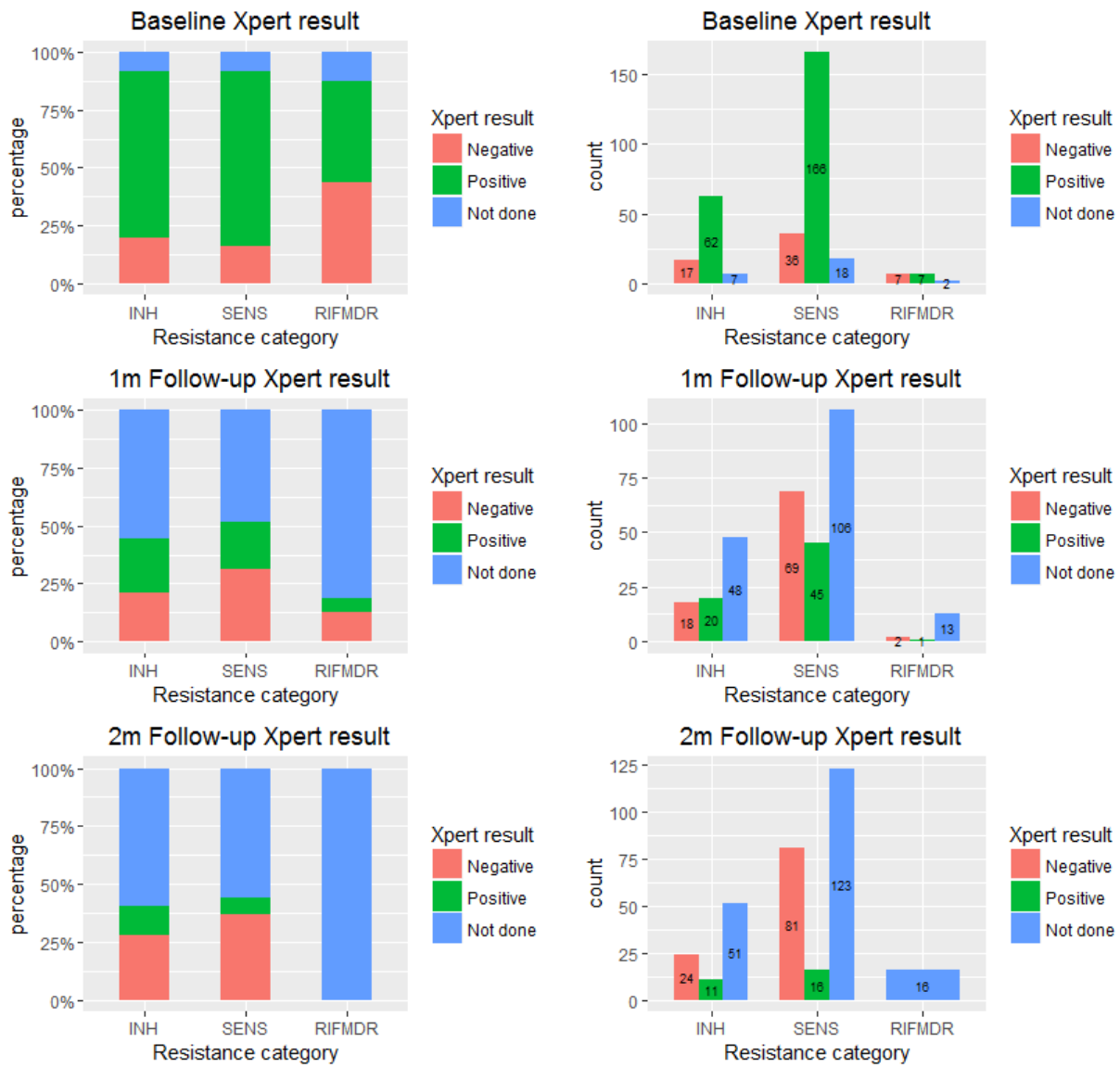


Figure 5.5 Baseline and follow-up CSF Xpert results

At baseline, 295 patients were tested for Xpert. After one month follow up, 286 patients were at risk; 79 in the INH-r group, 199 in the INH-s+RIF-s group and 8 in RIFMDR. Of those, 38, 114 and 3 were tested respectively. After two months of follow up, 271 patients were at risk; 77 in the INH-r group, 187 in the INH-s+RIF-s group and 7 in the MDR group. Of those, 35, 97 and 0 were tested respectively. Percentages shown in the first panel are based on the entire population (N=322) as the denominator, i.e. all patients not tested include those who were censored at the given time point.

There was no significant difference in Xpert positive results after one month of antituberculosis treatment between patients with isoniazid resistant TBM (20/38 (52.6%))and patients with isoniazid sensitive TBM (45/114(39.5%), OR 1.70, P=0.19) or in Xpert positivity after two months of treatment between the two resistance categories (OR 2.30, P=0.09).

Explorative univariate analysis of factors associated with Xpert positivity at one month are summarised in Table 5.3. Of 322 patients, 155 (48.1%) had available results of Xpert testing after one month. 89/155 (57.4%) had negative results, 66/155 (42.6%) had positive results i.e *M.tuberculosis* detected. HIV infection with severe immunosuppression, was the main baseline factor associated with Xpert positivity after one month of follow-up. A positive result on Xpert after one month of treatment was associated with a lower CSF white cell count after one month ($p<0.001$), however this finding may be confounded by HIV associated immunosuppression.

Table 5.3 Factors associated with Xpert positivity after one month of antituberculosis treatment

Baseline characteristic		**All patients (N=322)**		**One month Xpert negative (N=89)**		**One month Xpert positive (N=66)**	**Comparison**
	N	Summary statistic	n	Summary statistic	n	Summary statistic	(P-value)
Randomised treatment n(%)	322		89		66		0.33
- Standard		156(48.45%)		47(52.81%)		29(43.94%)	
- Intensified		166(51.55%)		42(47.19%)		37(56.06%)	
Resistance category n(%)	322		89		66		0.30
- INH-s and RIF-s		220(68.32%)		69(77.53%)		45(68.18%)	
- INH-r		86(26.71%)		18(20.22%)		20(30.3%)	
- RIFMDR		16(4.97%)		2(2.25%)		1(1.52%)	
Male sex n(%)	322	231(71.74%)	89	57(64.04%)	66	46(69.7%)	0.50
Age median (IQR)	322	34.00(29.00,41.00)	89	33.00(27.00,42.00)	66	33.00(29.25,38.75)	0.84
Weight median (IQR)	322	48.00(43.00,51.00)	89	49.00(45.00,54.00)	66	46.00(40.25,50.00)	0.09
Previous episode of TB n(%)	322	66(20.5%)	89	9(10.11%)	66	10(15.15%)	0.46
Duration of illness n(%)	322	15.00(10.00,30.00)	89	12.00(9.00,21.00)	66	15.00(8.00,21.00)	0.87
GCS baseline median (IQR)	322	14.00(11.00,15.00)	89	14.00(11.00,15.00)	66	13.00(10.25,15.00)	0.37
MRC grade n(%)	322		89		66		0.58
- 1		124(38.51%)		34(38.2%)		20(30.3%)	
- 2		129(40.06%)		36(40.45%)		29(43.94%)	
- 3		69(21.43%)		19(21.35%)		17(25.76%)	
chest X-ray n(%)	321		89		66		0.69
- abnormal consistent with TB		178(55.45%)		56(62.92%)		40(60.61%)	
- miliary TB		70(21.81%)		11(12.36%)		12(18.18%)	
- abnormal other		34(10.59%)		7(7.87%)		6(9.09%)	
- normal		39(12.15%)		15(16.85%)		8(12.12%)	
Baseline sodium level (mmol/l) median(IQR)	302	126.00(122.00,131.00)	88	126.50(123.00,132.00)	64	126.00(120.75,131.00)	0.27
HIV infected n(%)	322	185(57.45%)	89	36(40.45%)	66	42(63.64%)	0.006
CD4 cell count (cells/mm^3) median(IQR)	164	36.00(14.00,90.25)	36	42.50(21.25,87.00)	42	18.00(9.50,52.75)	0.026

Hospital HTD	322	174(54.04%)	89	87(97.75%)	66	66(100%)	0.51
Baseline CSF results							
- WCC	318	180.00(58.00,381.75)	89	292.00(122.00,502.00)	66	276.00(136.50,531.75)	0.93
- Neutrophils (%) median(IQR)	179	46.00(22.00,73.00)	83	42.00(20.00,65.50)	65	51.00(31.00,78.00)	0.09
- Lymphocytes (%) median(IQR)	309	78.00(44.00,95.00)	85	58.00(34.00,80.00)	65	49.00(22.00,68.00)	0.043
- Protein (g/l) median(IQR)	309	1.41(0.90,2.30)	88	1.61(1.20,2.70)	66	1.82(1.22,2.73)	0.55
- Glucose (mmol/l) median(IQR)	309	1.51(1.00,2.10)	88	1.48(0.92,1.88)	66	1.29(0.82,1.90)	0.37
- Lactate (mmol/l) median(IQR)	292	5.80(4.49,7.40)	88	6.59(4.80,7.94)	65	6.27(5.05,7.80)	0.75
- ZN stain positive n(%)	308	187(60.71%)	89	83(93.26%)	66	66(100%)	0.038
- Xpert positive n(%)	295	235(79.66%)	89	76(85.39%)	66	66(100%)	<0.001
- MGIT culture positive n(%)	310	280(90.32%)	89	86(96.63%)	66	66(100%)	0.26
One month CSF results							
- WCC	254	44.50(12.00,101.00)	89	79.00(33.00,159.00)	66	33.00(10.50,85.00)	<0.001
- Neutrophils (%) median(IQR)	110	20.50(6.25,39.00)	67	21.00(7.00,40.00)	36	21.50(6.00,37.25)	0.62
- Lymphocytes (%) median(IQR)	204	93.00(75.00,100.00)	69	79.00(60.00,93.00)	36	78.50(62.75,94.00)	0.69
- Glucose (mmol/l) median (IQR)	248	2.50(2.10,3.00)	89	2.46(2.16,2.82)	65	2.50(2.15,2.93)	0.91
- Lactate (mmol/l) median (IQR)	245	3.00(2.29,4.19)	87	3.10(2.41,4.07)	65	2.76(2.24,3.91)	0.18
- Protein (g/l) median (IQR)	248	1.19(0.66,2.09)	89	1.38(0.78,2.27)	65	1.18(0.71,1.69)	0.11
- ZN stain positive n(%)	181	62(34.25%)	88	8(9.09%)	66	54(81.82%)	<0.001
- MGIT culture positive n(%)	53	46(86.79%)	8	8(100%)	4	4(100%)	1.00

5.4.5 TREATMENT OUTCOME OF PATIENTS WITH DRUG RESISTANCE

Overall, 90 patients with a known drug resistance profile died during nine months follow up (90/322 (28.0%)); in the INH-s+RIF-s category 52/220 died (23.6%), in the INH-r category 27/86 patients died (31.4%) and in the MDR/RIF-r group 11/16 died (68.8%). Overall survival during 9 months per resistance category is shown in Figure 5.6.

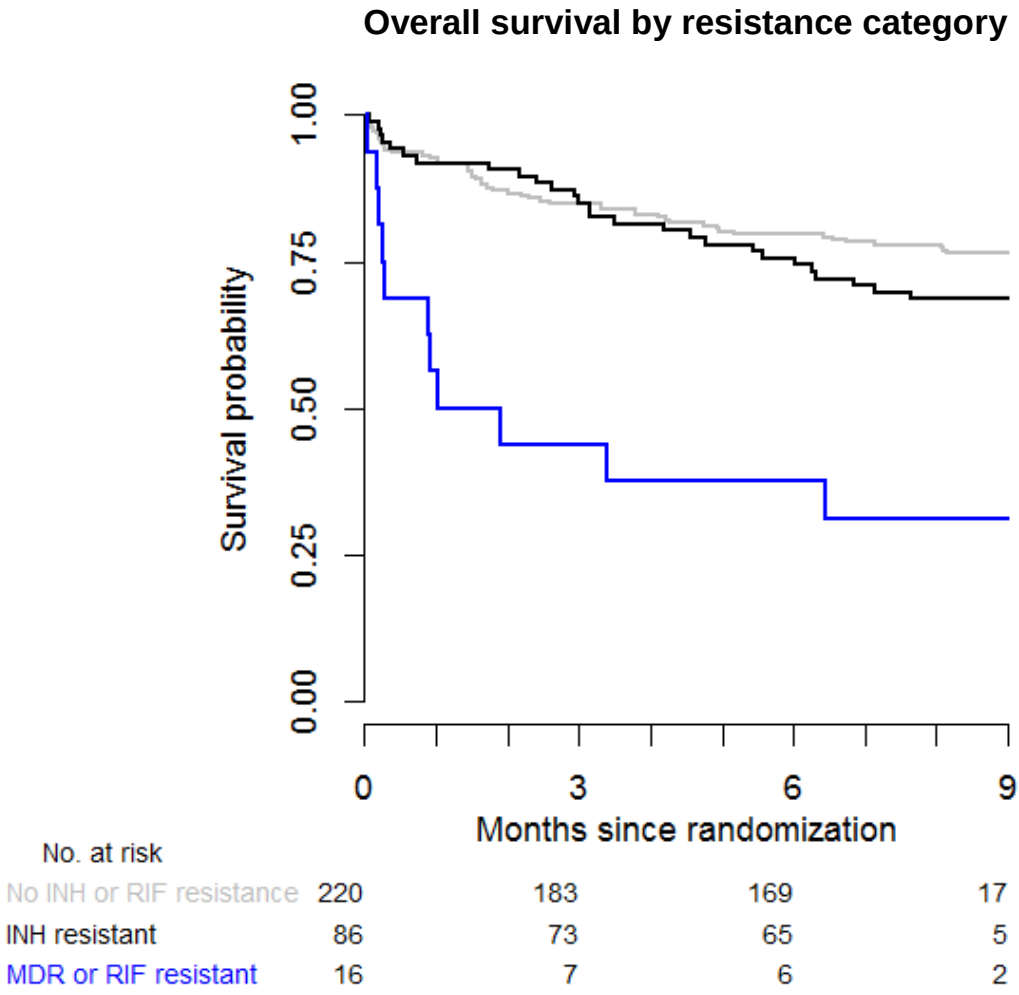


Figure 5.6 Overall survival by resistance category

Kaplan Meier estimated of survival during nine months by resistance category. The grey line represents patients with TBM with no INH or RIF resistance. The black line represents patients with TBM with INH resistance, but no RIF resistance. The blue line represents patients with TBM with RIF resistance or MDR. INH=isoniazid, RIF=rifampicin, MDR=multi-drug resistance.

Multivariate regression showed that HIV infection, disease severity grade 3 and MDR infection were independent predictors of death in the overall population, but not treatment arm or isoniazid resistance (HR=1.30 (95%CI 0.81-2.07), P=0.28) (Table 5.4), consistent with predictors found previously[264].

Table 5.4 Factors associated with nine-month outcome

Nine-month survival	Hazard ratio (95% CI)	P-value
Intensified treatment	0.92 (0.61-1.40)	0.70
INH resistance	1.30 (0.81-2.07)	0.28
RIFMDR	5.91 (3.00-11.64)	<0.001
BMRC grade 2	1.07 (0.62-1.84)	0.80
BMRC grade 3	4.54 (2.71-7.59)	<0.001
HIV infected	2.60 (1.62-4.17)	<0.001

Cox regression of factors associated with nine-month survival (number of patients included in the analysis=322, number of events=90).
INH=isoniazid, RIFMDR=rifampicin mono-resistance or multidrug resistance, BMRC=British Medical Research Council, CI=confidence interval.

The overall disability outcome at 9 months was not shown to be significantly affected by isoniazid resistance (Table 5.5). Infection with MDR in this study was not universally lethal, as in previous studies in our setting, nevertheless three of five survivors were severely disabled (Table 5.5). This is likely due to the current availability of second line anituberculosis treatment. Management and treatment response of MDR TBM patients is described in more detail in the following paragraph "Description of treatment outcome of MDR TBM patients".

Table 5.5 Disability status at nine months by resistance category

Disability n(%)	No or other resistance (n=220)	INH resistant (n=86)	RIF resistant or MDR (n=16)	Cumulative odds ratio* (95%CI) P-value
Good	91(42)	31(36)	2(12)	INH: 1.43
Intermediate	53 (25)	17(20)	0(0)	(0.89-2.30) P=0.14
Severe	20 (9)	11 (13)	3 (19)	RIFMDR: 10.49
Death	52 (24)	27 (31)	11 (69)	(3.44-31.95) P<0.001

** Cumulative odds ratio was obtained using a proportional odds logistical regression model depending on HIV status and TBM severity grade*
INH=isoniazid, RIF=rifampicin mono-resistance, MDR=multidrug resistance, CI= confidence interval

Time to new neurological events during treatment or death

In this section the combined endpoint of time to new neurological event or death is analyzed. Within the combined endpoint of new neurological events (as described in the methods section) and death, patients could have had a neurological event which was not followed by death, a neurological event followed by death after any period of time, but not on the same day or death not preceded by a clearly defined neurological event. Out of 322 patients, 154 (47.8%) patients met the combined endpoint. Overall, 64 (19.9%) had a neurological event, while surviving 9 months, 69 (21.4%) had a neurological event and did not survive 9 months, 21 patients (6.5%) had no neurological event recorded prior to death. The median time from randomization to neurological event was 6 days (IQR 2-43 days). If a neurological event was followed by death (although not on the same day), the median time from event to death was 15 days (IQR 2-79 days). If death was not preceded by a recorded neurological event, median time from randomization to death was 75 days (IQR 46-130 days). Table 5.6 shows the breakdown of events by resistance category. The survival curve of patients of this endpoint by resistance category is shown in Figure 5.7

Table 5.6 Neurological events and death by resistance category

Event type n (%)	**No or other resistance (n=220)**	**INH resistant (n=86)**	**Odds ratio (95% CI)**	**P-value***
Neurological event	39 (17.7)	24 (27.9)	1.79 (0.95-3.34)	0.06
Neurological event and death	40 (18.2)	19 (22.1)	1.28 (0.65-2.44)	0.43
Prior death	12 (5.5)	8 (9.3)	1.77 (0.60-4.93)	0.30

**P-value based on Fisher's exact test*
INH=isoniazid, RIF=rifampicin, MDR=multidrug resistance, 95% CI=95% confidence interval

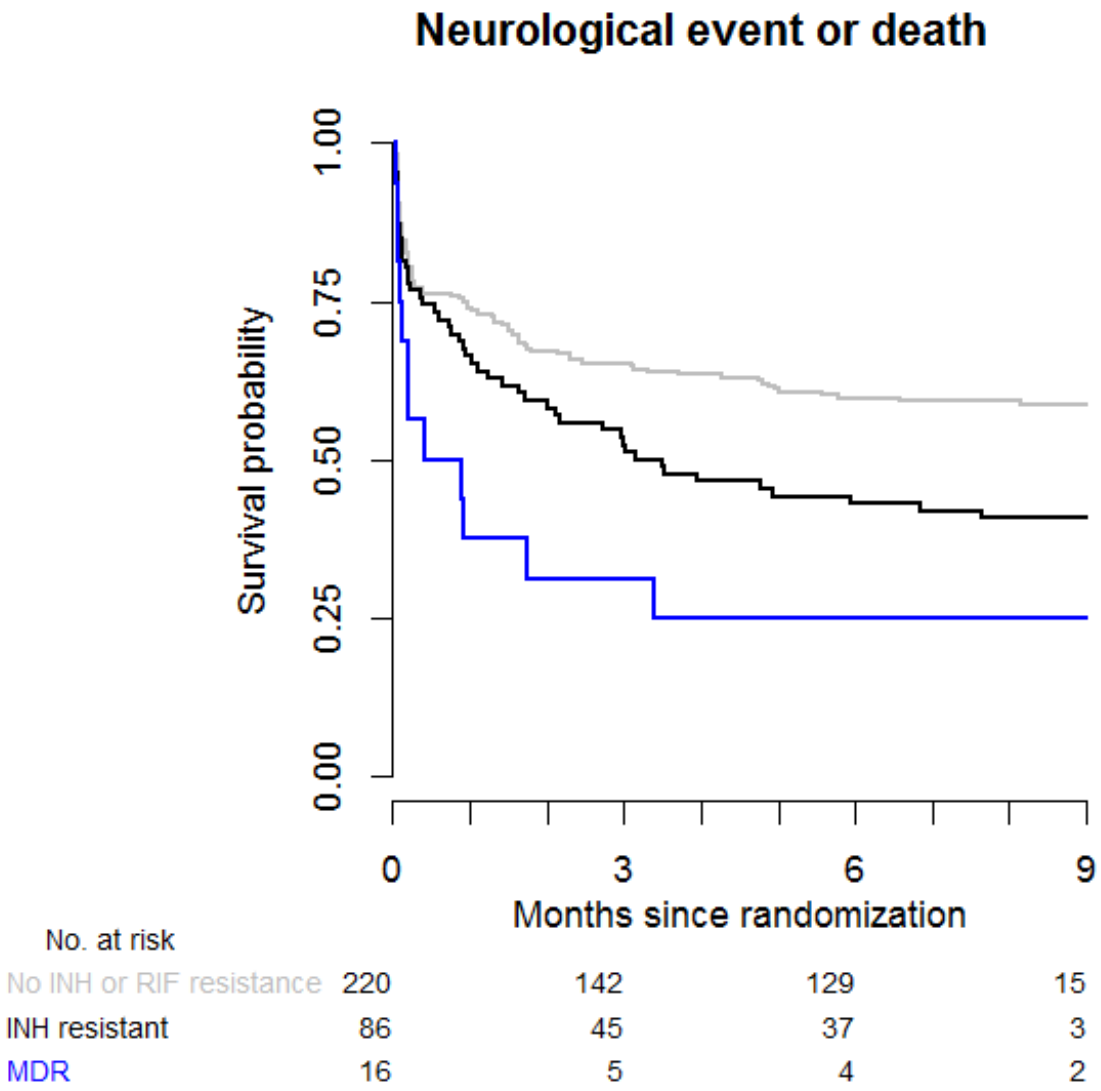


Figure 5.7 Time to new neurological event or death

B. Kaplan Meier estimates of combined endpoint of time to new neurological event or death by resistance category. The grey line represents patients with TBM with no INH or RIF resistance. The black line represents patients with TBM with INH resistance, but no RIF resistance. The blue line represents patients with TBM with RIF resistance or MDR. INH=isoniazid, RIF=rifampicin, MDR=multi-drug resistance.

Cox regression was performed to identify factors associated with the combined endpoint (Table 5.7). There was a significant effect of isoniazid resistance on the occurrence of any new neurological event or death when used as a combined endpoint (HR=1.57 (95% CI 1.1-2.23), P=0.01).

Table 5.7 Factors associated with combined endpoint

New neurological event or death	Hazard ratio (95% CI)	P-value
Intensified treatment	0.90 (0.66-1.24)	0.53
INH resistance	1.57 (1.11-2.23)	0.01
RIFMDR	3.29 (1.77-6.12	<0.001
BMRC grade 2	1.42 (0.95-2.11)	0.08
BMRC grade 3	4.63 (3.06-7.00)	<0.001
HIV infected	1.63 (1.66-2.28)	<0.001

Cox regression with new neurological event or death as a combined endpoint (N=322, number of events=154). INH=isoniazid, RIFMDR=rifampicin mono-resistance or multidrug resistance, BMRC= British Medical Research Council, CI=confidence interval.

5.4.6 POSITIVE XPERT RESULTS AFTER ONE MONTH TREATMENT AND OUTCOME

The significance of Xpert positive results after baseline was explored by comparing outcome parameters in both groups of patients with Xpert positive and Xpert negative results after one month of antituberculosis treatment (Table 5.8).

Table 5.8 Outcome by Xpert results after one month of antituberculosis treatment

Outcome parameter		All patients (N=322)		One month Xpert negative (N=89)		One month Xpert positive (N=66)	P-value*
New neurological event or death n (%)	322	154 (47.83%)	89	33 (37.08%)	66	37 (56.06%)	0.023
Last disability status n (%)	318		89		66		0.38
- Good		124 (38.99%)		34 (38.2%)		24 (36.36%)	
- Intermediate disability		70 (22.01%)		35 (39.33%)		21 (31.82%)	
- Severe disability		34 (10.69%)		8 (8.99%)		5 (7.58%)	
- Death		90 (28.3%)		12 (13.48%)		16 (24.24%)	
Patient died n (%)	322	90 (27.95%)	89	12 (13.48%)	66	16 (24.24%)	0.10

**Comparison made by Fisher's exact test*

Outcome of patients was similar in both groups (12/89(13.5%) vs 16/66(24.2%), P=0.10), but when new neurological events were considered, there was a significant difference between those with negative and positive Xpert results with more events occurring in the positive group (P=0.02). This univariate association may be confounded by a larger proportion of patients being severely immunosuppressed HIV infected patients.

5.4.7 Effect of intensified treatment on outcome of drug resistant TBM

In the overall population of the original study, it was suggested intensified treatment had a beneficial effect on outcome of patients with isoniazid resistant infection. In this population with confirmed TBM and a known resistance profile we reanalyse this effect and additionally evaluate the effect of regimen adjustments for patients after drug resistance was confirmed. The overall effect of intensified treatment as part of the randomised arm in INH-r and RIFMDR is shown in Figure 5.8A, B and C; A. In INH-r patients, the HR of intensified treatment, stratified by disease severity and HIV status, was 0.45 (95%CI 0.20-1.02, P=0.06). B. In MDR TBM, intensified treatment did not appear to impact mortality, (HR=0.63 (95%CI 0.15-2.69, P=0.54). C. For the combined outcome of new neurological event or death, the stratified HR of intensified treatment was 0.60 (95% CI 0.34-1.08, P=0.09). When only considering patients who were randomised to standard treatment, we can evaluate the effect of treatment adjustment with at least an additional fluoroquinolone after isoniazid resistance was established. This is discussed more elaborately in the next paragraph.

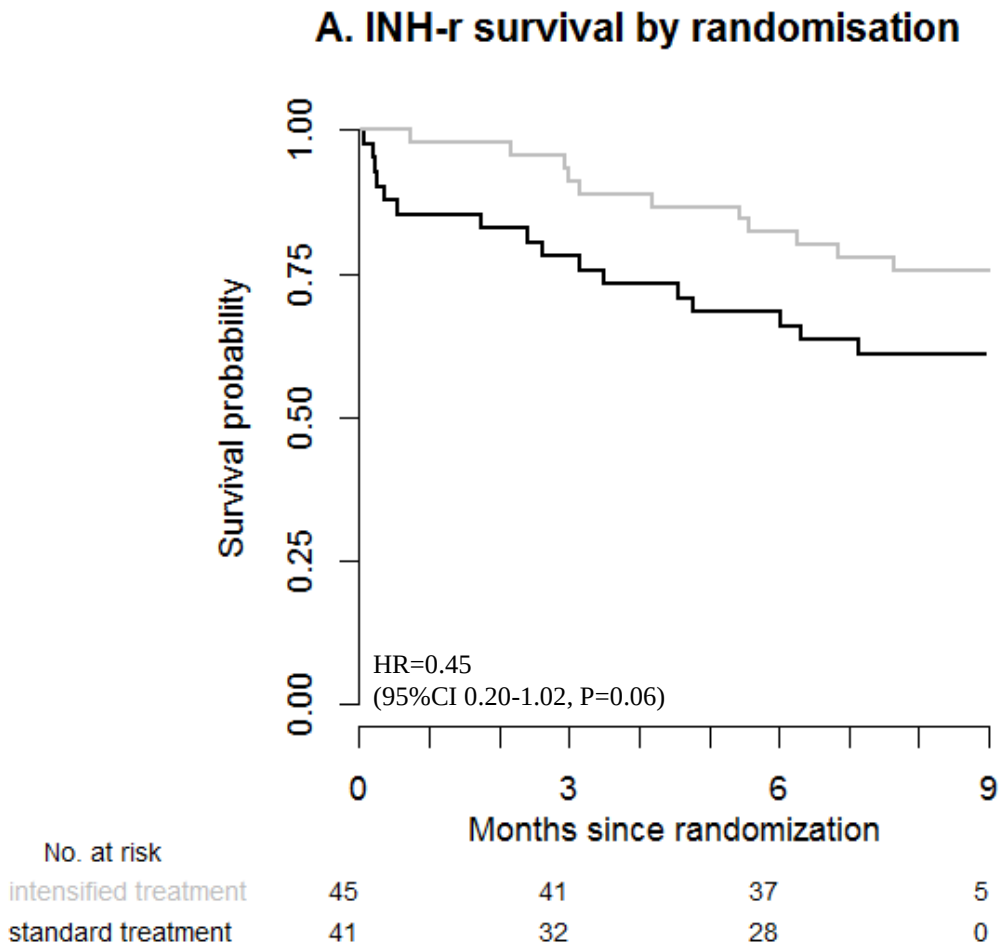
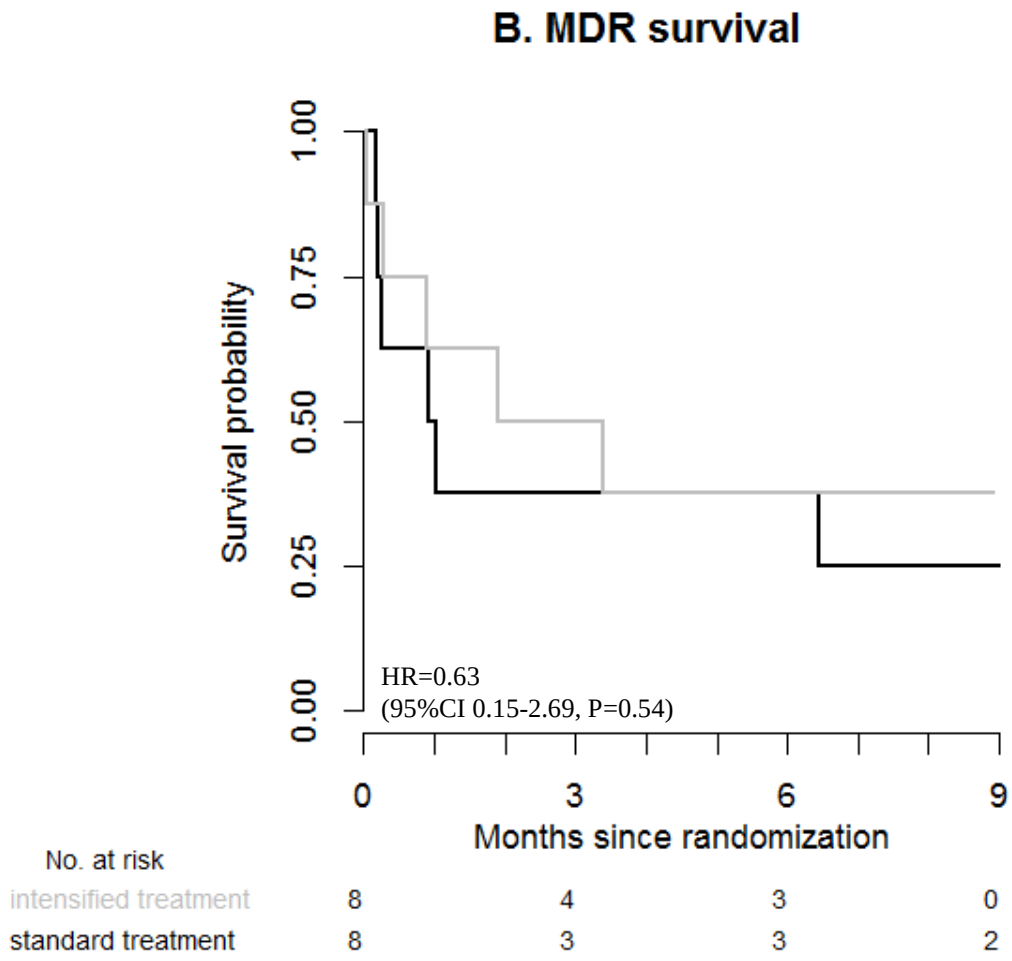
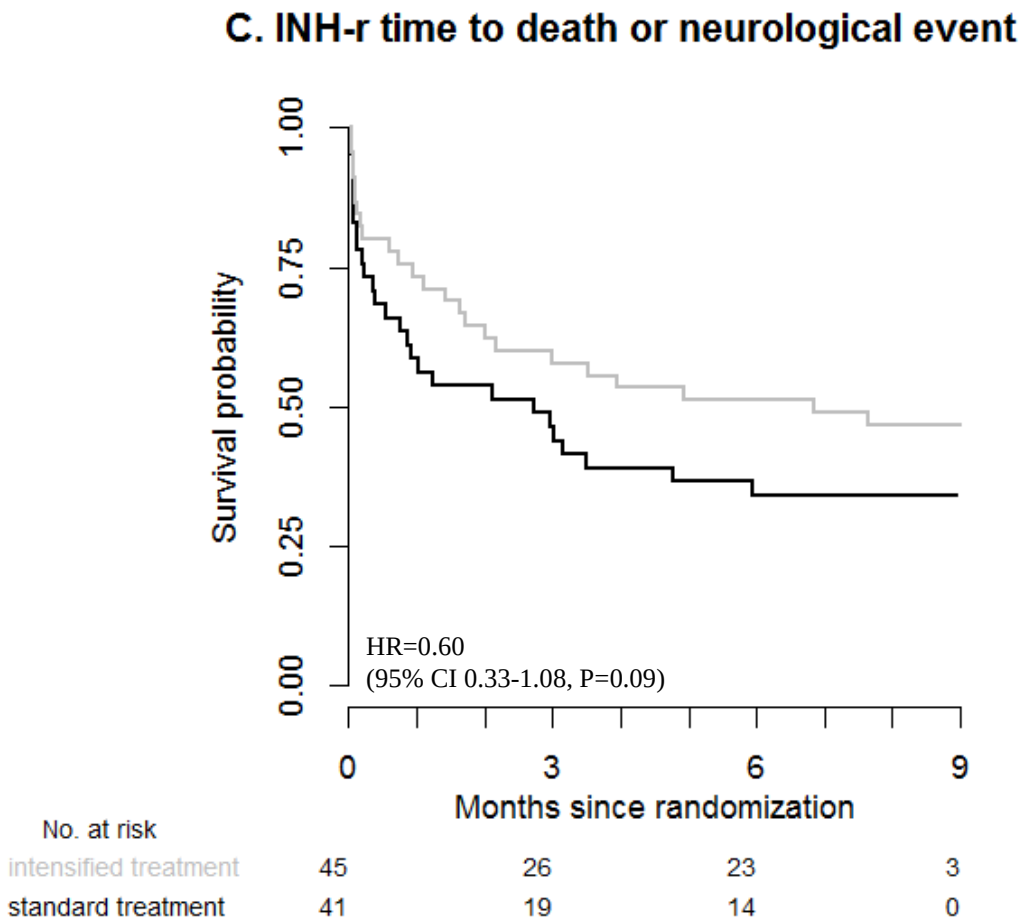


Figure 5.8 Treatment outcome of drug resistant TBM patients by randomised arm

A. Overall nine-month survival by randomised treatment arm of 86 patients in the INH-r category.



B. Overall nine-month survival by randomised treatment arm of 16 patients in the RIFMDR category.



C. Time to new neurological event or death by randomised treatment arm of 86 patients in the INH-r category. HR=adjusted hazard ratio, CI=confidence interval, levo=levofloxacin (in one case moxifloxacin was used)

5.4.8 EFFECT OF LATE TREATMENT ADJUSTMENTS IN ISONIAZID RESISTANCE

Within the INH-r category, 60 patients received treatment with a fluoroquinolone either as part of intensified treatment or targeted INH-r treatment, while 26 patients did not receive intensified treatment as part of randomization or any treatment adjustment throughout nine months of treatment. In case of isoniazid resistance, adjustments of treatment (i.e. addition of at least a fluoroquinolone) were made at the discretion of the treating physician. Hence, not all patients diagnosed with isoniazid resistance, received a fluoroquinolone. Additionally, adjustments depend upon the survival of the patient up to the moment of diagnosis. Treatment adjustments were also made for nine patients randomized to intensified treatment. Of those receiving intensified treatment or a fluoroquinolone 15/60 (25.0%) of patients died. In the standard treatment group 12/26 (46.1%) patients died. Since 15 patients received a fluoroquinolone based on DST results, without prior levofloxacin treatment as part of the treatment allocation, and initiation was dependent on survival up to more than 2 months after initial diagnosis, Cox regression was performed, including fluoroquinolone use as a time-dependent co-variate. Cox regression showed a significant benefit of fluoroquinolone use at any timepoint (0.38 (95%CI 0.18-0.80) P=0.01)), adjusted for disease severity grade and HIV infection (Table 5.9A). This benefit seems to be predominantly driven by the intensified treatment started at baseline. Only 15 patients in the standard treatment arm received a fluoroquinolone later in the course of treatment. Benefit of fluoroquinolone use in this group could not be demonstrated (HR 0.59 (95%CI 0.18-1.91), P=0.38) (Table 5.9B).

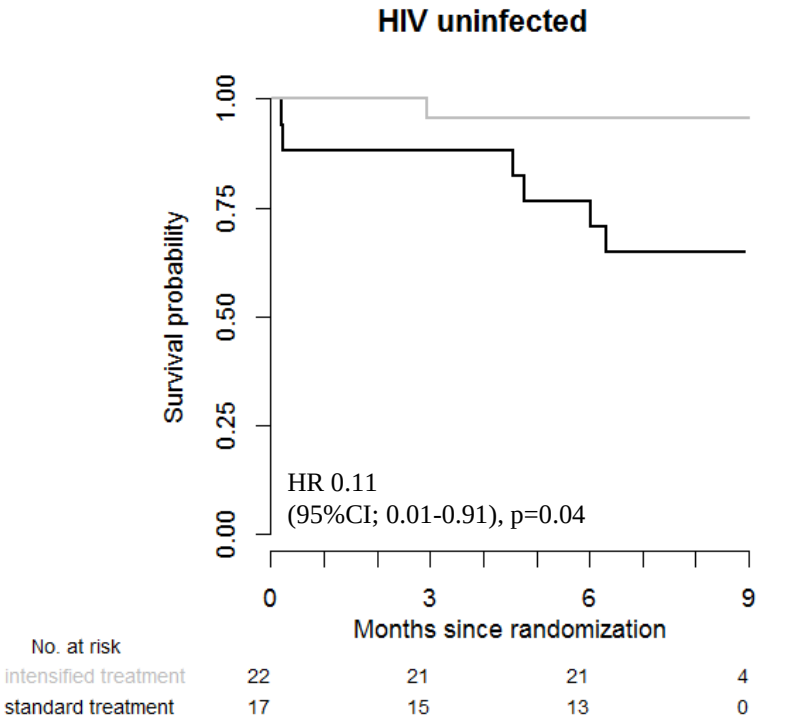
Table 5.9 Effect of intensified and adjusted treatment in patients in INH-r category

A. Fluoroquinolone use at any time point	**Hazard ratio (95% CI)**	**P-value**
Intensified or adjusted treatment (Tdc)	0.38 (0.18-0.80)	0.01
MRC grade		
-1	1 (reference category)	
-2	1.60 (0.67-3.82)	0.29
-3	2.96 (1.11-7.89)	0.03
HIV infection		
-negative	1 (reference category)	
-positive	2.93 (1.31-6.59)	0.01
B. Effect of later adjustments		
Late adjustment regimen (Tdc)	0.59 (0.18-1.91)	0.38
Randomised arm		
-standard treatment	1 (reference category)	
-intensified treatment	0.34 (0.15-0.76)	0.01
MRC grade		
-1	1 (reference category)	
-2	1.57 (0.66-3.75)	0.31
-3	3.03 (1.14-8.08)	0.03
HIV infection		
-negative	1 (reference category)	
-positive	2.82 (1.25-6.39)	0.01

A. *Cox regression including treatment enrichment with a fluoroquinolone as a time-dependent co-variate (Tdc), adjusted for TBM severity grade (MRC grade) HIV status. B. Cox regression including only late treatment adjustment as a time-dependent co-variate, while treatment allocation is treated as a baseline co-variate, corrected for TBM severity grade and HIV status. 86 patients in the INH-r category were included in the analysis. MRC=Medical Research Council, CI=confidence Interval.*

5.4.9 THE EFFECT OF HIV INFECTION ON INTENSIFIED TREATMENT RESPONSE

We explored the differential effect of intensified treatment in patients with HIV infection and uninfected patients in order to estimate which patients with isoniazid resistant TBM may benefit from intensified treatment. Intensified treatment appeared to be predominantly beneficial in the HIV uninfected patients with isoniazid resistant infection (Figure 5.9). Of the HIV uninfected patients, 6/17 (35.3%) died in the standard treatment arm compared with 1/22 (4.6%) in the intensified treatment arm (HR 0.11 (95%CI; 0.01-0.91), p=0.04). In contrast, 10/24 (41.7%) of HIV infected patients died in the standard treatment arm, compared with 10/23 (43.5%) in the intensified treatment arm (HR 0.69 (95% CI; 0.27-1.77), p=0.44).



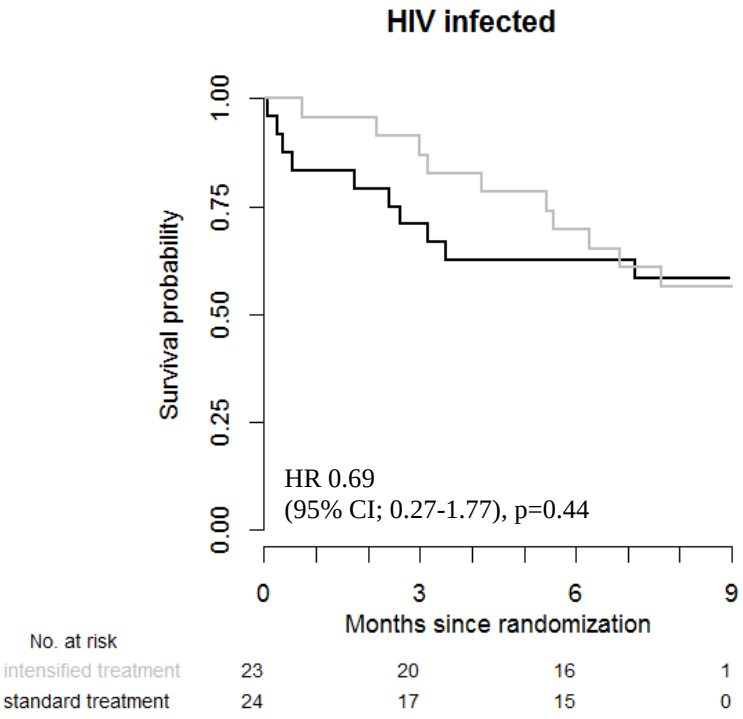


Figure 5.9 Survival by treatment arm in INH-r stratified by HIV status

Hazard ratio (HR) adjusted for TBM severity grade

5.4.10 THE EFFECT OF PATHOGEN ISONIAZID RESISTANCE MUTATION ON OUTCOME

From patients with isoniazid resistance, 78 isolates were available for molecular analysis of resistance mutations. 55/78 Isolates (70.5%) had mutation in the KatG region, 15/78 (19.2%) had mutation in the inhA promoter region. 10/78 (12.8%) had no mutation identified. There were dual mutations in both inhA and KatG regions in three isolates. In order to estimate whether there was a survival benefit associated with resistance mutations and estimated resistance level, dual mutations were classified as KatG (associated with high level resistance) and unknown mutations were grouped with inhA (associated with intermediate level resistance). Survival by resistance mutation is shown in figure 6. Resistance mutations were not associated with mortality by Cox regression adjusted for MRC grade, HIV status and treatment arm (HR 0.80 (95%CI 0.31-2.06, P=0.66)).

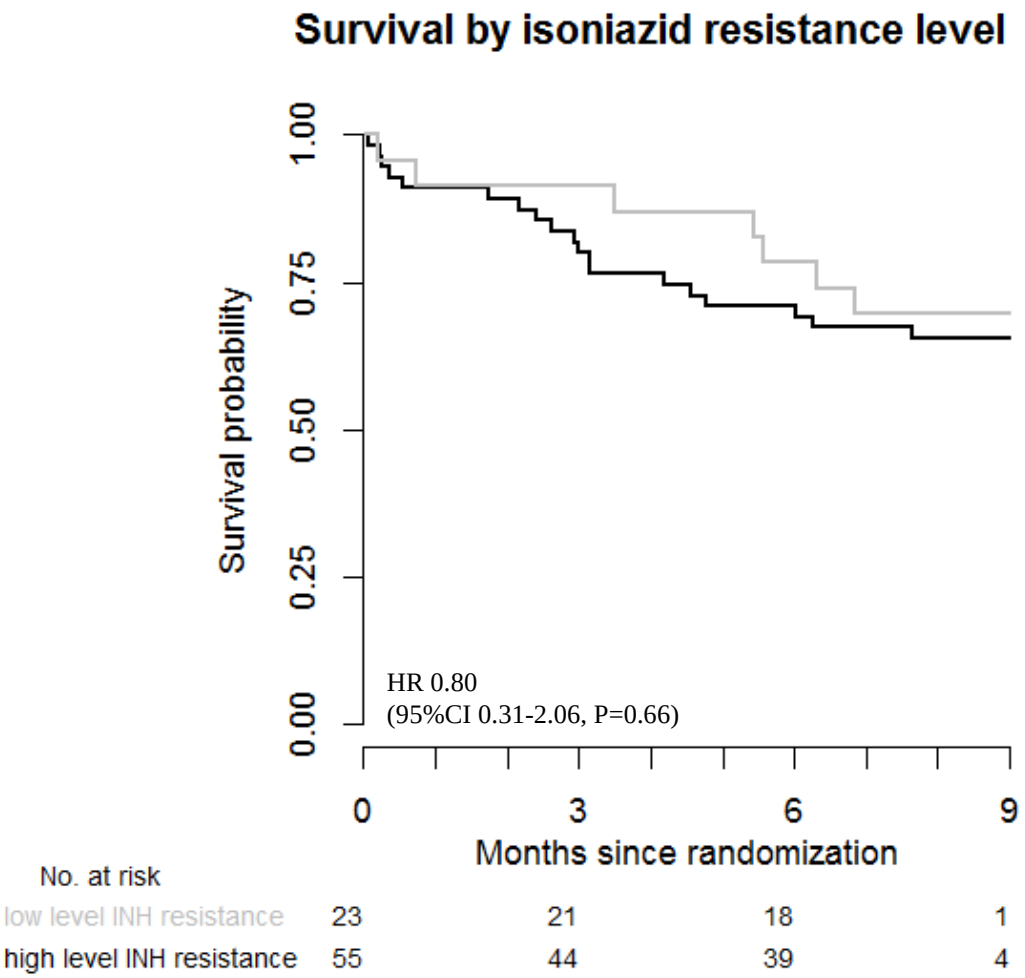


Figure 5.10 Survival by isoniazid resistance mutation

Low level resistance is defined as inhA mutations and unknown resistance mutations, high level resistance is defined as mutations in KatG region. Hazard ratio (HR) adjusted for TBM severity grade and HIV infection

5.4.11 DESCRIPTION OF TREATMENT OUTCOME OF MDR TBM PATIENTS

Xpert MTB/RIF detection of rifampicin resistance

Rifampicin resistance was detected early by Xpert MTB/RIF testing in five patients with MDR TBM and one patient with rifampicin mono-resistant TBM. Of these patients, two died before treatment adjustments to their regimen could be arranged. Two patients received second-line treatment according to the local guidelines after diagnosis. For two patients, the first-line regimen was enriched with levofloxacin with or without kanamycin. These patients were enrolled to the study prior to availability of second-line treatment for patients with TBM.

Xpert detected rifampicin resistance in two cases in which DST showed no resistance to rifampicin or isoniazid. One patient was HIV positive, presented with grade 1 TBM disease and was randomized to standard treatment. Study treatment was interrupted due to the Xpert result and open-label levofloxacin was added to the regimen, however discontinued after 5 days. At that time second-line treatment was not accessible to TBM patients. This patient died after three months of standard antituberculosis treatment. The other patient was HIV negative, presented with grade 3 TBM disease and was randomized to standard treatment. He survived 9 months without any alterations to the standard antituberculosis regimen.

Presentation and outcome of MDR TBM

Multidrug resistance was detected by DST in 15 patients. One patient had rifampicin mono-resistance. A summary of clinical presenting factors and treatment regimen are given in Table 5.10. The patients were predominantly male (14/16(87.5%)), with a median age of 34 years (IQR 31-36 years) and weight of 50.0kg (IQR 42.8-49.1kg). The majority of patients were HIV infected (13/16(81.3%)), with severe immunosuppression with a median CD4 cell count of 25 cells/mm^3 (IQR 11-55 cells). Most patients had signs of tuberculosis on chest X-ray (12/16(75%)), of which three had miliary TB. Five patients (31.2%) survived, of which two (12.5%) had a good neurological outcome, and three were severely disabled. Four of the survivors received second line antituberculosis treatment. None of

the patients who died received second line treatment. The median time to death of MDR patients was 27 days (IQR 7-45 days).

Three of the four patients receiving second line treatment had been randomized to intensified treatment. For two of the patients receiving second line treatment, the switch occurred relatively late (after 1 month of treatment with the first line regimen). As part of the study, these patients had received intensified treatment during that initial period. This may have been beneficial to initial survival until commencement of a full second line regimen (after 7 (patient 12024) ,9 (patient 11080), 35 (patient 42059) and 57 (patient 12026) days after randomization).

One patient survived nine months, while he did not receive second line treatment. This patient received first line treatment during admission, he was grade 1 on the MRC TBM severity scale when diagnosed. He was discharged after 37 days and subsequently lost to follow up. After nine months, upon telephone contact, his relatives told us he was severely disabled and bedridden. He had been taking antituberculosis drugs, but the regimen was unknown. He had also received traditional medicine. He had been randomized to standard treatment so had not received levofloxacin as part of the study.

Table 5.10 Summary of patients with multidrug resistant or rifampicin mono-resistant TBM

Patient	Time to death (days)	Disability outcome	Randomised treatment	Sex	Age	Previous TB	TBM grade	CD4 cell count/ mm3	HIV status	ARV status	SM	INH	RIF	Xpert RIF detection	EMB	Category	Treatment regimen
1	58	death	Intensified	male	28	yes	1	8	positive	not on ARV	R	R	R	*NA*	R	MDR	23dRHZE*'/ 35dSZH
2	8	death	Standard	male	33	yes	2	5	positive	not on ARV	R	R	R	*NA*	R	MDR	8dRHZSE
3	5	death	Standard	male	35	yes	1	34	positive	not on ARV	R	R	R	R	R	MDR	6dHRZSE
4	269	good	Intensified	male	31	no	1	57	positive	on ARV	R	R	R	R	R	MDR	8dHRZE/ 2EKmLfxZEthCSH/ 4KmLfxEthCs/ 2LfxEthCs
5	1	death	Intensified	male	39	yes	2	305	positive	on ARV	sens	R	R	R	sens	MDR	1dRHZSE
6	289	severe	Standard	male	34	yes	2	15	positive	on ARV	sens	sens	R	R	sens	RIF-r	8dHRZSE/ >9EZKmLfxCSPto
7	271	severe	Intensified	male	45	yes	2	214	positive	on ARV	R	R	R	*NA*	R	MDR	3HRZSE/ >6KmLfxEZPtoCs
8	103	death	Intensified	male	47	yes	1	*NA*	negative	*NA*	R	R	R	*NA*	R	MDR	39dRHZSE*/ 1RHZSE
9	289	severe	Standard	male	53	yes	1	*NA*	negative	*NA*	sens	R	R	*NA*	R	MDR	37dRHZSE
10	31	death	Standard	male	35	no	1	23	positive	on ARV	R	R	R	R	sens	MDR	28dRHZE/ 3dLfx
11	28	death	Standard	femal e	29	no	1	55	positive	on ARV	R	R	R	ind	R	MDR	28dRHZE
12	27	death	Intensified	male	31	no	1	11	positive	not on ARV	R	R	R	*NA*	R	MDR	27dRHZE
13	6	death	Standard	male	32	no	2	25	positive	not on ARV	R	R	R	*NA*	R	MDR	6dRHZE
14	196	death	Standard	femal e	33	no	2	10	positive	not on ARV	R	R	R	*NA*	R	MDR	46dRHZE
15	9	death	Intensified	male	25	no	3	48	positive	not on ARV	R	R	R	R	sens	MDR	6dRHZEAmkLfx
16	267	good	Intensified	male	49	no	2	*NA*	negative	*NA*	R	R	R	*NA*	sens	MDR	1RHZSE/ 4KmLfxZECsEthPas/ 2KmLfxECsEthPas/ >2KmLfxECsPas

ARV=antiretroviral treatment, S or SM=streptomycin, H or INH=isoniazid, R or RIF=rifampicin, E or EMB=ethambutol, MDR=multidrug resistant, Z=pyrazinamide, Km=kanamycin, Lfx=levofloxacin, Eth=ethionamide, Cs=cycloserine, Pto=prothionamide, Pas=para-aminosalicylic acid, NA=data missing

5.5 Discussion

The trial which forms the basis of this analysis is the largest intervention trial investigating targeted antituberculosis treatment for TBM. To date, no clinical trials have explored optimal treatment regimens for drug resistant TBM. In this sub-group analysis of patients with a known resistance profile, mortality was significantly reduced in patients in the INH-r category, when receiving a regimen intensified with a fluoroquinolone and high dose rifampicin, provided the analysis was corrected for later treatment adjustment with fluoroquinolones (HR 0.34 (95%CI; 0.15-0.76) p=0.01). In a stratified analysis, not corrected for later treatment adjustments, the effect of intensified treatment was only statistically significant in HIV uninfected patients (p=0.04). Improving treatment is crucial, since isoniazid resistance is increasingly prevalent. Isoniazid resistance is associated with worse outcome and improving treatment regimens for this group of patients will have a significant impact on mortality of TBM overall. Although a fluoroquinolone is generally considered the best option, there has been no evidence to demonstrate benefit due to the rarity of confirmed isoniazid resistant TBM cases with robust clinical data. A considerable proportion of isolated mycobacterial strains were found to be isoniazid resistant (26.7%). This reflects the high incidence of isoniazid resistant TB in Vietnam and it is likely that in other high isoniazid resistant incidence areas such as Eastern Europe and China, TBM follows the same pattern[271]. Clinicians should be alerted to the possibility of drug resistance when a patient reports a history of a TB episode or is HIV infected. We could not identify other baseline clinical parameters that were clearly associated with drug resistance. Although paraplegia was more frequently found in patients with isoniazid resistant infection, the symptom is relatively rare and the clinical importance of this finding is questionable. After treatment initiation, patients with isoniazid resistant TBM tended to more often experience a neurological event than isoniazid susceptible patients. However, there was no difference found in lumbar puncture results up to two months of follow up between these two resistance categories, nor in disability outcome. In general, low sodium levels are associated with worse outcome, reflecting neurological compromise. We found no difference in baseline sodium levels between resistance categories, but during the first two months of treatment, mean sodium levels were significantly lower in patients with MDR TBM, but not in

those with isoniazid resistant TBM. These findings illustrate the difficulty clinicians are faced with in determining which patients are failing treatment due to resistance to first line antituberculosis drugs. Other factors may cause patients to deteriorate during treatment, including paradoxical enlargement of tuberculoma, malabsorption of drugs, non-adherence to treatment or misdiagnosis.

In patients not receiving early intensified treatment, the majority of deaths occurred within 2 months of diagnosis. We propose that improved early bacterial killing is of increased importance to outcome in the absence of isoniazid susceptibility in TBM, even though the intervention did not show a benefit in the overall population. Mortality in isoniazid resistant infection may be mitigated by low level resistance in *M.tuberculosis* mutations in the promoter region of inhA, contrasting with higher level resistance in strains with mutation in the KatG region, however this was not demonstrated in our analysis.

We could not show an effect of fluoroquinolone use on outcome in patients for whom treatment adjustments were made after diagnosis of drug resistance through conventional methods. Although the number of patients included in this analysis was too low to be conclusive, the bactericidal effect of isoniazid is known to occur early in treatment, subsequently to substitute early killing, it is plausible that effective treatment adjustment should be initiated as early as possible. Moreover, since the continuation phase of treatment in TBM only consists of rifampicin and isoniazid, prolonged treatment with a third effective drug, possibly a fluoroquinolone into the continuation phase should be considered.

In settings with a high burden of isoniazid resistance, in patients at increased risk of being infected with an isoniazid resistant strain or with a known resistance profile from other biological samples (sputum or gastric fluid), the treating clinician may consider intensifying treatment with increasing the dose of rifampicin to 15mg/kg and adding a fluoroquinolone to the treatment regimen, despite lack of confirmation on the CSF sample, taking into account the clinical circumstances and provided the patient is not on a failing regimen already. The intervention is considered safe, as there was no overall increase in toxicity found with the intensified regimen in the original trial.

11 of 16 MDR/RIF-R patients died in this trial. Of the 5 survivors, 3 received second-line treatment. None of the patients who died received second-line treatment. Early diagnosis of MDR TBM allows the possibility to apply second line regimens but few data are available to guide regimen tailoring. In the near future, rapid whole genome sequencing may provide rapid specific data on the second line resistance profile of Mtb isolates to guide regimen design, but treatment of MDR TBM is further complicated by relative blood:brain barrier penetration. Pharmacokinetic and treatment trials for MDR TBM remain essential to improve outcomes and are a research priority. In other trials in this setting, prior to the availability of second-line treatment, mortality in MDR TBM was invariably lethal[53, 64].

The limitations of this study are that it is not a trial designed to target treatment for drug resistant TBM. Treatment with fluoroquinolone as part of treatment arm was initiated prior to diagnosis of drug resistance and in combination with high dose rifampicin. The impact of individual treatment with a fluoroquinolone as part of the regimen or the benefit of fluoroquinolones when introduced after diagnosis cannot be estimated from our data. It is plausible that the treatment effect in isoniazid resistant cases is due to the use of levofloxacin rather that an increase in rifampicin dose. Early bactericidal activity of rifampicin is appreciably lower than that of levofloxacin. Although higher doses of rifampicin are associated with increased early bacterial killing[265] possibly the dose increase in rifampicin in this trial was too modest to exert a clinical effect [272]. However, the design of the trial does not allow us to exclude the possibility. Pharmacokinetic analysis of the samples will provide us with more insight on the relationship between rifampicin levels in the CSF and clinical outcome. Additionally, inter-patient variability in isoniazid levels may influence treatment outcome, caused by differences in acetylator state and HIV status for example. Acetylator status and pharmacokinetic data were not yet available to be included in this analysis.

Conclusion: Early intensified treatment improves survival of patients with isoniazid resistant TBM. Second line treatment in MDR TBM may be crucial to survival. Clinical awareness and early diagnosis are key to positive outcome. Prospective intervention trials are needed.

CHAPTER 6

6 DISCUSSION

The aims of this thesis were to:

1. Improve diagnosis of TBM by evaluating the performance of a novel molecular diagnostic test; Xpert MTB/RIF
2. Improve treatment of TBM by evaluating an intensified antituberculosis treatment regimen.
3. Improve management of drug resistant TBM by exploring factors associated with drug resistance and evaluating response to intensified treatment.

The extent to which these questions have been answered will be addressed in this chapter. This chapter will also provide the context of current developments in TBM clinical research in which the significance of these results may be interpreted.

6.1 Improved diagnosis

Early diagnosis is critical to optimal outcome of treatment in TBM [47, 273]. Xpert MTB/RIF has been a major advance in early diagnosis of TBM. Sensitivity in our study compared to a clinical gold standard was 59.3% (95% CI; 51.8-66.5%) and 85.1% (95%CI 77.5-90.9%) compared to liquid culture as a gold standard. These and results from studies from other settings have led to a WHO recommendation, in which Xpert MTB/RIF is recommended as the initial diagnostic test in TBM[179]. To a certain extent this recommendation is reasonable, as in the context of TBM (and in most laboratories) smear is extremely insensitive (10-20%) and replacement of smear microscopy by Xpert MTB/RIF testing will impact early confirmation of disease. However, some limitations of this recommendation and the use of Xpert MTB/RIF as a single diagnostic test in the context of diagnosis

of TBM have been postulated[171]. The NPV of Xpert MTB/RIF based on the data used for the recommendation was 84%. Even in studies with large volume of centrifuged CSF examined for Xpert MTB/RIF testing, the NPV was at most 94%, in a high HIV prevalence setting [274]. Boyles and Thwaites suggested a NPV of at least 99% is needed for a diagnostic test to be satisfactory in its ability to exclude disease [180]. Which is a high target, however currently, there is no single diagnostic test that can exclude the possibility of TBM. Recently, a group of TBM specialists proposed a more comprehensive diagnostic approach, in which Xpert MTB/RIF is used as part of a diagnostic panel, in order to prevent wrongful omission of treatment. In particular, exclusion of other causes of subacute meningitides, such as cryptococcal meningitis using a highly sensitive lateral flow antigen assay on CSF, is an important aspect of this addition to the WHO recommendation [171]. High a priori clinical suspicion based on clinical determinants should already prompt clinicians to initiate treatment. The extent to which Xpert MTB/RIF impacts the number of patients for whom treatment would have been withheld if test results were not available, remains to be established. Such depends on the treatment threshold, i.e. the point at which the clinician is at equipoise regarding the decision to gather additional data or to rule in disease and initiate treatment [275, 276]. Clinical prediction rules have been proven to be quite sensitive and helpful in guiding clinicians when to start treatment [143, 144, 277]. Therefore, possibly in the majority of cases, a positive Xpert MTB/RIF result may reassure clinicians in their decision to treat rather than tipping the decision over from no treatment to treatment. Further evaluation of the sway of Xpert MTB/RIF results on clinical decision making is needed.

Other avenues for diagnosis of TBM have been explored, but none with overwhelming promise. Biomarkers of disease in the CSF, such as *M.tuberculosis* specific antigens or cytokines, have the ability to confirm disease, but absence does not rule-out disease. An ELISA to detect lipoarabinomannan (LAM), a cell wall lipopolysaccharide antigen of mycobacteria, had a sensitivity of 50% (95%CI: 27-73%) in HIV infected patients with severe immunosuppression [278]. Although sensitivity improved when combined with a clinical prediction rule, this performance still compares unfavorable with performance of Xpert MTB/RIF in our study. Unstimulated IFNγ levels or IFNγ

release assays (IGRAs) have some value in predicting the presence of disease. In particular, unstimulated levels of IFNγ were shown to be equally sensitive as IGRAs performed on CSF. IFNγ is a crucial cytokine in the immune response against *M.tuberculosis*. IGRAs may be helpful in diagnosing active disease in the brain, since the production of IFNγ of local mononuclear cells is higher than that of peripheral blood mononuclear cells (PBMCs) [279]. However, direct measurement of IFNγ levels is more rapid, less elaborate and equally sensitive (92% (95%CI: 78-98%)) when a cut-off of 0.244IU/ml is used [280]. A Chinese group tested also evaluated the use of an IGRA directly on CSF. They used the enzyme-linked immunospot assay (T SPOT.TB (Oxford Immunotec Ltd., Oxford, United Kingdom)) which detects IFN-γ secreting T-cells specific for two antigens, early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), which are present in MTB, but absent from *Mycobacterium bovis* BCG vaccine and most environmental mycobacteria. The population was relatively small, only 12 patients had definite or highly probable TBM, 28 had no TBM. The sensitivity and specificity of T SPOT.TB against clinical diagnosis on CSF directly were 92% (95% CI: 62–100%) and 93% (76–99%) respectively[281].

Adenosine deaminase (ADA) has been of interest for many years in TB-diagnosis. ADA is ubiquitously present in the body, but especially in lymphoid tissue, levels are especially high in active T-lymphocytes, hence it is associated with disorders that induce T-cell mediated immune responses. It has been shown to be of value in the distinction of tuberculous pleural-effusions [282]. The use in differentiating between TBM and other forms of meningitis is attractive, because it is a relatively cheap and easy test, especially of importance in low-income settings. Numerous studies have been published regarding the usefulness in TBM. A meta-analysis concluded that the mean sensitivity and specificity of ADA-assays were respectively 79% and 91% [283]. However, publication bias may have resulted in overestimation of diagnostic accuracy. Generally, ADA-assays may be useful in confirming TBM, but raised levels may also be seen in other CNS disorders (sarcoidosis, meningeal lymphoma, subarachnoid haemorrhage, neurobrucellosis), rendering it too non-specific [284-286]. It is not a useful test in HIV positive patients [285]. Combined with negative results for other tests, IGRA's and ADA may be useful rule-in tests, but are insufficient to rule out disease.

M. tuberculosis has a unique cell wall, formed by a complex of peptidoglycans, arabinogalactans and mycolic acids, which plays a significant role in the pathogenesis of tuberculosis[287-289]. There are three publically available databases of *M. tuberculosis* lipids - *Mtb*LipidDB, *MycoMass* and *MycoMap* - which describe more than 5,000 molecular features of the cell envelope lipids of *M. tuberculosis*[290-292]. Many of these lipids are determinants in the signaling events leading to host invasion and the control by immune response[291, 293, 294]. They are also potential markers of diagnosis and treatment response [295]. Recent advances in mass spectrometry (MS) and nuclear magnetic resonance (NMR) means the detection of extremely low concentrations in clinical samples is now a reality. Studies are underway that investigate novel lipid signatures in the CSF of patients with TBM using these new techniques, to determine whether they can be used as markers of both diagnosis and treatment response. However, the application of these as a primary diagnostic will be a long time away and application in resource limiting settings is uncertain.

Despite the low sensitivity, still ZN smear microscopy is the mainstay for diagnosis and is usually performed in all settings when TBM is suspected. Achievable modifications to the ZN staining procedure which may improve the performance of the test, can be implemented readily in existing laboratory infrastructure. Centrifugation of large volumes of the CSF, and meticulous slide examination are simple examples. Recently, Chinese investigators have devised a modification to the conventional CSF ZN stain which improves the intracellular staining of mycobacteria in CSF. The modified technique involves two additional steps to conventional smear, namely cytospinning the slide and an additional triton processing step. These simple modifications are achievable in most settings. In a pilot study conducted in China, including 29 patients with culture confirmed TBM (and 48 samples of their CSF), all samples (100%) stained positive using the modified smear technique, compared to only 8 samples (27.6%) positive by conventional ZN stain. In the modified smears, the integrity of the leucocytes was maintained and both intracellular and extracellular staining of mycobacteria was enhanced. In the conventional smear, no intracellular bacteria were visualised[296].

The results of this pilot study suggested that the modified smear was more sensitive than conventional smear and a larger study was undertaken. The method was assessed in 280 patients with a clinical diagnosis of TBM and the findings affirmed the results of the pilot study. Against a clinical diagnostic gold standard the sensitivity of conventional CSF ZN stain was 3.3% (95% CI: 1.6 to 6.7%), compared to 82.9% (95% CI: 77.4 to 87.3%) for modified ZN stain. The modified ZN stain revealed intracellular bacteria in 87.8% of the positive specimens. However, AFB were also seen in 6 patients with cryptococcal (n=4) and pyogenic bacterial meningitis (n=2), yielding a diagnostic specificity of 85.0% (34/40)[297].

These results are extremely promising, but need replication in a different population with the inclusion of larger numbers of patients without TBM in order to substantiate the high sensitivity and determine the specificity of the test. Furthermore, data are required on how the sensitivity of the method declines after the start of anti-tuberculosis drugs and whether it can be used as an early marker of treatment success or failure. The new method also needs to be assessed against high-quality conventional CSF ZN stain and microscopy, liquid culture, and the Xpert MTB/RIF assay.

The interpretation of diagnostic study results relies greatly on the use of appropriate clinical case definition, since the microbiological gold standard is suboptimal. For this purpose, a uniform case definition for use in clinical research was developed. However, the accuracy of the criteria for probable and possible TBM have not been ascertained. In children, retrospective evaluation of the clinical criteria used in the case definition, showed excellent discrimination between bacterial meningitis and TBM, but low specificity in patients classified as possible TBM [298]. In particular, in patients with early stage disease and little neurological compromise, clinical factors are non-specific. It is therefore important for any new diagnostic tool to be highly sensitive, also in early stages of disease. In our study, we found no difference in performance of Xpert MTB/RIF by disease grade. Further prospective evaluation of the clinical case definition in all stages of disease is necessary. Additionally, the accuracy of the clinical criteria should be evaluated in both HIV infected and uninfected patients, since differences in CSF results and neurological imaging have been reported [55].

The effective roll-out of Xpert MTB/RIF has led to an impulse in TB diagnostic development. Newer generations of commercial NAATs may have improved sensitivity and expanded drug resistance detection, potentially in a format of a point of care test, against reasonable costs.

6.2 Improved treatment

Our trial showed unequivocally that the intensified antituberculosis regimen used in this population of TBM patients had no effect on 9-month survival. We were able to establish that the intensified regimen with high dose rifampicin and levofloxacin added during the first two months of treatment, was safe, however ineffective. This is particularly disappointing, since overall mortality (28%), is still unacceptably high. There may be several explanations for the failure of this regimen, one of which has been a suboptimal dose of rifampicin [272, 299]. In a relatively small set of patients from Indonesia (n=60), a higher intravenous dose (~13mg/kg) during the first two weeks of treatment, did show a significant reduction in mortality (adjusted HR 0·42 (95% CI: 0·20–0·910, P=·03))[109]. Patients who received intravenous rifampicin had significant higher exposure to rifampicin (ratio of AUC_{0-6} (mg.h/l) of intravenous over oral dose; 3.0 (95%CI: 2.2-4.2), P<0.001). In an associated pharmacokinetic study, when patients were categorized in 3 separate exposure groups ('low', 'medium' and 'high'), there was a significant difference in 6-month mortality between these exposure groups: 'low': 11/18 (61%), 'medium': 7/18 (39%), 'high': 3/16 (19%), P<0.05[248]. Moreover, they report a concentration-response analysis, which reveals a target exposure of rifampicin of AUC_{0-6} of ca. 70 mg.h/l, which had not yet been reached with the doses used in their regimen, implying higher doses may be needed for optimal outcome [248]. Pharmacokinetic data from patients from our trial should reveal whether exposure in our population was indeed too low. Besides the dose, the route of administration of drugs may be critical. TBM patients may be severely ill on presentation and in particular in comatose patients, mortality is high (mortality grade 1; 14.5%, mortality grade 2; 28.6, mortality grade 3; 55.6%). In critically ill patients, enteral administration of drugs may significantly reduce bioavailability, with reduced absorption due to altered gastric emptying, altered gastric pH or

blood flow[300]. With intravenous administration, bioavailability is by definition 100%. Indeed, in the Indonesian trial, fewer patients had grade 1 disease on presentation (7% in Indonesia vs. 39% in Vietnam), which may have augmented the effect of intravenous treatment in this population. It has been proposed that doses of all first-line antituberculosis drugs may be suboptimal for severe forms of TB, such as pericardial TB and TBM[301]. Rather than higher doses of rifampicin only, possibly overall intravenous treatment in the first period of admission should be a focus of future intervention trials focused on improving antituberculosis treatment for TBM. The drawback of this is that intravenous formulations are often not available in settings where the burden of TBM is greatest.

Since the alteration to the intensified regimen consisted of both an increased dose of rifampicin and the addition of levofloxacin, we can conclude that neither contributed to improved survival. It has been suggested that fluoroquinolones may rather serve as a substitute for isoniazid, rather than add to improved sterilization in an isoniazid containing regimen[299]. Which is also evidenced by the disappointing results for fluoroquinolone containing regimens in shortening treatment of pulmonary TB [212, 213, 250]. In two small Indian trials evaluating the use of fluoroquinolone in TBM, firstly levofloxacin was substituted for rifampicin, which led to a significant reduction of 6-month mortality (HR 2.13 (95% CI: 1.04-4.34, P = 0.04)), but survivors were more severely disabled in the levofloxacin arm[263]. In the second trial, addition of levofloxacin to a standard first-line four drug regimen had a non-significant result; HR of death of the standard regimen compared with a levofloxacin enriched regimen was 2.61 (95% CI: 0.73-9.36, P = 0.14))[302]. The power of these small trials (sample size n=60 and n=57, respectively), may have been too limited. The addition of moxifloxacin (either 400mg or 800mg) was not associated in with improved survival in the Indonesian study, although this study was not powered for survival[109]. Considering these results, fluoroquinolones have no substantial role in treatment of drug susceptible TBM, unless when other agents of the regimen are not tolerated or contraindicated.

Besides inadequate levels of effective antituberculosis drugs in the brain, other factors may contribute to the high mortality of TBM. An appropriate immune response is essential in curtailing intracranial infection. This has been underscored by the reduced mortality seen when dexamethasone is used

adjunctively to the treatment regimen[32]. In the Vietnamese trial, dexamethasone treatment was associated with a 31% relative risk reduction (from 41.3% to 31.8%) [32]. Dexamethasone did not prevent disability among survivors, and long term effect of dexamethasone, until 5 years after randomization was only preserved in those with mild disease[59]. The rationale for the use of dexamethasone is that the host immune response is disproportionate to the infection and this leads to unacceptable intracranial inflammation, formation of exudate, leading to enclosure of vessels, compromise of neuronal tissue and subsequent damage through increased intracranial pressure and ischemia. Since the effect of dexamethasone is most pronounced in early stage disease, it may be that dexamethasone is able to prevent further damage by reducing inflammation, however exerts little effect on lesions that are in further stages of development[303]. A serial MRI study on the effect of dexamethasone on the cerebral pathology of TBM showed a tendency to reduced occurrence of hydrocephalus and stroke, but was underpowered[304]. The mitigated effect of corticosteroids on outcome of TBM may be further explained by the recently discovered polymorphism in the LTA4H gene, controlling the balance of pro- and anti-inflammatory eicosanoids, which influences response to treatment with dexamethasone[118]. In short, depending on the polymorphism, patients may exhibit an exaggerated immune response (TT genotype), a reduced immune response (CC genotype) or in case of heterozygous alleles, an intermediate immune response (CT genotype), as demonstrated by the recruitment of inflammatory cells in the CSF[118]. In 182 Vietnamese patients with TBM, 25 had the hyperinflammatory genotype (TT) and indeed these patients appeared to reap the greatest benefit of dexamethasone adjunctive treatment. Only a relatively small proportion of patients possess the hyperinflammatory polymorphism (14% in this study). These findings illustrate the importance of more a more sophisticated approach of employing adjunctive treatment to aid the immune response. Genotype dependent use of immunosuppressive corticosteroids needs to be further explored in larger prospective clinical trials. Conversely, the immune response may need to be augmented in patients with a genetic predisposition of balance towards an anti-inflammatory response.

Management of patients with further advanced brain compromise is a crucial but more challenging issue. Besides appropriate anituberculosis treatment and inflammatory control, the brain tissue needs

to be protected from the effects of raised intracranial pressure and ischemia. This involves advanced intensive and neurosurgical care; however both are often not accessible in resource limited settings. Proper management of hydrocephalus and hyponatremia, both associated with severe outcome [134, 305, 306], are vital to the brain parenchyma, but often neglected[132]. Some basic interventions are however achievable in most clinical locations, and may be indispensable to survival. Differentiation between communicating and non-communicating hydrocephalus is often difficult on imaging only, but the low-invasive procedure of air-encephalography is achievable in most resource limited settings, can be performed by clinicians and is highly informative [307]. Mostly (in children estimated around 75-83%) hydrocephalus is of the communicating type [50] and may be managed medically, without the need for neurosurgical intervention, with reasonable chance of success [132]. There are no trials evaluating medical treatment of hydrocephalus, but a combination of furosemide and acetazolamide led to success in around 80% of pediatric patients [131]. Hyponatremia is extremely common in patients with TBM and contributes to brain oedema and raised intracranial pressure [135, 308]. Although there has been debate about the underlying mechanism of hyponatremia in TBM, the commonest causes are probably SIADH and CSW, which are not easily distinguishable [309]. Differentiation is perhaps not as important as appropriate correction, which may be achieved with hypertonic saline in both syndromes [132]. It is probably advisable to take a pragmatic approach, and hypertonic saline may also alleviate raised intracranial pressure [132]. It is also preferred over mannitol, because it serves a dual purpose, as many patients with TBM both are hyponatremic and have increased intracranial pressure. Although in the context of TBM not evidence based, these achievable interventions have the potential to prevent irreversible brain damage.

Increased intracranial pressure may cause clinical deterioration through brain herniation or tissue ischemia. Ischemia and stroke prevention also lie at the core of brain care. Optimal oxygen supply and blood pressure control are key to ensure proper brain perfusion and tissue oxygenation. Early aggressive reversion of hypoxia has been implicated to have prevented infarction in a severe case of TBM[142]. Even in the absence of invasive continuous intracranial oxygenation monitoring, maintaining optimal arterial oxygen saturation, blood pressure and hemoglobin levels are basic interventions that can be taken to improve supportive care.

Vasculitis, due to perivascular engorgement of exudate, contributes to luminal caliber loss of small and medium sized vessels and subsequent infarcts. Meticulous histopathological descriptions of the vascular changes in TBM by were published by Hektoen in the late 19th century. He concluded that the changes could be either explained by an endarteritis with subendothelial tubercles, proposed to be caused by direct haematogenous invasion of bacilli in the vessel wall, or tuberculous proliferation affected the arteries from the adventitia inward to reach the media and the intima[137]. Other stenosing or damaging mechanisms are thought to be intimal proliferation, vessel wall necrosis, thrombosis or vasospasms[36, 41]. Although the exact mechanism of vasculitis is under debate, aspirin, either low dose (anti-thrombotic effect) or high dose (anti-inflammatory effect) may have a role in preventing stroke in TBM and results of a phase 2 safety trial in TBM are expected early 2017.

Improved treatment may be achieved by better delivery of antituberculosis drugs, especially in severely ill patients, targeted immunomodulation, stroke prevention and proactive supportive care. More intricate and standardized phenotyping patients and severity classification may aid clinicians in identifying those patients benefiting from the before mentioned interventions and evaluating prognosis.

6.3 Drug resistant TBM

This cohort and subgroup analysis has added to limited existing data on drug resistant TBM. The resistance rate found in this population was high, with 45.3% of isolates exhibiting resistance to at least one of four first line antituberculosis drugs and a rate of isoniazid resistance of 26.7%. In contrast, a multi-cohort European study reported that of 142 *M.tuberculosis* strains from CSF, 122 (85.9%) were fully susceptible to all first line drugs, and 12 (8.5%) were resistant to INH (not MDR)[254]. Mortality in the resistance categories were comparable to rates found in Vietnam; 23.8 % (n = 29) of fully susceptible cases, 33.3 % (n = 3) of cases who exhibited monoresistance to INH, and in 40 % (n = 2) of cases with MDR-TBM[254].

Our trial is the first to evaluate the effect of intensified treatment on outcome of drug resistant TBM. A puzzling aspect of the results presented here was that there was no significant effect of isoniazid resistant infection on survival, but benefit of intensified treatment in HIV uninfected individuals (p=0.04). Potentially this may be due to reduced virulence of isoniazid resistant strains. Most of the isolates were katG mutants, which have reduced catalase activity and thus may be more susceptible to reactive oxygen species within the phagosome [310]. Evidence of reduced fitness of isoniazid resistant mycobacterium is conflicting however and in vitro study results may not adequately reflect in vivo activity [311]. Virulence may also vary according to region and immuno-competence of the host. This may be a reason for differences in outcome found in varying studies [69, 70, 255, 261].

Early initiation of effective antituberculosis treatment for patients with TBM is essential for survival, therefore, and our data support this, treatment adjustment in drug resistant TBM should be made timely. Early treatment with the regimen used in our study appears beneficial in particular in HIV uninfected TBM patients. The intensified treatment we used is unlikely to be the optimal regimen for treatment of isoniazid resistant TBM. In children in South Africa, when isoniazid resistance, with no rifampicin resistance is confirmed, a fluoroquinolone and terizidone (a bacteriostatic antimycobacterial agent, less toxic derivative of cycloserine, used in second line treatment regimens), are added to the regimen for the remainder of the treatment course, regardless of HIV status [68]. A reasonable approach to adjust regimens would be to add at least one agent with good early bactericidal activity and CSF penetration to the regimen, for the duration of the entire treatment, to substitute for the loss of isoniazid activity.

We found very few clinical parameters to suggest resistant infection. A prior history of TB treatment is associated with drug resistance and HIV co-infection is associated with MDR TB. Clinicians may be alerted to consider resistance to isoniazid in patients for whom clinical improvement is hampered by new neurological events The benefit of intensified treatment depends on early treatment initiation and is more pronounced in HIV uninfected patients. The lack of clinical parameters and the benefit of adjusted treatment stress the importance of sensitive diagnostic tests targeted at identification of isoniazid resistance. The widespread use of rapid molecular testing of pulmonary TB by Xpert

MTB/RIF has impacted early diagnosis of rifampicin resistance and early second line treatment initiation[312]. Xpert MTB/RIF has been recommended by the WHO for direct use on CSF as the initial diagnostic test[179, 268]. However, isoniazid resistance cannot be detected by the current Xpert MTB/RIF cartridge. Xpert MTB/RIF Ultra (In sputum samples spiked with *Mtb* H37Rv, Ultra had an limit of detection of 5 CFU/ml compared to an limit of detection of 50 CFU/ml for Xpert (p=0.001))[313], to be released in 2016, is likely to increase sensitivity of the assay in detection of *M.tuberculosis* and an isoniazid-resistance cartridge is under development. The line-probe assays, such as Genotype MTBDR (Hain Life-Science, Nehren, Germany) can detect both rifampicin and isoniazid resistance. The bacillary load in CSF is likely too low for direct use, but they may be used on bacteria isolated from culture confirmed CSF samples, which will reduce time to diagnosis, since the second inoculation for conventional DST testing may take up to two weeks.

The outcome of MDR TBM is dismal, but in our trial, four lives were saved with second line treatment. Appropriate treatment was initiated after 7-57 days after randomization, and initial intensified treatment with levofloxacin was allocated to three patients. What constitutes appropriate treatment in MDR TBM is unclear. Second line drugs have varying antimycobacterial activity and CSF penetration. Of the drugs that may be used in second line treatment schedules, besides the second generation fluoroquinolones, moxifloxacin and levofloxacin and pyrazinamide, ethionamide, prothionamide and cycloserine have reasonable ability to penetrate the CSF[105]. Recently bedaquiline, a novel diarylquinoline which selectively inhibits the mycobacterial ATP synthase complex, has been approved for restricted use in MDR TB. One case report exists on its use in MDR TBM. The authors measured bedaquiline in serum and CSF, after 11 weeks of antituberculosis treatment, and could not detect bedaquiline in the CSF [314]. Penetration may potentially be improved in earlier stages of meningeal inflammation, but its prolonged use in TBM is doubtful, considering these findings.

Optimal treatment regimens for drug resistant still need to be determined. The design of trials addressing this will need rigorous consideration.

6.4 Conclusion

While the microbiological confirmation of TBM has been improved with the advent of the Xpert MTB/RIF, there has been little advance in treatment of TBM since the demonstration of the benefits of dexamethasone in 2004. Disappointingly, intensification of the antituberculosis regimen with oral high dose rifampicin and levofloxacin does not improve the outcome of TBM, although it may be beneficial in patients with isoniazid resistance and diagnostic tests to rapidly identify such resistance would allow targeted therapy. Diagnosis and treatment of drug resistance in TBM is a pressing problem. In particular, MDR TBM is associated with a grave outcome. Early treatment with effective antituberculosis drugs remains the most crucial aspect of management of this devastating condition.

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APPENDIX

This appendix contains the following documents:

Evaluation of GeneXpert MTB/RIF for the Diagnosis of Tuberculous Meningitis
Nguyen Thi Quynh Nhu, Dorothee Heemskerk, Do Dang Anh Thu, Tran Thi Hong Chau, Nguyen Thi Hoang Mai, Ho Dang Trung Nghia, Pham Phu Loc, Dang Thi Minh Ha, Laura Merson, Tran Thi Van Thinh, Jeremy Day, Nguyen van Vinh Chau, Marcel Wolbers, Jeremy Farrar and Maxine Caws
J. Clin. Microbio. 2014; 52(1):226.

Intensified treatment with high dose Rifampicin and Levofloxacin compared to standard treatment for adult patients with Tuberculous Meningitis (TBM-IT): protocol for a randomized controlled trial
Dorothee Heemskerk, Jeremy Day, Tran Thi Hong Chau, Nguyen Huy Dung, Nguyen Thi Bich Yen, Nguyen Duc Bang, Laura Merson, Piero Olliaro, Thomas Pouplin, Maxine Caws, Marcel Wolbers, Jeremy Farrar
Trials. 2011; 12:25

Sample size requirements for separating out the effects of combination treatments: randomised controlled trials of combination therapy vs. standard treatment compared to factorial designs for patients with tuberculous meningitis.
Wolbers M, Heemskerk D, Chau TT, Yen NT, Caws M, Farrar J, Day J.
Trials. 2011; 12:26.

Intensified Antituberculosis Therapy in Adults with Tuberculous Meningitis
A. Dorothee Heemskerk, Nguyen D. Bang, Nguyen T.H. Mai, Tran T.H. Chau, Nguyen H. Phu, Pham P. Loc, Nguyen V.V. Chau, Tran T. Hien, Nguyen H. Dung, Nguyen T.N. Lan, Nguyen H. Lan, Nguyen N. Lan, Le T. Phong, Nguyen N. Vien, Nguyen Q. Hien, Nguyen T.B. Yen, Dang T.M. Ha, Jeremy N. Day,Maxine Caws, Laura Merson, Tran T.V. Thinh, Marcel Wolbers, Guy E. Thwaites, and Jeremy J. Farrar
N Engl J Med. 2016;374:124-34

Therapy for Tuberculous Meningitis
A. Dorothee Heemskerk, Nguyen D. Bang, Guy E. Thwaites
(Response to Reinout van Crevel, Martin J. Boeree, Praveen Sudhindra et al.)
N Engl J Med. 2016; 374:2187-2189

Clinical Outcomes of Patients With Drug-Resistant Tuberculous Meningitis Treated With an Intensified Antituberculosis Regimen
A. Dorothee Heemskerk, Mai Thi Hoang Nguyen, Ha Thi Minh Dang, Chau Van Vinh Nguyen, Lan Huu Nguyen, Thu Dang Anh Do, Thuong Thuy Thuong Nguyen, Marcel Wolbers, Jeremy Day, Thao Thi Phuong Le, Bang Duc Nguyen, Maxine Caws, and Guy E. Thwaites
Clin Infect Dis. 2017; May 4

Statistical analysis plan for the 05TB study (ISRCTN61649292)
"Intensified treatment with high dose rifampicin and levofloxacin compared to standard treatment for adult patients with tuberculous meningitis (TBM-IT)"

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Evaluation of GeneXpert MTB/RIF for Diagnosis of Tuberculous Meningitis

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Tuberculous meningitis (TBM) is the most severe form of tuberculosis. Microbiological confirmation is rare, and treatment is often delayed, increasing mortality and morbidity. The GeneXpert MTB/RIF test was evaluated in a large cohort of patients with suspected tuberculous meningitis. Three hundred seventy-nine patients presenting with suspected tuberculous meningitis to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, between 17 April 2011 and 31 December 2012 were included in the study. Cerebrospinal fluid samples were tested by Ziehl-Neelsen smear, mycobacterial growth indicator tube (MGIT) culture, and Xpert MTB/RIF. Rifampin (RIF) resistance results by Xpert were confirmed by an MTBDR-Plus line probe assay and all positive cultures were tested by phenotypic MGIT drug susceptibility testing. Overall, 182/379 included patients (48.0%) were diagnosed with tuberculous meningitis. Sensitivities of Xpert, smear, and MGIT culture among patients diagnosed with TBM were 59.3% (108/182 [95% confidence interval {CI}, 51.8 to 66.5%]), 78.6% (143/182 [95% CI, 71.9 to 84.3%]) and 66.5% (121/182 [95% CI, 59.1 to 73.3%]), respectively. There was one false-positive Xpert MTB/RIF test (99.5% specificity). Four cases of RIF resistance (4/109; 3.7%) were identified by Xpert, of which 3 were confirmed to be multidrug-resistant (MDR) TBM and one was culture negative. Xpert MTB/RIF is a rapid and specific test for the diagnosis of tuberculous meningitis. The addition of a vortexing step to sample processing increased sensitivity for confirmed TBM by 20% ($P = 0.04$). Meticulous examination of a smear from a large volume of cerebrospinal fluid (CSF) remains the most sensitive technique but is not practical in most laboratories. The Xpert MTB/RIF represents a significant advance in the early diagnosis of this devastating condition.

Tuberculous meningitis (TBM) is the most devastating consequence of infection with *Mycobacterium tuberculosis*. Approximately a third of patients die soon after presenting to hospital, and many of those surviving are left with severe neurological sequelae (1, 2). In patients with HIV coinfection, mortality exceeds 60% (3). Early diagnosis and treatment for TBM have been shown in numerous studies to be the best predictor of survival (4–8). However, many patients are diagnosed late because initial signs are aspecific, and rapid and sensitive diagnostic tests are lacking. Many patients are initially treated empirically with broad-spectrum antibiotics until clinical deterioration warrants adjustment of the differential diagnosis (9). In low-resource settings, limited access to health care, limited diagnostic capacity, and economic constraints frustrate early treatment initiation. In high-resource settings, clinical suspicion is often low, and lack of recognition may lead to treatment delay. A microbiologically confirmed TBM diagnosis is rare in most laboratories. Ziehl-Neelsen (ZN) microscopy staining of cerebrospinal fluid (CSF) is the most widely applied rapid diagnostic technique; however, sensitivity for TBM rarely exceeds 20% (10). Previous work has shown that testing a large volume (>7 ml) of CSF and meticulously examining ZN slides for up to 30 min before recording a negative result improve smear and culture sensitivity significantly (11). However, in our experience, this time investment is not feasible in most busy routine diagnostic laboratories, where it has been shown increasing the examination time of sputum smears to just 10 min can increase case detection by up to 70% compared with examination times routinely applied (12–14). Liquid culture techniques, including the mycobacterial growth indicator tube (MGIT; Bactec) and the mycobacterial observation drug susceptibility assay (MODS) culture offer improved sensitivity over solid culture, to a sensitivity of almost 60% (15). The clinical value of culture techniques is limited to diagnostic confirmation and drug susceptibility testing, because they take 1 to 4 weeks to return a positive result, and negative results cannot be used to exclude a TBM diagnosis. In addition, molecular typing of *M. tuberculosis* isolates can provide insights into epidemiology and immunopathogenesis.

Studies to identify useful biomarkers for TBM in CSF and blood are ongoing. Tests such as the adenosine deaminase assay (ADA) have been evaluated and may be used as an aid in diagnosis; however, they are not specific enough to differentiate TB meningitis from other forms of bacterial meningitis (16, 17).

A meta-analysis of nucleic acid amplification techniques (NAAT) showed wide variability for performance of in-house tests and sensitivity for commercial tests of below 60% (18). Individual reports of the use of in-house PCR have reported higher sensitivities, particularly with multiplex PCR techniques; however, these tests can be difficult to implement with appropriately

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rigorous quality controls in resource-limited, high-burden health care centers, where the need is greatest (16).

The GeneXpert MTB/RIF test (Cepheid) is a closed-cartridge-based system that is easy to operate by minimally trained staff and gives results in approximately 2 h (19). The Xpert MTB/RIF test was approved by the WHO in 2010 for the diagnosis of pulmonary TB following extensive evaluation projects in six countries led by the Foundation for Innovative New Diagnostics (FIND) (20).

The test is based on a real-time heminested PCR test which detects the presence of *M. tuberculosis* complex bacilli (21). By using 5 molecular beacons which span the *rpoB* gene 81-bp rifampin resistance-determining region (RRDR), the test simultaneously determines susceptibility to rifampin, which can be used as a surrogate marker for multidrug resistance (MDR) (21). The closed-cartridge system makes it possible for the assay to be used outside the laboratory environment, and studies assessing biosafety have suggested that the use of Xpert MTB/RIF carries a smaller biohazard risk than smear microscopy (19). The risk of cross-contamination is also reduced with the closed cartridge system (19). The test has shown a sensitivity above 90% for culture-positive tuberculosis, with high specificity in sputum samples. Sensitivity in individuals with HIV coinfection is over 80% (22–24). A recent Cochrane review concluded that the Xpert MTB/RIF as an initial replacement for sputum smear showed a pooled sensitivity of 88% (95% credible interval [CrI], 83 to 92%) and a pooled specificity of 98% (95% CrI, 97 to 99%) (25).

Several studies have reported successful use of the Xpert MTB/RIF test on extrapulmonary samples, with overall sensitivities of over 80% and specificity reaching 100% (26–30). However, the number of CSF samples in these studies combined was low, including only a total of 62 specimens. Due to the urgency of diagnosis in suspected TBM cases because of a rapid decrease of survival chances with the increase of severity (mortality for grade 1 patients is approximately 20%; for grade 3 it reaches 55% [31]), a rapid, accurate diagnostic test which also is able to identify rifampin resistance could have a great impact on survival.

The aim of the present study was to prospectively determine the diagnostic accuracy of Xpert MTB/RIF in a large consecutive series of samples from patients presenting to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, with suspected TBM. A preliminary review of the data in September 2011 resulted in a minor modification of the sample processing, with the addition of a brief vortexing step after addition of sample reagent.

MATERIALS AND METHODS

Ethical approval. Ethical approval for this study was obtained from the Ethical Review Board of the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, and from the Oxford Tropical Research Ethics Committee (OxTREC).

All adult patients (>18 years) presenting to the Hospital for Tropical Diseases (HTD), Ho Chi Minh City, Vietnam between 17 April 2011 and 31 December 2012 with suspected TBM and who underwent lumbar puncture as part of screening for enrollment in a randomized controlled trial of intensified treatment for TBM were included in the study. The full protocol of that trial is reported elsewhere (International Standard Randomized Controlled Trial Number ISRCTN61649292) (32). At HTD, clinicians are encouraged to draw at least 8 ml of CSF when possible, in order to improve microbiological confirmation rates (11). Approximately 1 ml of sample is sent to microbiology and biochemistry laboratories, and the remainder is sent to the TB laboratory. Upon receipt in the TB laboratory, CSF samples were centrifuged at 4,000 × *g* for 15 min. Supernatant was removed to leave a 0.5-ml deposit, which was then used for Ziehl-Neelsen smear preparation (100 μl), inoculation of MGIT culture (100 μl), and Xpert testing (200 μl). The remaining deposit was stored at −20°C. All tests were performed by one of three technicians highly experienced in microbiological tests for TBM diagnosis. Clinical data and results of biochemical investigations were not available to the technicians at the time of the test; technicians were aware of smear results.

Ziehl-Neelsen smear. Ziehl-Neelsen smears were prepared using standard methods with two modifications. First, the smear was layered, with two drops of CSF deposit applied. The layered smear was then stained according to standard procedures. Second, the ZN smear was meticulously examined for up to 30 min under a ×1,000 magnification before being recorded as negative. Observation of a single acid-fast bacillus was considered a positive result (11).

Xpert MTB/RIF. A 200-μl portion of the deposit was resuspended in phosphate-buffered saline to a 500-μl volume. The sample reagent supplied with the test (1.5 ml) was then added. Prior to August 2011, the mixture was then shaken by hand according to test instructions. Following a preliminary review of the Xpert data from 1 August, the mixture was vortexed for 30 s to ensure all bacteria were resuspended. The sample was left to stand for 15 min, as per the manufacturer's instructions, with intermittent manual shaking. The solution was then transferred to the Xpert cartridge using a Pasteur pipette, and the cartridge was loaded onto the Xpert machine for analysis. Results are reported as positive or negative for *M. tuberculosis*. Positive results were placed in one of four categories; very low, low, medium, or high. Rifampin resistance results were reported as susceptible or resistant.

MGIT culture. A 100-μl portion of the deposit was used to inoculate a MGIT tube containing 0.8 ml MGIT supplement (PANTA antibiotics [polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin] and growth supplements). MGIT tubes were incubated in a MGIT 960 machine until they were automatically identified as positive or for 56 days. All positive cultures were tested for susceptibility to rifampin, isoniazid, streptomycin, and ethambutol using a Bactec MGIT SIRE kit (Becton, Dickinson) according to the manufacturer's instructions (33).

Line probe assay. Cases of rifampin resistance detected by Xpert were confirmed using the MTBDRplus line probe assay (Hain Lifesciences, Germany) (34) on DNA extracted from a positive MGIT culture isolated from the same CSF sample. DNA was extracted from positive MGIT cultures using the cetyltrimethylammonium bromide (CTAB) method (35), and the purified DNA was then used for the MTBDRplus test using the manufacturer's instructions (36).

Other investigations. All patients underwent routine investigations for diagnosis of meningitis, including CSF biochemistry, cell counts, India ink stain for fungi, Gram stain, culture (on blood, chocolate, MacConkey, and Sabouraud dextrose agar), viral PCR (for herpes simplex virus [HSV] and varicella zoster virus [VZV]), and IgM and IgG serology for Japanese encephalitis (JE).

Diagnostic classification. For this study, patients were classified as having TBM if no other diagnosis was made and the attending physician made the decision to treat for TBM based on the clinical algorithm in Table 1 . In addition, patients diagnosed with TBM were classified as having definite, probable, or possible TBM using this standardized case definition (37). Xpert MTB/RIF results were not included in the case definition, because it was the test under evaluation. Definite TBM was defined as a clinical syndrome consistent with TBM, with acid-fast bacilli seen on CSF smear or *M. tuberculosis* isolated in CSF MGIT culture. Patients in the "probable TBM" group had a diagnostic score of 10 or more without cerebral imaging (MRI or CT scan) or 12 or more with cerebral imaging, with at least 2 points from CSF or cerebral imaging criteria. Patients in the "possible TBM" group had a diagnostic score of between 6 and 9 if cerebral imaging was not performed or between 6 and 11 if cerebral imaging was performed (37). All patients who did not meet the criteria or did not receive treatment for TBM and received an alternative discharge diagnosis were classified as not having TBM.

TABLE 1 Clinical case definition[a]

Category (maximum category score)	Criterion	Diagnostic score
Clinical criteria (6)	Symptom duration of more than 5 days	4
	Systemic symptoms suggestive of tuberculosis (one or more of the following): wt loss (or poor wt gain in children), night sweats, or persistent cough for more than 2 weeks	2
	History of recent (within the past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
	Focal neurological deficit (excluding cranial nerve palsies)	1
	Cranial nerve palsy	1
	Altered consciousness	1
CSF criteria (4)	Clear appearance	1
	Presence of 10–500 cells per μl	1
	Lymphocytic predominance (>50%)	1
	Protein concn greater than 1 g/liter	1
	CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concn less than 2.2 mmol/liter	1
Cerebral imaging criteria (6)	Hydrocephalus	1
	Basal meningeal enhancement	2
	Tuberculoma	2
	Infarct	1
	Precontrast basal hyperdensity	2
Evidence of tuberculosis elsewhere (4)	Chest radiograph suggestive of active tuberculosis: signs of tuberculosis = 2; miliary tuberculosis = 4	2/4
	CT/MRI/ultrasound evidence for tuberculosis outside the CNS	2
	AFB identified or *Mycobacterium tuberculosis* cultured from another source—i.e., sputum, lymph node, gastric washing, urine, blood culture	4
	Positive commercial *M. tuberculosis* NAAT from extraneural specimen	4
Exclusion of alternative diagnoses	An alternative diagnosis must be confirmed microbiologically (by stain, culture, or NAAT when appropriate), serologically (e.g., syphilis), or histopathologically (e.g., lymphoma); the list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include pyogenic bacterial meningitis, cryptococcal meningitis, syphilitic meningitis, viral meningoencephalitis, cerebral malaria, parasitic or eosinophilic meningitis (*Angiostrongylus cantonensis*, *Gnathostoma spinigerum*, toxocariasis, cysticercosis), cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging), and malignancy (e.g., lymphoma)	The individual points for each criterion (one, two, or four points) were determined by consensus and by considering their quantified diagnostic value as defined in studies.
Clinical entry criteria	Symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy	
Tuberculous meningitis classification		
Definite tuberculous meningitis (patients should meet one set of criteria)	Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF, *M. tuberculosis* cultured from the CSF, or a CSF positive commercial nucleic acid amplification test	
	Acid-fast bacilli seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy)	
Probable tuberculous meningitis	Clinical entry criteria plus a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnoses; at least 2 points should come from either CSF or cerebral imaging criteria	
Possible tuberculous meningitis	Clinical entry criteria plus a total diagnostic score of 6–9 points (when cerebral imaging is not available) or 6–11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses; possible tuberculosis cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging	
Not tuberculous meningitis	Alternative diagnosis established, without a definitive diagnosis of tuberculous meningitis or other convincing signs of dual disease	

[a] Modified with permission from reference 3. CNS, central nervous system; TST, tuberculin skin test; IGRA, interferon-gamma release assay; NAAT, nucleic acid amplification test; AFB, acid-fast bacilli; CT, computed tomography; MRI, magnetic resonance imaging.

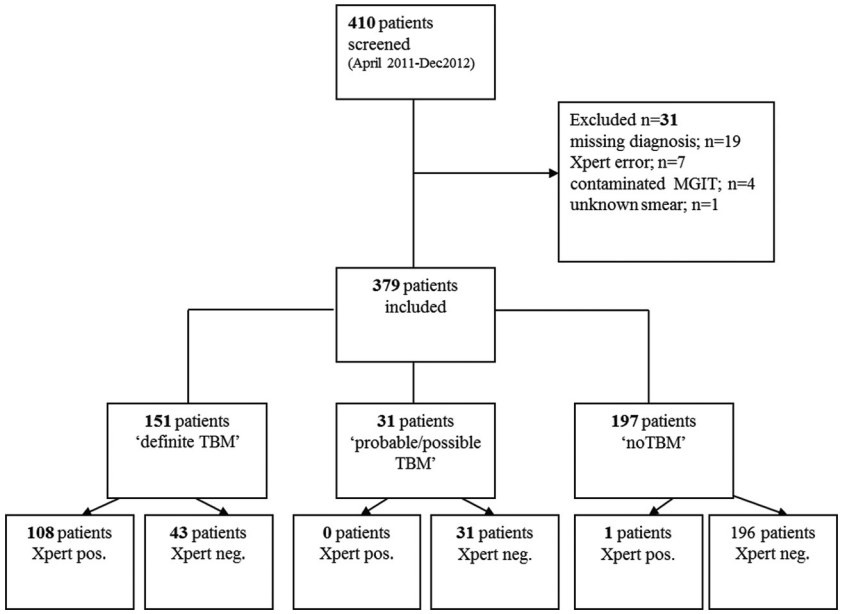

FIG 1 Flow chart of diagnosis for patients included in the study, showing final TBM diagnosis and Xpert MTB/RIF results. Neg., negative; pos., positive.

Statistical analysis. Sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals were calculated. The proportion of positive results for each test (smear, MGIT culture, and Xpert MTB/RIF) was compared using McNemar's test for paired samples. To determine if the introduction of a vortexing step after addition of the sample reagent altered sensitivity of the Xpert test, we also analyzed sensitivity and specificity in samples processed before and after 1 August 2011. The sensitivity of Xpert MTB/RIF stratified by CSF volume was also analyzed.

All statistical analyses were done using R version 2.15.1 (The R foundation for Statistical Computing) with the package epiR.

RESULTS

A total of 410 patients presented to the Hospital for Tropical Diseases with suspected TBM during the study period. A total of 31 patients were excluded: 19 for whom no final diagnosis could be made or clinical information was missing, 7 with an Xpert "error" result, 4 with contaminated cultures, and 1 with an unknown smear result. Thus, 379 eligible patients were included in the analysis. Of these, 151 were finally classified as having definite TBM, 18 as having probable TBM, 13 as having possible TBM, and 197 as not having TBM (Fig. 1).

Patients in the "not TBM" group were diagnosed with viral meningoencephalitis ($n = 95$), bacterial meningitis ($n = 41$), eosinophilic meningitis ($n = 15$), cerebral vascular event ($n = 12$), cryptococcal meningitis ($n = 10$), sepsis ($n = 9$), pneumonia ($n = 7$), cerebral toxoplasmosis ($n = 2$), psychiatric disorder ($n = 1$), cerebral tumor ($n = 1$), prolonged fever of unknown origin ($n = 1$), progressive multifocal leukoencephalopathy ($n = 1$), dengue ($n = 1$), and cerebral abscess ($n = 1$).

Overall, 79/379 (20.8%) patients were HIV infected, 108 (28.5%) were not HIV infected, and 192 (50.7%) had an unknown HIV status (i.e., they declined consent to an HIV test or were discharged before testing).

Of those classified as having definite, probable, or possible TBM ($n = 182$), 66 (36.3%) were HIV infected, 94 (52.6%) were not HIV infected, and 22 (12.1%) had an unknown HIV status.

Diagnostic accuracy for TBM. Overall, the sensitivity of Xpert was 59.3% (108/182; 95% confidence interval [CI], 51.8 to 66.5) compared to clinical diagnosis of TBM (definite, probable, and possible TBM). Specificity was 99.5% (95% CI, 97.2 to 100).

The sensitivity of smear relative to final clinical diagnosis was 78.6% (143/182 [95% CI, 71.9 to 84.3]), and that of MGIT culture was 66.5% (121/182 [95% CI, 59.1 to 73.3]) (Table 2). Since smear and MGIT culture were the reference microbiological tests for diagnosis of TBM, specificity of these tests could not be determined; however, all patients positive by smear or MGIT had a clinical picture consistent with TBM and had no other organisms isolated from the CSF.

The sensitivity of Xpert MTB/RIF relative to smear was 73.4% (105/143 [95% CI, 65.4 to 80.5]), and that relative to MGIT sensitivity was 85.1% (103/121 [95% CI, 77.5 to 90.9]).

The sensitivity of Xpert MTB/RIF relative to clinical diagnosis was significantly lower than the sensitivity of smear relative to clinical diagnosis (−19.3%; $P < 0.001$) and slightly lower than that of MGIT culture relative to clinical diagnosis (−7.2%; $P =$

TABLE 2 Results of smear, MGIT culture, and Xpert MTB/RIF testing by final diagnosis

Test	Result	No. (%)		
		TBM	Not TBM	Total
Xpert MTB/RIF	Positive	108 (59.3)	1 (0.5)	109
	Negative	74 (40.6)	196 (99.5)	270
	Total	182 (100)	197 (100)	379
Ziehl-Neelsen smear	Positive	143 (78.6)	0	143
	Negative	39 (21.4)	197 (100)	236
	Total	182 (100)	197 (100)	379
MGIT culture	Positive	121 (66.5)	0	121
	Negative	61 (33.5)	197 (100)	258
	Total	182 (100)	197 (100)	379

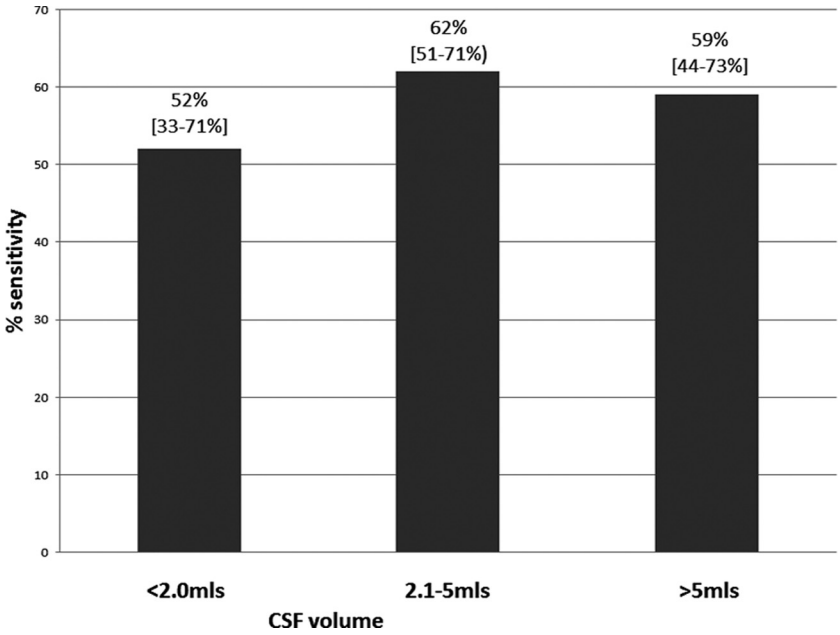

FIG 2 Sensitivity of Xpert MTB/RIF by volume of CSF processed for TB testing.

0.024). The sensitivities of smear and MGIT culture were also significantly different (−12.1%; $P < 0.001$).

The positive and negative predictive value of Xpert against final clinical diagnosis of TBM were 99.1% (108/109 [95% CI, 95.0 to 100]) and 72.5% (196/270 [95% CI, 66.9 to 77.8]), respectively.

Diagnostic accuracy with addition of a vortexing step. Prior to 1 August 2011, there were 48 patients included in the study, 26 of whom were finally diagnosed with TBM and 22 diagnosed as not having TBM. The sensitivity of Xpert for these samples was 50.0% (13/26 [95% CI, 29.9 to 70.1]), and that of smear was 88.5% (23/26 [95% CI, 69.8 to 97.6]). MGIT culture had a sensitivity of 57.7% (15/26 [95% CI, 36.9 to 76.6]). The sensitivity of Xpert for the "definite TBM" result was 54.2% (13/24 [95% CI, 32.8 to 74.4]).

After the introduction of the vortexing step on 1 August 2011, 331 patients were included in the study. Of these, 156 were finally classified as TBM cases and 175 as not having TBM. The sensitivity of Xpert for these samples was 60.9% (95/156 [95% CI, 52.8 to 68.6]), and the sensitivities of smear and MGIT, respectively, were 76.9% (120/157 [95% CI, 69.5 to 83.3]) and 67.9% (106/156 [95% CI, 60.0 to 75.2]). The sensitivity of Xpert for the "definite TBM" result was 74.8% (95/127 [95% CI, 66.3 to 82.1]).

The increase in sensitivity of Xpert for the diagnosis of definite TBM with the addition of the vortexing step to sample processing was 20.6% ($P = 0.04$).

Diagnostic accuracy of Xpert MTB/RIF by CSF volume. The volume of CSF received in the TB laboratory was recorded. Of all 379 CSF samples received, 65 (17.2%) were low volume (≤2.0 ml), 230 (60.6%) were medium volume (2.1 to 5.0 ml), and 84 (22.2%) samples were high volume (>5 ml). The sensitivities of Xpert MTB/RIF were 51.7% (15/29) (95% CI, 32.5 to 70.6) for low-volume samples, 61.5% (64/104) (95% CI, 44.2 to 73.0) for medium-volume samples, and 59.2% (29/49) (95% CI, 44.2 to 73.0) for high-volume samples (Fig. 2). Although the sensitivities for medium- and high-volume samples were greater than those for low-volume samples, this difference did not reach statistical significance ($P = 0.341$).

Qualitative estimation of bacterial load. The majority of Xpert results were categorized by Xpert as very low (54/109; 49.5%) or low (46/109; 42.2%), with 9 medium results (8.3%). Xpert did not report a high bacterial load for any CSF sample.

Diagnostic accuracy by HIV status. Sensitivity of Xpert for TBM against clinical diagnosis was significantly higher for HIV-infected patients (odds ratio = 4.01 [95% CI, 3.65 to 4.36; $P < 0.001$]) than for non-HIV-infected patients.

Among HIV patients, sensitivity was 78.8% (52/66 [95% CI, 77.6 to 79.7]), while it was 47.9% (45/94 [95% CI, 47.0 to 48.7]) in non-HIV-infected patients (Fig. 3).

Detection of rifampin resistance. Rifampin resistance was detected in four cases during the study by Xpert MTB/RIF. In three cases, the result was confirmed to be MDR TBM by an MTBDRplus line probe assay performed on DNA extracted from a positive MGIT culture. One case did not have a positive MGIT culture result. Xpert testing for rifampin resistance showed an "indeterminate" result in two cases. In one case, rifampin resistance was detected using the MGIT SIRE kit. Overall, phenotypic drug resistance testing of all MGIT-positive cultures using the MGIT SIRE kit showed 104 rifampin (RIF)-susceptible results and 5 RIF-resistant cases, 3 of which were detected by Xpert MTB/RIF.

However, it is not possible to draw robust conclusions about the sensitivity of Xpert for the diagnosis of MDR TBM given the low prevalence of MDR TBM in this study.

DISCUSSION

We have shown that Xpert MTB/RIF is a rapid, specific test for the diagnosis of TBM. As with other tests for TBM, a negative result cannot exclude a diagnosis of TBM. While smear microscopy is a more sensitive test in our laboratory, this exceptional sensitivity compared to contemporary published reports from other laboratories is consistent with early reports of TBM diagnosis using

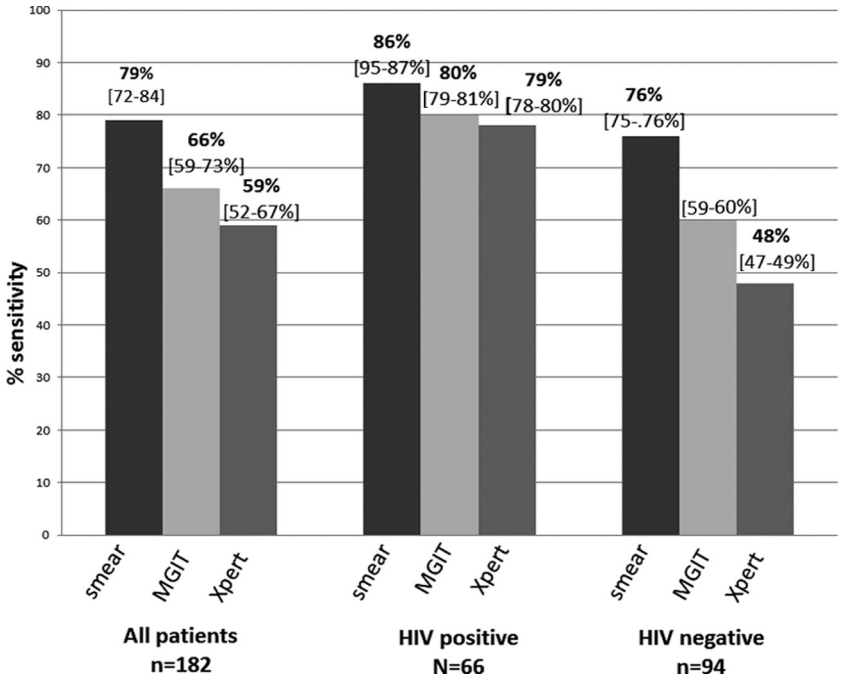

FIG 3 Sensitivities of ZN smear, MGIT culture, and Xpert MTB/RIF against the clinical gold standard for the diagnosis of TB meningitis in all patients and by HIV status. Values in brackets are 95% confidence intervals.

smear (38) and previous publications from our laboratory. We believe that this exceptional sensitivity depends upon the meticulous examination of individual slides for 30 min by a highly skilled and experienced technician. This may be difficult to replicate outside a dedicated research setting due to the work burden in public health laboratories of resource-limited countries (11). The lower sensitivity of Xpert MTB/RIF compared to this meticulous smear process raises an important question regarding the inability of Xpert to detect bacilli visualized on the slide in some samples. Generally it is accepted that the lower limit of detection for standard sputum smear is around 10,000 CFU/ml, whereas that of Xpert MTB/RIF is reported to be 100 CFU/ml. PCR inhibitors in the CSF are unlikely to be the culprit; the Xpert MTB/RIF test contains an internal processing and amplification control (*Bacillus atrophaeus* subsp. *globigii* spores) which should lead to an error result if inhibitors are present in the sample. An alternative explanation may be the fact that unlike nonautomated PCR tests, the Xpert MTB/RIF depends upon capture of intact bacilli from the sample within the cartridge, and it is probable given the reported limits of detection that not all bacilli are captured and lysed during the process. Therefore, in high-volume laboratories with low sensitivity for CSF smear microscopy, Xpert MTB/RIF is likely to substantially improve the diagnostic confirmation of TBM, since it is less dependent on the skill and time of individual technicians.

A limitation of this study regarding the comparison of the sensitivities of Xpert MTB/RIF and MGIT culture is the difference in volumes of CSF deposit used for each test (200 μl for Xpert versus 100 μl for MGIT), which is likely to have decreased the sensitivity of MGIT culture in comparison with Xpert MTB/RIF. However, MGIT culture is not directly useful in making a decision to treat for TBM due to the time required for a positive result; TBM is a medical emergency, and delayed treatment is strongly associated with mortality in every case series. Further comparative study of the optimal sampling processing and inoculation volumes for each test to maximize early diagnosis while also obtaining *M. tuberculosis* isolates for drug susceptibility testing (DST) is required.

The sensitivity of Xpert reported here is similar to the sensitivity of other molecular techniques for TBM diagnosis. Xpert has two significant advantages: the closed-cartridge-based format and the ability to simultaneously detect *M. tuberculosis* and RIF resistance. The cartridge-based format removes the need for manual DNA extraction processing, and the closed system dramatically reduces any potential for cross-contamination of samples with PCR amplicons. The addition of a brief vortexing step after addition of the sample reagent improved sensitivity of Xpert in these paucibacillary samples, and further optimization of sample processing for extrapulmonary samples may be required to improve detection rates. The overall increase in sensitivity for TBM was 10%, with a 20% increase for definite TBM cases ($P = 0.04$).

The Xpert test system depends upon capture and lysis of whole bacilli (21), and therefore, as for other microbiological tests for TBM, high volumes (>7 ml) of CSF are crucial to achieving high sensitivity (11). Bacterial loads are higher in HIV-infected TBM patients, and this is reflected in the higher sensitivity for HIV-associated TBM of all the tests (Fig. 2); therefore, settings with a lower HIV prevalence among TBM patients will have correspondingly lower TBM confirmation rates. This is the inverse of the situation with pulmonary TB, where HIV-positive individuals with TB are less likely to be smear positive.

The costs of smear microscopy are substantially lower than the costs of an Xpert MTB/RIF test (consumable and reagent costs, approximately $2 [U.S. dollars] versus $15), but the hands-on time required to achieve high sensitivity in smear testing is greater (approximately 40 min for smear versus 20 min for Xpert). Additionally, in four cases, Xpert detected rifampin resistance within 2.5 h; we were unable to confirm rifampin resistance in one of

these cases due to negative culture. Rapid detection of drug resistance in the paucibacillary CSF has been a major challenge to improving outcome for patients with MDR TBM. Without rapid diagnosis and administration of second-line regimens, mortality is 100% (39). However, rare false-positive results for rifampin resistance have been reported with Xpert (40), and the consequences of mistakenly treating a patient with rifampin-susceptible TBM with weak second-line regimens would be grave. It will be extremely difficult to accumulate sufficient data on MDR TBM diagnosis to demonstrate robustly the accuracy of the test for this condition due to its rarity, and accuracy must be inferred from other paucibacillary forms of TB. Therefore, a rifampin-resistant TBM diagnosis by Xpert should be evaluated in the context of the clinical information and response to treatment and, wherever possible, should be confirmed by a second rapid test, such as a line probe assay. An *M. tuberculosis* isolate remains necessary to confirm susceptibility patterns for all drugs, including rifampin, since Xpert detects *rpoB* mutations, which are present in only 95% of phenotypically rifampin-resistant *M. tuberculosis* isolates (41). Liquid culture methods, where available, have the highest sensitivity and speed for *M. tuberculosis* isolation (42). However, for patients with rifampin resistance detected by Xpert MTB/RIF and a clinical suspicion of MDR TBM, second-line drugs with appropriate CSF penetration should not be withheld until the results from conventional DST become available.

One patient in our cohort had a false-positive result for *M. tuberculosis* detection. This specificity is consistent with results reported for pulmonary TB. The patient was diagnosed with viral meningoencephalitis and did not meet the clinical criteria for TBM (scoring three points for the published case definition; with cranial imaging available, the minimum score required for a TBM diagnosis is six points). The patient was treated with antiviral drugs and antibiotics, but not with antimycobacterial drugs, and made a full recovery. The patient was still alive and well when contacted 10 months after presentation (9 July 2013). Without treatment, TBM is invariably fatal; therefore, the patient could not have had TBM.

In conclusion, the Xpert MTB/RIF test is able to rapidly confirm a diagnosis of TBM with 59% sensitivity and 99% specificity when large volumes of concentrated CSF and an additional vortexing step are used. This represents a significant advance in the early diagnosis of this devastating condition.

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TRIALS

STUDY PROTOCOL Open Access

Intensified treatment with high dose Rifampicin and Levofloxacin compared to standard treatment for adult patients with Tuberculous Meningitis (TBM-IT): protocol for a randomized controlled trial

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Abstract

Background: Tuberculous meningitis is the most severe form of tuberculosis. Mortality for untreated tuberculous meningitis is 100%. Despite the introduction of antibiotic treatment for tuberculosis the mortality rate for tuberculous meningitis remains high; approximately 25% for HIV-negative and 67% for HIV positive patients with most deaths occurring within one month of starting therapy. The high mortality rate in tuberculous meningitis reflects the severity of the condition but also the poor antibacterial activity of current treatment regimes and relatively poor penetration of these drugs into the central nervous system. Improving the antitubercular activity in the central nervous system of current therapy may help improve outcomes. Increasing the dose of rifampicin, a key drug with known poor cerebrospinal fluid penetration may lead to higher drug levels at the site of infection and may improve survival. Of the second generation fluoroquinolones, levofloxacin may have the optimal pharmacological features including cerebrospinal fluid penetration, with a ratio of Area Under the Curve (AUC) in cerebrospinal fluid to AUC in plasma of >75% and strong bactericidal activity against *Mycobacterium tuberculosis*. We propose a randomized controlled trial to assess the efficacy of an intensified anti-tubercular treatment regimen in tuberculous meningitis patients, comparing current standard tuberculous meningitis treatment regimens with standard treatment intensified with high-dose rifampicin and additional levofloxacin.

Methods/Design: A randomized, double blind, placebo-controlled trial with two parallel arms, comparing standard Vietnamese national guideline treatment for tuberculous meningitis with standard treatment *plus* an increased dose of rifampicin (to 15 mg/kg/day total) and additional levofloxacin. The study will include 750 patients (375 per treatment group) including a minimum of 350 HIV-positive patients. The calculation assumes an overall mortality of 40% vs. 30% in the two arms, respectively (corresponding to a target hazard ratio of 0.7), a power of 80% and a two-sided significance level of 5%. Randomization ratio is 1:1. The primary endpoint is overall survival, i.e. time from randomization to death during a follow-up period of 9 months. Secondary endpoints are: neurological disability at 9 months, time to new neurological event or death, time to new or recurrent AIDS-defining illness or death (in HIV-positive patients only), severe adverse events, and rate of treatment interruption for adverse events.

Discussion: Currently very few options are available for the treatment of TBM and the mortality rate remains unacceptably high with severe disabilities seen in many of the survivors. This trial is based on the hypothesis that

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current anti-mycobacterial treatment schedules for TBM are not potent enough and that outcomes will be improved by increasing the CSF penetrating power of this regimen by optimising dosage and using additional drugs with better CSF penetration.

Trial registration: International Standard Randomised Controlled Trial Number ISRCTN61649292

Background

Driven in part by the HIV epidemic, tuberculosis (TB) is a major global health problem. Of all the syndromes caused by *Mycobacterium tuberculosis* (Mtb), tuberculous meningitis (TBM) is the most severe. Almost all patients with untreated TBM die. Since the introduction of antibiotic treatment with streptomycin for TB in the 1950's the death rate has declined [1]. However morbidity and mortality overall are still high. In Vietnam the death rate in HIV negative patients treated according to current guidelines is 25%, and a further 30% of patients suffer long term neurological sequelae [2]. 50% of adult patients who are admitted with TB meningitis in Ho Chi Minh City are HIV positive. HIV significantly worsens outcome, with a mortality rate of 67% [3]. In our previous studies, the majority (75%) of deaths occurred within 1 month of starting treatment, and almost all deaths occurred within 6 months [3]. Across the globe similar high rates of mortality and disability are reported for TBM [4]. Although TBM predominantly occurs in developing countries, 320 TBM cases were reported in the UK in 2007 making it one of the three leading causes of meningitis in the UK. These numbers are likely to rise, due to the global increase of drug resistant TB, increasing use of immunosuppressive therapies, and the HIV epidemic. Along with increased awareness and vigilance for the disease, it is of the utmost importance to improve treatment for TBM based on sound scientific evidence.

Pathogenesis of TBM

The exact pathogenesis is still largely unknown and current knowledge is based on original pathological studies performed in the early 1930's by Rich and McCordock who postulated that the development of TBM is a two step process [1]. The first step is believed to be a short bacteraemia following pulmonary infection that enables the mycobacteria to seed elsewhere in the body, including the meninges and brain parenchyma. Small foci are believed to form subpially or subependymally, the so called Rich foci. The second step is the rupture of these foci in the subarachnoid space, causing the onset of meningitis. Three general processes are thought to cause the subsequent neurological pathology; adhesion formation, obliterative vasculitis, and encephalitis or myelitis. Immunopathology of TBM is still poorly understood. As a result of a large randomized controlled trial in Vietnam [2], guidelines now recommend all patients with TBM should receive corticosteroids. This trial showed a 31% reduction in risk of death. TB treatment is complicated by a significant risk of adverse events, in particular liver toxicity [5]. In the patient group receiving dexamethasone there was a significantly lower incidence of adverse events compared with placebo. Even though adjunctive immunomodulatory treatment has a beneficial impact on outcome, mortality is still high, especially for the intermediate and high grade severity groups (MRC grade 2 and 3) and HIV co-infected patients. The host-response must be supplemented with appropriate anti-mycobacterial agents in order to facilitate rapid bacterial clearing and prevent a cascade of intracerebral events that will lead to clinical deterioration.

Treatment of TBM

There is a lack of good quality evidence on TBM treatment, in particular for the anti-tubercular chemotherapy regimens. In the past decades, no randomized controlled trials have been published which compared different anti-tubercular regimens and there are no good quality cohort studies. Treatment schedules for TBM globally are not uniform and are mostly extrapolated from those used for pulmonary TB. The National Institute for Health and Clinical Excellence UK (NICE) has published guidelines for the UK in collaboration with the British Thoracic Society (BTS) in 2006, acknowledging the lack of evidence NICE classify the recommendation as Level 4 which indicates a weak evidence-base.

The current treatment guidelines for TBM in Vietnam recommend treatment in the intensive phase with rifampicin (10 mg/kg max 750 mg/day), isoniazid (5 mg/kg, max 300 mg/day), pyrazinamide (25 mg/kg, max 2 g/day) and streptomycin (20 mg/kg, max 1 g/day) for 3 months. All drugs are given orally once daily, with the exception of streptomycin which is administered intra-muscularly. This is followed by rifampicin and isoniazid for 6 months in the consolidation phase. In HIV-positive patients, streptomycin is replaced with ethambutol (15-20 mg/kg, max 1.2 g/day). However the variability of CSF penetration of the different first-line TB-drugs may warrant a need for adjustment of these regimens accordingly. In particular, the penetration of rifampicin, the key drug, is poor, as is that of ethambutol [6-10]. The mortality rate of TBM patients may reflect both poor antibacterial

activity of current treatment regimes and poor penetration of these drugs into the central nervous system. For HIV patients the excess death rate may be a reflection of even less efficient uptake of antimycobacterial drugs due to malabsorption and subsequent low drug levels [9-11], combined with a severely impaired immune system. Improving the sterilising power of current therapy may result in improved outcomes of all TBM patients.

We propose a randomised placebo controlled trial to test this hypothesis in patients with TB meningitis. The study will compare standard anti-tuberculous treatment with anti-tuberculous treatment intensified with high dose rifampicin and levofloxacin.

High Dose Rifampicin for TBM

Rifampicin is a semisynthetic derivative of rifamycin and is a key drug in the treatment of all forms of TB, demonstrated by the fact that in tuberculous meningitis resistance to this drug is associated with high rates of relapse and death [12]. Rifampicin is used throughout the whole of the 9 month treatment period in TBM. The recommended dose is 10 mg/kg/day. Dosage is administered according to weight categories. It is not recommended to divide tablets and hence many patients in these weight categories in fact receive less than the recommended 10 mg/kg.

Rifampicin penetrates well into cells, and is active against intra-cellular bacteria, but CSF concentrations are reported to be low [13-15]. There are few data comparing the Area Under the Curve (AUC) in the cerebrospinal fluid and plasma compartments [AUC_c/AUC_p], but the ratio is probably in the order of 10-20%. Penetration may be influenced by the level of damage to the blood brain barrier and the serum protein binding of rifampicin which approaches 80% [14]. The therapeutic range of rifampicin lies between 8 - 24 μg/ml and levels below 4 μr/ml are considered very low [7,9]. Huge inter-individual variation in metabolism and rifampicin drug-levels have been reported, with worrying numbers of patients with low to very low levels. HIV infection has been associated with even lower plasma levels of Rifampicin [10,11]. Consequently CSF levels are expected to be in the low or very low ranges.

Recently an Indonesian study has been published concluding that a dose increase from 10 to 13 mg/kg/day is associated with a 65% increase in mean plasma $AUC_{0\text{-}24\ h}$ and 49% increase in plasma C_{max} without a significant increase in the rate of serious adverse events [16,17]. This study was not powered for outcome, but the increase in the drug levels in the CSF associated with a relatively small increase in the doses are fascinating and may be of clinical importance for TBM patients.

Rifampicin is relatively non-toxic. The most noticeable side effect is red staining of body secretions, also known as the "red man syndrome" [15]. Other side effects include rash, flushing and gastrointestinal disturbances (usually mild). Drug-induced hepatitis (DIH) is a well recognised side-effect of TB treatment, with a frequency of between 5 and 33% [5]. The drugs most usually implicated are isoniazid and pyrazinamide. However, transient elevation of transaminases (and less commonly bilirubin) is reported with rifampicin use. DIH usually responds well to treatment interruption. A gradual sequential re-introduction of each drug is usually tolerated without recurrence of hepatitis [5].

Based on the data presented in this section we propose an increased dose of rifampicin of 15 mg/kg for patients with TBM, to increase serum levels and we anticipate increase levels of rifampicin at the site of infection. With this strategy we aim to improve the sterilising power of the anti tubercular regimen in the brain.

Levofloxacin for TBM

Of the anti-tubercular reserve drugs, fluoroquinolones, in particular levofloxacin, are an attractive candidate in the treatment of TBM. Especially the later generation drugs such as levofloxacin, moxifloxacin and gatifloxacin have improved *in vitro* activity, and there is evidence of good sterilising activity in sputum in pulmonary TB [18,19]. Despite demonstration of *in vitro* activity of various drugs against Mtb, there has been little progress in drug development or assessment of alternative antimycobacterial treatment regimes in TBM [20]. Trials in pulmonary TB however, have demonstrated the safety of prolonged treatment with fluoroquinolones [21,22].

Fluoroquinolones are an attractive option for the treatment of TBM because of their demonstrable *in vitro* activity, tolerability, good bioavailability and ease of administration [23-37]. Our centre recently completed a pharmacokinetic study comparing oral ciprofloxacin (750 mg/12 hours), levofloxacin (500 mg/12 hours) or gatifloxacin (400 mg/24 hours) for the first 60 days in patients with TBM, and examining their pharmacokinetic interaction with rifampicin. We found levofloxacin to have excellent CSF penetration, with AUC_c/AUC_p = 75%. This compared favourably with gatifloxacin (35%) and ciprofloxacin (14%). Of the second generation fluoroquinolones, levofloxacin has the greatest Early Bactericidal Activity (EBA), comparable to that of isoniazid. The MIC of drug sensitive isolates is in the order of 0.25 - 1 μg/ml [34,38]. Plasma levels of levofloxacin in Vietnamese patients are comfortably in excess of this, with $AUC_{0\text{-}12}$ of 80 mg/hr/L (G. Thwaites Personal Communication).

Fluoroquinolone resistance has been identified in strains from Vietnam, but currently is rare in TBM cases (<1%) and less frequent than rifampicin resistance (M. Caws Personal Communication). Levofloxacin has performed well in human studies using surrogate markers of efficacy such as EBA (rate of fall of colony

forming units in sputum) [18]. This is probably a reflection of its favourable pharmacokinetic profile resulting in high plasma and intracellular concentrations.

Levofloxacin has the advantages of a favourable toxicity profile, affordable cost and an extensive amount of available safety data from clinical trials examining its prolonged use in pulmonary TB. We propose levofloxacin as an additional drug in the highly active treatment arm combined with a high dose of rifampicin in this randomised placebo controlled trial.

Hypothesis

Current antimycobacterial regimes are not potent enough to treat TBM effectively, as most of the antimycobacterial drugs have very low cerebrospinal fluid penetration. Increasing levels of effective anti-mycobacterial drugs in the cerebrospinal fluid and hence at the site of infection will we hope improve treatment outcome.

Aims

The primary aim of this study will be to reduce mortality by intensifying the induction phase of anti-tuberculous treatment of TBM. Secondary aims are to assess the effect on morbidity and disability of intensifying standard treatment, to assess the safety and tolerability of the intensified treatment.

Methods/Design

Design

This is a randomized, double blind, placebo-controlled trial with two parallel arms, comparing standard antitubercular treatment for tuberculous meningitis (according to national guidelines) with standard treatment *plus* an increased dose of rifampicin and additional levofloxacin. We aim to enhance the antimycobacterial efficacy of current treatment for TB meningitis in Vietnam by adding levofloxacin 20 mg/kg/day to the intensive phase of treatment and increasing the dose of rifampicin to 15 mg/kg/day during the intensive phase of treatment for the duration of 2 months (Figure 1)

Inclusion and exclusion criteria

All adult patients (aged ≥ 18 years) with a clinical diagnosis of TBM (Additional File 1) presenting to the Hospital for Tropical Diseases (HTD), HCMC, or Pham Ngoc Thach Hospital (PNT), HCMC, will be eligible to enter the study. Exclusion criteria are: a positive CSF Gram or India Ink stain, pregnancy, known hypersensitivity/intolerance to fluoroquinolones or rifampicin, creatinine >3 ULN, laboratory contraindications to antituberculous therapy (bilirubin > 2.5 × ULN, AST or ALT > 5 × ULN), diagnosis of multi-drug resistant TBM or lack of informed consent

Primary endpoint

The primary endpoint will be overall survival, i.e. time from randomization to death during a follow-up period of 9 months. Survivors will be censored at the date they were last known to be alive (i.e. date of last follow-up visit, loss to follow-up or withdrawal).

Secondary endpoints

The secondary endpoints are:

a) neurological disability at 9 months, assessed using the "two simple questions" and Rankin score (Additional File 2).

b) time to new neurological events or death (Neurological events are defined as: any of the following adverse events: cerebellar symptoms, coma, hemiplegia, neurological deterioration, paraplegia, seizures, cerebral herniation or cranial nerve palsy or a fall in Glasgow coma score by ≥ 2 points for ≥ 2 days from highest previously recorded Glasgow coma score).

c) any grade 3 or 4 adverse event (Additional File 3).

d) rate of treatment interruption for adverse events.

e) the rates of asymptomatic transaminitis and symptomatic hepatitis.

f) time to new or recurrent AIDS defining illness or death (in HIV positive patients only).

g) time to undetectable viral load (in HIV positive patients only).

h) CD4 count at completion of therapy (in HIV positive patients only).

Randomization procedure

Randomization will be 1:1 and patients will be stratified according to hospital site (HTD and PNT), HIV status and TBM disease severity at presentation (TBM severity will be graded according to the modified MRC system, Additional File 4). Enrolment logs specific to site, HIV positivity and severity of TBM will be used to assign patients to the next available sequential number within the appropriate stratification group. The assigned number will correspond to two pre-packaged bottles which contain a 2 month supply of additional doses of rifampicin and levofloxacin or visually matched placebos of each. Bottles will be prepared centrally by an unblinded study pharmacist and distributed to the sites in batches as required. Only two central study pharmacists who will hold the master randomization list will know the contents of each bottle. This list will be accessed only in the case of emergency unblinding authorized by an investigator as per standard operating procedures. Within strata, we will use block randomization with variable block size. Stratified randomization will ensure that almost equal numbers of patients with equivalent prognosis are included in the two treatment arms.

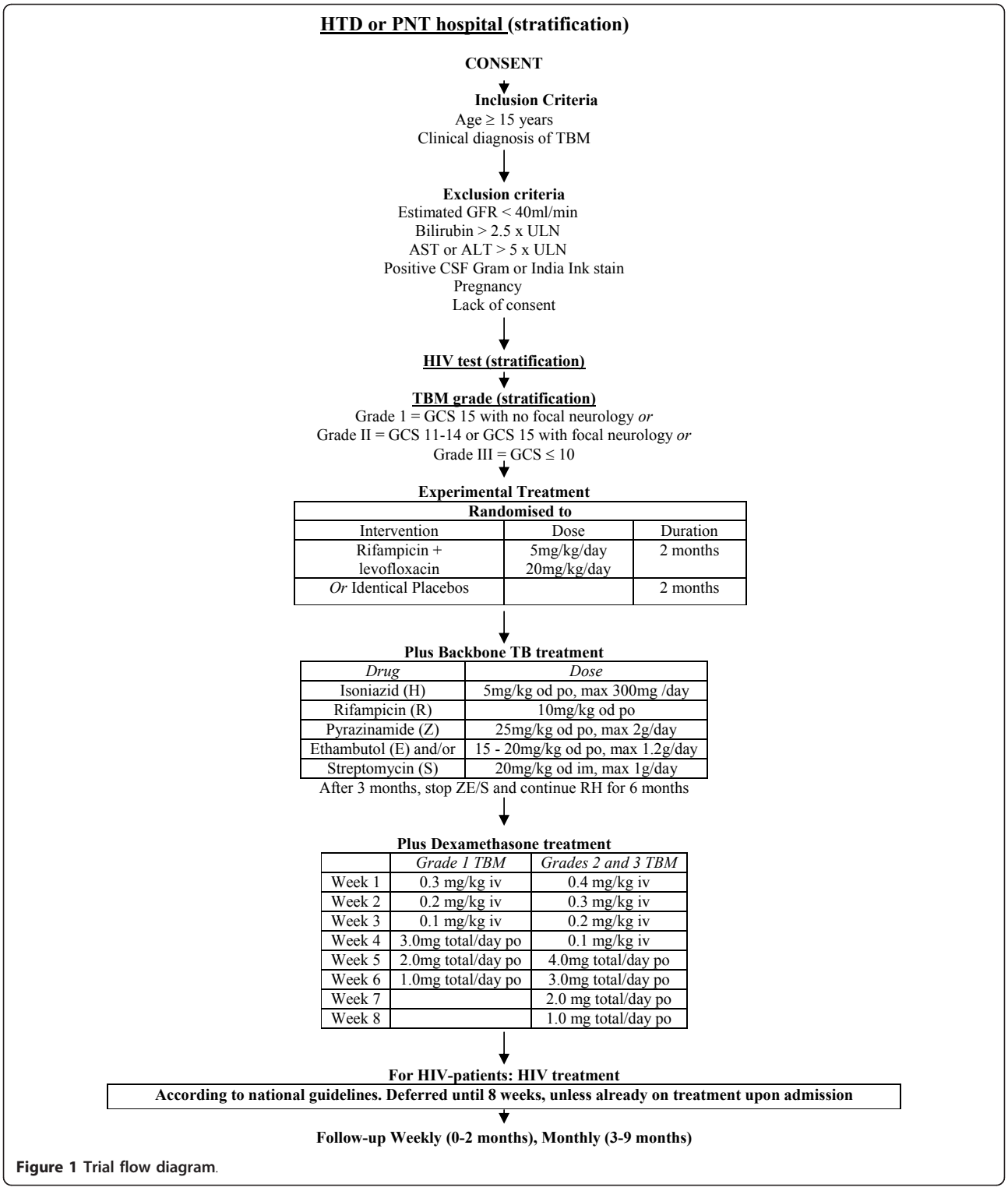

Figure 1 Trial flow diagram.

Enrolment and blinding

For each group, tablets of intensified treatment or placebo will be placed in bottles in coded sealed packages, which are labeled with the randomization number of the patient. Drug appearance and administration schedules will be identical to maintain blinding amongst the attending physicians and nurses. The admitting physician will be responsible for ensuring the patient satisfies the entry criteria, completes informed consent and starts a study drug treatment

package. Clinical details will be recorded in individual patient case record forms (CRFs).

Additional treatment for all TBM patients

All patients will receive backbone treatment with standard antituberculous therapy (Additional File 5) and adjunctive dexamethasone (Additional File 6) on study entry, according to Pham Ngoc Thach Hospital and Vietnamese National TB Programme guidelines. All patients receiving isoniazid will also receive pyridoxine (vitamin B6). Patients, who develop TBM while on treatment for pulmonary TB, will be eligible to enter the study. According to Vietnamese hospital guidelines, these patients will receive TBM-treatment with 5 first line TB-drugs (SRHZE) which will be the "backbone" or standard TB treatment. If patients consent to take part in the trial, they will be randomized to intensified TB treatment or placebo as described previously. If the patient is comatose, the drugs can be given by nasogastric tube.

Second-line antituberculous therapy

Patients with a definite or clinical diagnosis of multidrug resistant (MDR) TBM will be excluded from the trial and referred to the MDR-TBM department for second- line MDR treatment according to NTP guidelines. Change to: Patients who have been randomized and are subsequently diagnosed with MDR-TB will be referred for second-line therapy according to Vietnamese guidelines. They will continue to be followed up in the study and included in the ITT analysis.

Anti-retroviral therapy

Antiretroviral therapy will be provided for HIV infected patients within the current Vietnamese guidelines. Antiretroviral therapy is available free of charge through the US Government PEPFAR programme for in-patients with life-threatening opportunistic infections from 2 weeks after admission. HIV positive patients will be referred to the HIV Outpatient Clinic (OPC). To ensure that treatment naïve HIV-positive patients receive ARV treatment at 8 weeks and continue their treatment, patients will be enrolled either at the hospital OPC or through local specialized OPC services, following standard local practice. For ARV-treatment naïve patients, ARV therapy will be initiated after 8 weeks of TB therapy. This is consistent with the results of the recent trial of immediate or deferred antiretroviral therapy in TB meningitis, carried out by our group and consistent with local practice guidelines (E. Torok Personal Communication). There are currently 4 different treatment schedules for first line ARV treatment in Vietnam, all containing 2 NRTI's and 1 NNRTI. Patients already receiving ARVs at the time of diagnosis of TBM will continue ARV therapy. The majority of patients will be on schedules containing nevirapine (NVP). According to Vietnamese guidelines NVP will be changed to efavirenz for HIV positive patients that require a TB-regimen containing rifampicin.

Reports show good clinical outcome for patients on a 600 mg dose of efavirenz who are on TB-regimens containing rifampicin [39]. Accordingly and following National treatment guidelines, the dose of efavirenz will not be increased for patients on TB-regimens containing rifampicin. Second line ARV treatment is rarely prescribed in Vietnam. Very few patients will have a PI in their treatment schedule. Decisions on dose or schedule adjustments for these patients will be made on an individual basis, following the National guidelines. Liver function tests will be monitored in all patients.

Prophylaxis for opportunistic infections (for HIV positive patients)

Patients will receive prophylaxis for opportunistic infections according to Vietnamese national guidelines. If the CD4 count is less than 200 cells/uL, patients will receive prophylaxis against *Pneumocystis jirovecii* pneumonia and cerebral toxoplasmosis with cotrimoxazole 960 mg/day.

Data on concomitant medications

At each visit, information on other medications, including start dates and indications, will be documented in the case record forms.

Data collection

Baseline evaluation

On admission all patients will have a full clinical assessment and examination to determine TBM MRC grade (Additional File 4), and assess any neurological symptoms and signs. The following laboratory tests will be performed at study entry: haematology (full blood count), biochemistry (total protein, albumin, creatinine and liver function tests), cerebrospinal fluid (cell count, protein, glucose, lactate, Gram stain, Ziehl - Neelsen (ZN) stain, India Ink stain, cryptococcal antigen, bacterial and mycobacterial culture), HIV test. Additional tests for HIV positive patients will include immunology (CD4 count) and virology (confirmatory HIV test, plasma HIV-1 RNA, HbsAg, HBV DNA, HCV Ab test, HCV RNA). A baseline chest radiograph will be performed for all patients. A CT or MRI brain scan will be performed if there is evidence of raised intracranial pressure or focal neurological abnormalities.

In-patient monitoring

Patients will have daily review until discharge from hospital at 2 months (this period may be adjusted according to clinical findings) for neurological, drug-related adverse events (Additional File 3) and new or recurrent

AIDS defining illnesses (HIV positive patients only). In-patients will have weekly routine laboratory monitoring of haematology (full blood count) and biochemistry (creatinine and liver function tests). Cerebrospinal fluid analysis will be done routinely according to local guidelines at 4 and 8 weeks.

A subgroup of patients recruited to the pharmacokinetics study will have additional blood and CSF samples taken. Other investigations may be performed if clinically indicated. Uniform management of patients and recording of data will be ensured by the principal investigator who will make a daily round of all study participants. Following discharge, patients will be followed up as part of the National Tuberculosis Programme. Formal outpatient review will occur monthly until the completion of treatment, at 9 months.

Out-patient monitoring

Out-patients will pay monthly visits to the out-patient department (OPD) for clinical evaluation and laboratory monitoring until completion of treatment at 9 months. Haematology (full blood count) and biochemistry (creatinine, liver function tests) will be checked monthly. Final cerebrospinal fluid analysis will be at 9 months. HIV positive patients will have additional samples taken for immunology (CD4, CD8) and virology (plasma HIV-1 RNA) every 3 months until the end of treatment. A subgroup of HIV positive patients who started ARV-treatment at week 8 of TBM-treatment will have an additional cerebrospinal fluid analysis at the 3 month OPD-visit.

Imaging

Chest and brain imaging will be performed as clinically indicated - i.e. in the event of pulmonary or neurological deterioration.

Clinical trial specimens

All clinical trial specimens will be labeled with the patient's trial number. Samples will be transferred to the laboratories at the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital for initial processing. Investigation results will be issued to the investigators in a timely manner and a hard copy of the results will be retained in the laboratory for verification. Samples will be stored securely in freezers at the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital prior to transfer to the Oxford University Clinical Research Unit for further investigations and long term storage.

Management of adverse events and toxicities

Management of antituberculous toxicity

A symptom checklist will be used to determine clinical toxicity. Routine laboratory tests will be performed weekly as an inpatient and monthly as an outpatient. Clinicians may also request additional tests if clinically indicated. Common side effects of first-line TB-drugs are given in Additional File 3). Therapy may need to be interrupted for severe (grade 3 or 4) adverse events. Once clinical and laboratory features resolve, drugs may be reintroduced sequentially. Details of management are given in Additional Files 7,8,9.

Reporting adverse events

According to the ICH Guidelines for Clinical Safety Data Management: definitions and Standards for Expedited Reporting (1994), a serious adverse event (SAE) is defined as "any untoward medical occurrence that a) results in death, b) is life threatening, c) requires unplanned inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect, e) any other important medical condition, which, although not included in the above, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed."

If the patient dies or experiences an adverse event (serious, grade 3 or 4, or one leading to modification of treatment, see Additional File 3 Common Toxicity Criteria) the investigator should inform the principal investigator as soon as possible and complete the specific case report form. When applicable, adverse events will be treated as per the management guidelines in Appendix 2.0. All SAEs will be recorded on the SAE form and reported to the principal investigator, the Oxford Tropical Research Ethics Board and the Ethical Committee of the Ministry of Health Vietnam within 72 hours of the event. Unblinded adverse event and mortality summaries will be reviewed by the trial's independent Data and Safety Monitoring Committee at regular time points (see section "ethical issues" for details.) If there is a protocol violation for any reason this will be fully recorded. Protocol violations which affect patient safety will be reported to the Oxford Tropical Research Ethics Board and the Ethical Committee of the Ministry of Health Vietnam.

Statistical considerations

Sample size and power calculations

The trial is powered for the primary endpoint, i.e. overall survival during the 9 month follow-up period. Based on previous publications from our research group, the 9-month mortality in the control arm is expected to be 60-65% in HIV-positive and around 25% in HIV-negative TBM patients [2]. Approximately 50% of TBM patients in the participating hospitals are HIV-positive; we therefore expect an overall 9-month mortality rate of around 40% in the control arm of our trial. An absolute risk

reduction of 10% in 9-month mortality from 40% to 30% due to intensified treatment was judged as both realistic and clinically relevant.

Assuming proportional hazards, these mortality estimates translate into a hazard ratio of 0.7 [= log(1-0.3)/log(1-0.4)], i.e. a 30% risk reduction due to intensified treatment on the hazard ratio scale. Based on Schoenfeld's formula, a total of 247 deaths are required to detect a hazard ratio of 0.7 based on a two-sided test at the 5% significance level with 80% power; assuming an overall mortality rate of 35% in the trial, this translates into 706 required patients. In order to account for potential deviations from our assumptions and losses to follow-up, a safety margin of 6% was added to this number leading to a total sample size of 750 patients (375 per treatment group).

HIV-positive TBM patients with a very high mortality are a particularly important subgroup of our study population and we aimed to have sufficient power to also detect a benefit in this subgroup of patients alone. If intensified treatment reduces 9-month mortality by 15% in HIV-positive patients (from 65% to 50%), corresponding to a hazard ration of 0.67, a total of 196 deaths in HIV-positive patients are required to detect this difference with 80% power; approximately 350 HIV-positive patients are necessary to observe 196 deaths during follow-up.

To guarantee both sufficient power in the subgroup of HIV-positive TBM patients and a sufficiently high event rate in the total population, the trial will continue recruitment until both a total of 750 patients and a minimum of 350 HIV-positive patients have been recruited.

Primary and secondary endpoint analysis

The primary endpoint of this trial is overall survival, i.e. time from randomization to death, during the entire follow-up period of 9 months. Overall survival will be analyzed with a log-rank test stratified by HIV status (positive/negative) and TBM disease severity at presentation (modified MRC grade I, II or III). Kaplan-Meier plots and explicit survival estimates at 3, 6 and 9 months of follow-up will also be calculated for the full populations and in the subgroups defined by HIV status and TBM disease severity separately.

In a second stage, overall survival will be modeled using the Cox proportional hazards regression model and the following covariates (in addition to the treatment group): TBM disease severity (grade I, II, or III), HIV status (positive/negative), participating hospital (PNT/HTD), previous TB treatment (yes/no), drug resistance (drug sensitive/MDR-TB/Isoniazid resistant non-MDR). A separate analysis for HIV positive patients only will be performed which will include prior antiretroviral therapy (yes/no), CD4 cell count and log10-HIV viral load at baseline as additional covariates.

The homogeneity of the treatment effect on overall survival in the subgroups defined by TBM grade (I, II, or III), HIV status (positive/negative), prior TBM treatment (yes/no), drug resistance (drug sensitive/MDR-TB/isoniazid resistant non-MDR) respectively, will be examined and tested using tests of interaction between treatment and the grouping variable.

For the secondary endpoints concerning neurological disability, the disability score at month 3, 6, and 9 of follow-up is defined as the higher (worse) of the "simple question" and the Rankin score assessed at that time point as previously described [2]. Disability score will be defined as 4 (worst outcome) if the patient died prior to the respective time point. The score of primary interest is the month 9 score which will be compared between the two arms with the generalized Cochran-Mantel-Haenzsel test as described in Mantel's generalized statistics [40] taking into account that the disability score is ordinal. The test will be stratified by HIV status and TBM disease severity at presentation. Patients lost to follow up will be analyzed according to their last recorded disability status. If the rate of patients lost to follow-up exceeds 10%, we will also perform an alternative analysis based on multiple imputation of missing values.

Time-to-event endpoints, i.e. time to new neurological event or death and time to new or recurrent AIDS defining illness or death (in HIV positive patients only), will be analyzed with a log-rank test, Kaplan-Meier curves and Cox regression models as described for the primary endpoint above.

All reported serious and grade 3&4 adverse reactions will be listed; their overall frequencies and the rate of treatment interruptions due to adverse events will be compared between the two treatment groups using a generalized Cochran-Mantel-Haenszel test stratified by HIV status and TBM disease severity at presentation.

Analysis populations

All patients will be analyzed in the primary analysis according to their randomization arm (intention-to-treat, ITT). The primary endpoint, overall survival, will in addition be analyzed on the per-protocol (PP) population which excluded the following patients: patients with a final diagnosis other than TBM, major protocol violations and those receiving less than 2 months of administration of the randomized study drug for reasons other than death.

Ethical issues

Ethical approval

This protocol, the patient information sheet, the patient consent form has been reviewed and approved by the

Oxford Tropical Research Ethics Committee (OxTREC) and the Institutional Review Boards of the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital. The study and study materials have also been submitted for approval by the Ethical Committee of the Ministry of Health Vietnam.

Informed consent and information sheet

A patient cannot enter the trial without informed consent.

Written informed consent will be sought for all patients entering the trial. When written consent is not possible verbal consent will be considered acceptable in the presence of a witness who can attest to the accurate reading of the informed consent form and the agreement of the patient. The doctor entering the patient into the trial is responsible for obtaining informed consent. If the patient is unconscious, the consent of the relatives or family members is acceptable. If there are no relatives, the consent of two independent physicians will be considered acceptable. In this case consent from the patient will be sought as soon as the patient regains the ability to give or refuse consent.

Withdrawal from the trial

Patients may voluntarily withdraw from the trial for any reason. If this occurs, the trial researchers are under no obligation to provide treatment. The withdrawal of the patient from the trial will not affect their access to the best standard of care within the national health system. Clinical and laboratory assessment should be performed and recorded at the time of withdrawal.

Confidentiality

A unique trial number will be assigned to each patient entering the trial and will be used to identify all laboratory specimens and the case record forms. All records will be stored securely on the wards or in the OUCRU. Clinical information will not be released without written permission of the patient.

Interim analysis and role of the Data and Safety Monitoring Committee (DSMC)

An independent DSMC will oversee the trial. Interim analyses are planned after 20 deaths have been observed, after 6 and 12 months of recruitment and yearly thereafter until the completion of the trial. The DSMC will be provided with unblinded summary tables of grade 3&4 and serious adverse events and an analysis of overall survival. These analyses will be performed by an independent statistician not otherwise involved with the trial.

Based on these data, the committee will make one of the following recommendations:

- Continue the trial without modification
- Continue the trial with modification
- Stop the trial due to safety concerns

Unless the benefit of intensified treatment is shown "beyond reasonable doubt" at an interim analysis, no formal stopping for efficacy is foreseen. The Haybittle-Peto boundary, requiring $p < 0.001$ at interim analysis to consider stopping for efficacy, should be used as a guidance. However, the DSMB recommendation should not be based purely on statistical tables but also requires clinical judgment. As the dissemination of preliminary summary data could influence the further conduct of the trial and introduce bias, access to interim data and results will be confidential and strictly limited to the involved independent statistician and the monitoring board and results (except for the recommendation) will not be communicated to the outside and/or clinical investigators involved in the trial.

Discussion

Currently very few options are available for the treatment of TBM. There are 5 "first-line drugs" and a small number of "second-line drugs". With the exception of fluoroquinolones, the second-line drugs are relatively toxic and apart from ethionamide, cycloserine and some of the fluoroquinolones, penetration into the CSF is poor. Several new agents are now in the early stages of clinical evaluation, but will not be evaluated in treating TBM in the immediate future. The amplification of MDR-TB strains globally and the exceptionally high mortality among MDR-TBM patients are worrying signs of insufficient TB and TBM treatment globally. This trial is based on the hypothesis that current anti-mycobacterial treatment schedules for TBM are not potent enough and that outcomes will be improved by increasing the CSF penetrating power of this regimen by optimising dosage and using additional drugs with better CSF penetration. We acknowledge the fact that this trial is testing this hypothesis by essentially including two interventions in one arm of the trial. From a clinical point of view the main interest of this research is improving treatment, however scientifically it would be satisfying to know, if positive results are observed, to which drug they can be attributed or which modifications are strictly necessary in this treatment regimen.

In order to assess the effect of either intervention alone and the combined effect it is necessary to either perform a series of trials or do a 2×2 factorial trial. In a companion paper to the present study protocol [41] we show that an adequately powered 2×2 factorial design would require an eight-fold increase in sample size that would transform our study protocol from what will be the largest trial ever conducted in TBM to an impossible study. Currently 40% of all adult patients

with TBM die from the disease. In view of this high mortality we argue for a pragmatic approach. The quest for optimal treatment should no longer be postponed. Subsequent trials to further refine the optimal treatment can be initiated if the present working hypothesis proves successful.

Additional material

Additional file 1: Diagnostic criteria for tuberculous meningitis. Diagnosis and grading of tuberculous meningitis, including outcome and disability.

Additional file 2: Outcome and disability grading.

Additional file 3: Toxicity grading and management. Table of common toxicity criteria.

Additional file 4: Modified MRC grading for tuberculous meningitis.

Additional file 5: Standard TBM treatment. First-line antituberculous therapy.

Additional file 6: Dexamethasone therapy.

Additional file 7: Guide to management of toxicities.

Additional file 8: Management of common adverse effects of antituberculous medications.

Additional file 9: Reintroduction of antituberculous therapy. Based on British Thoracic Society Guidelines for chemotherapy and management of tuberculosis (Thorax 1998; 53: 536-548).

Abbreviations

Ab: Antibody; AIDS: Acquired immune deficiency syndrome; ALT: Alanine aminotransferase; ARV: Antiretroviral; AST: Aspartate transaminase; AUC: Area Under the Curve; BTS: British Thoracic Society. CRF: Case record form; CSF: Cerebrospinal fluid; CT: Computed tomography; DIH: Drug Induced hepatitis; DNA: Deoxyribonucleic acid; DSMC: Data and safety monitoring committee; E: Ethambutol; EBA: Early bactericidal Activity; H: Isoniazid; HbsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCMC: Ho Chi Minh City; HCV: Hepatitis C virus; HIV: Human Immunodeficiency virus; HTD: Hospital for Tropical Diseases; ICH: International Conference on Harmonization; MDR TB: Multi-drug resistant tuberculosis; MIC: Minimum inhibitory concentration; MRC: Medical Research Council; MRI: Magnetic resonance imaging; Mtb: Mycobacterium tuberculosis; NICE: National Institute for Health and Clinical Excellence UK; NNRTI: Non- Nucleoase reverse transcriptase inhibitor; NRTI: Nucleoase reverse transcriptase inhibitor; NTP: National Tuberculosis Programme of Vietnam; NVP: Nevirapine; OPC: Out Patient clinic; OPD: Out Patient department; OUCRU: Oxford University Clinical Research Unit; OXTREC: Oxford Tropical Research Ethics Committtte; PNT: Pham Ngoc Thach Hospital for Tuberculosis and Lung Diseases; R: Rifampicin; PEPFAR: President's Emergency Plan for AIDS Relief; RNA: Ribonucleic acid; S: Streptomycin; SAE: Severe adverse event; TB: Tuberculosis; TBM: Tuberculous meningitis; UK: United Kingdom; ULN: Upper limit of normal; US: United States; Z: Pryrazinamide; ZN: Ziehl-Neelsen.

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Authors' contributions

JF, DH, JD, TTHC, NTBY, MC, MW conceived the study. All authors discussed the development of the protocol and the manuscript and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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TRIALS

METHODOLOGY Open Access

Sample size requirements for separating out the effects of combination treatments: Randomised controlled trials of combination therapy vs. standard treatment compared to factorial designs for patients with tuberculous meningitis

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Abstract

Background: In certain diseases clinical experts may judge that the intervention with the best prospects is the addition of two treatments to the standard of care. This can either be tested with a simple randomized trial of combination versus standard treatment or with a 2 × 2 factorial design.

Methods: We compared the two approaches using the design of a new trial in tuberculous meningitis as an example. In that trial the combination of 2 drugs added to standard treatment is assumed to reduce the hazard of death by 30% and the sample size of the combination trial to achieve 80% power is 750 patients. We calculated the power of corresponding factorial designs with one- to sixteen-fold the sample size of the combination trial depending on the contribution of each individual drug to the combination treatment effect and the strength of an interaction between the two.

Results: In the absence of an interaction, an eight-fold increase in sample size for the factorial design as compared to the combination trial is required to get 80% power to jointly detect effects of both drugs if the contribution of the less potent treatment to the total effect is at least 35%. An eight-fold sample size increase also provides a power of 76% to detect a qualitative interaction at the one-sided 10% significance level if the individual effects of both drugs are equal. Factorial designs with a lower sample size have a high chance to be underpowered, to show significance of only one drug even if both are equally effective, and to miss important interactions.

Conclusions: Pragmatic combination trials of multiple interventions versus standard therapy are valuable in diseases with a limited patient pool if all interventions test the same treatment concept, it is considered likely that either both or none of the individual interventions are effective, and only moderate drug interactions are suspected. An adequately powered 2 × 2 factorial design to detect effects of individual drugs would require at least 8-fold the sample size of the combination trial.

Trial registration: Current Controlled Trials ISRCTN61649292

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Background

Tuberculous meningitis (TBM) is the most severe form of *M. tuberculosis* infection, and kills or disables more than half of those affected [1]. Effective new intervention strategies are thus urgently needed. The study hypothesis of a new randomized clinical trial whose protocol is published along with the current manuscript [2] is that current anti-mycobacterial regimes are not potent enough and that increasing the levels of effective anti-mycobacterial drugs in the cerebrospinal fluid will improve clinical outcome. This hypothesis is tested by simultaneously increasing the dose of rifampicin and adding levofloxacin to the standard treatment and performing a two-group comparison of intensified versus standard treatment. This approach allows testing of the primary study hypothesis but it does not allow quantification of the individual effects of each drug or facilitate exploration of potential synergistic or antagonistic interactions between them.

A 2 × 2 factorial design would potentially answer these questions by simultaneously randomizing patients to one of two levels of factor 1 (e.g. standard treatment vs. standard treatment + treatment A [intensified rifampicin]) and to one of two levels of factor 2 (e.g. standard treatment vs. standard treatment + treatment B [levofloxacin]) such that one quarter of the patients receive each of the possible combination treatments. A typical analysis of factorial designs estimates the overall treatment effect of treatment A by pooling across levels of factor 2, i.e. the estimate corresponds to the average of the treatment effects of treatment A in patients randomized to standard treatment for factor 2 and those randomized to treatment B for factor 2, respectively. Crucially, all randomized patients are included in the estimation of the effect of treatment A and, thus, a 2 × 2 factorial design has essentially the same power as a corresponding simple randomized trial which would only randomize factor 1. Factorial designs thus have the potential to answer two (or multiple) questions for the "price" of one and appear to be ideal designs for evaluating combination treatments.

One major complication of studying combination treatments is that they may interact, i.e. that the effect of treatment A is different depending on whether it is added to standard treatment or standard treatment +treatment B (or, equivalently, that the effect of adding both treatments A and B is not equal to the sum of their individual effects). Both synergistic and antagonistic (negative) interactions are possible. While interactions can be studied in the framework of factorial designs, they complicate their analysis and interpretation. In particular, the interpretation of the treatment effect estimate from the standard analysis outlined above is problematic [3-6].

The need to improve outcomes in diseases with high morbidities and mortalities is clear. In rare diseases, such as TBM, the pace of progress is slowed because of the time needed to recruit sufficient numbers of patients to studies powered to appropriate clinical endpoints. Clinical experts may judge that the intervention with the best prospects to improve outcomes is the addition of a combination of several drugs to the standard of care. This new intervention could either be tested with a simple randomized trial of combination therapy versus standard of care or with a factorial design which evaluates each intervention component separately. The objective of the present manuscript is to compare these approaches in terms of their statistical power using our study in TBM as a representative example. We find that in certain situations performing the pragmatic combination trial is the preferred approach.

Methods

Sample size calculation for the new TBM trial

The primary endpoint of our proposed study in TBM is overall survival during a follow-up period of 9 months. We expect a 9-month mortality rate of approximately 40% in the control arm and an absolute risk reduction of 10% (from 40% to 30%) due to intensified combination treatment was judged as both realistic and clinically relevant. Assuming proportional hazards, these mortality estimates translate into a hazard ratio of 0.7 [= log(1-0.3)/log(1-0.4)], i.e. a 30% risk reduction due to intensified treatment on the hazard ratio scale. Using Schoenfeld's formula [7], a total of 247 deaths are required to detect a hazard ratio of 0.7 based on a two-sided test at the 5% significance level with 80% power; assuming an overall mortality rate of 35% in the trial, this translates into a need to enroll 706 patients. In order to account for potential deviations from our assumptions and losses to follow-up, a safety margin of 6% was added to this number leading to a total sample size of 750 patients (375 per treatment group). Further details of the sample size calculation are described in the study protocol [2].

Factorial designs without interactions

We first assumed that the combination treatment effect is as described above, i.e. that the hazard ratio of combination treatment versus control is 0.7, and that there is no interaction present, i.e. that the total combination treatment effect (as measured on the log-hazard ratio scale) is equal to the sum of the individual contributions of treatment A [intensified rifampicin] and B [levofloxacin].

For our investigations we varied the total sample size of a hypothetical factorial trial from the size of the two-group trial (i.e. 750 patients) to 8-fold its size (i.e. 6'000 patients) and assumed that the total observed number

of deaths per 750 included patients was 247 (as in the sample size calculation above). In addition, we varied the contribution of the more potent of the two individual treatments to the combination treatment effect from 50-100%. The analysis was assumed to be a Cox regression analysis with treatment indicators for each treatment as covariates. We then calculated the statistical power of the following comparisons of the factorial design:

- Probability that the two tests for the effects of individual treatment A and B both reach statistical significance.
- Probability that the test of the more potent of the two treatments reaches statistical significance.
- Probability that at least one of the two tests for individual treatment A and B effects reaches statistical significance.
- Probability that the comparison of combination treatment versus standard of care reaches statistical significance in the factorial design.
- Power of a two-arm trial of combination treatment versus standard of care with the same sample size as the factorial design.

Details regarding the power calculation are provided in Additional file 1: appendix. Of note, the formulas in the appendix are approximations. In addition, we performed a simulation study where we estimated exact power based on results from Cox regression analyses of simulated trial data assuming exponentially distributed survival times and averaging results over 10'000 simulated trials for each parameter setting. As results from this simulation study were qualitatively identical to the approximations, the simulation results are not reported here.

Factorial designs with interactions

In a second step, we investigated the impact of interactions assuming that the effect of either treatment A or B alone leads to a reduction in the hazard of 0.84, i.e. identical effects of each drug alone and a combination treatment effect corresponding to a hazard ratio of 0.84 0.84 = 0.7 in the absence of an interaction. For our investigations we varied the total sample size of a hypothetical factorial trial from 4-fold the size of the two-group trial (i.e. 3'000 patients) to 16-fold its size (i.e. 12'000 patients) and varied the strength of the interaction effect from -200% to + 200% of the effect of either drug alone. An interaction of -200% corresponds to an effect of combination treatment of zero, i.e. the effect of either drug vanishes when combined with the other, an interaction of size -100% indicates that either drug alone works as well as their combination, whereas an interaction of +200% corresponds to a strongly synergistic interaction where the combination treatment effect is twice the sum of the individual effects of A and B alone. The analysis was assumed to be a Cox regression analysis with treatment indicators for each therapy as covariates plus an interaction term. For each scenario, we calculated the power of the following comparisons:

- Probability that the interaction test reaches statistical significance and probability that the one-sided p-value is ≤ 10% (indicating mild evidence for an interaction).
- Probability that the main effect corresponding to treatment A reaches statistical significance.
- Power of a two-arm trial of combination treatment versus standard of care with the same sample size as the factorial design.

Of note, the main effect of treatment A corresponds to an averaged effect of treatment A when added to either standard of care or standard of care plus treatment B and may be difficult to interpret clinically.

Other conventions and statistical software

Statistical significance was determined as significance at the one-sided 2.5% significance level. We used one-sided tests in the direction of the true (simulated) effect throughout because power formulas are simpler for the connected rejection areas of one-sided tests. No adjustment for multiple testing was performed. All calculations were performed with the statistical software R version 2.9.1 [8].

Results

Power curves for the factorial design assuming no statistical interactions depending on the sample size and the contribution of the more potent individual treatment are displayed in Figure 1. It is apparent from these curves that if only one drug contributes to the total treatment effect, the power of the factorial design to detect individual treatment effects is essentially equal to the power of the combination treatment trial to detect the combination effect. However, if both drugs contribute to the total effect, power is much diminished. For example, the right lower panel of Figure 1 shows that in order to get 80% power to detect an effect for both individual treatments simultaneously assuming the split-up in effects between the two drugs is between 35:65 to 65:35, one would need an 8-fold increased sample size. Moreover, the figure shows that a factorial trial with suboptimal sample size has a much higher chance of concluding that one of the treatment works than concluding that both jointly work, i.e. that the combination treatment is the optimal treatment, even if both treatments have similar effects. For example, with 1'500 patients and equal effects of both individual treatments, the probability that exactly one of them is significant is 2-times higher than the probability that both jointly reach statistical significance (chances of approximately 50% vs. 25%). Finally, for the comparison

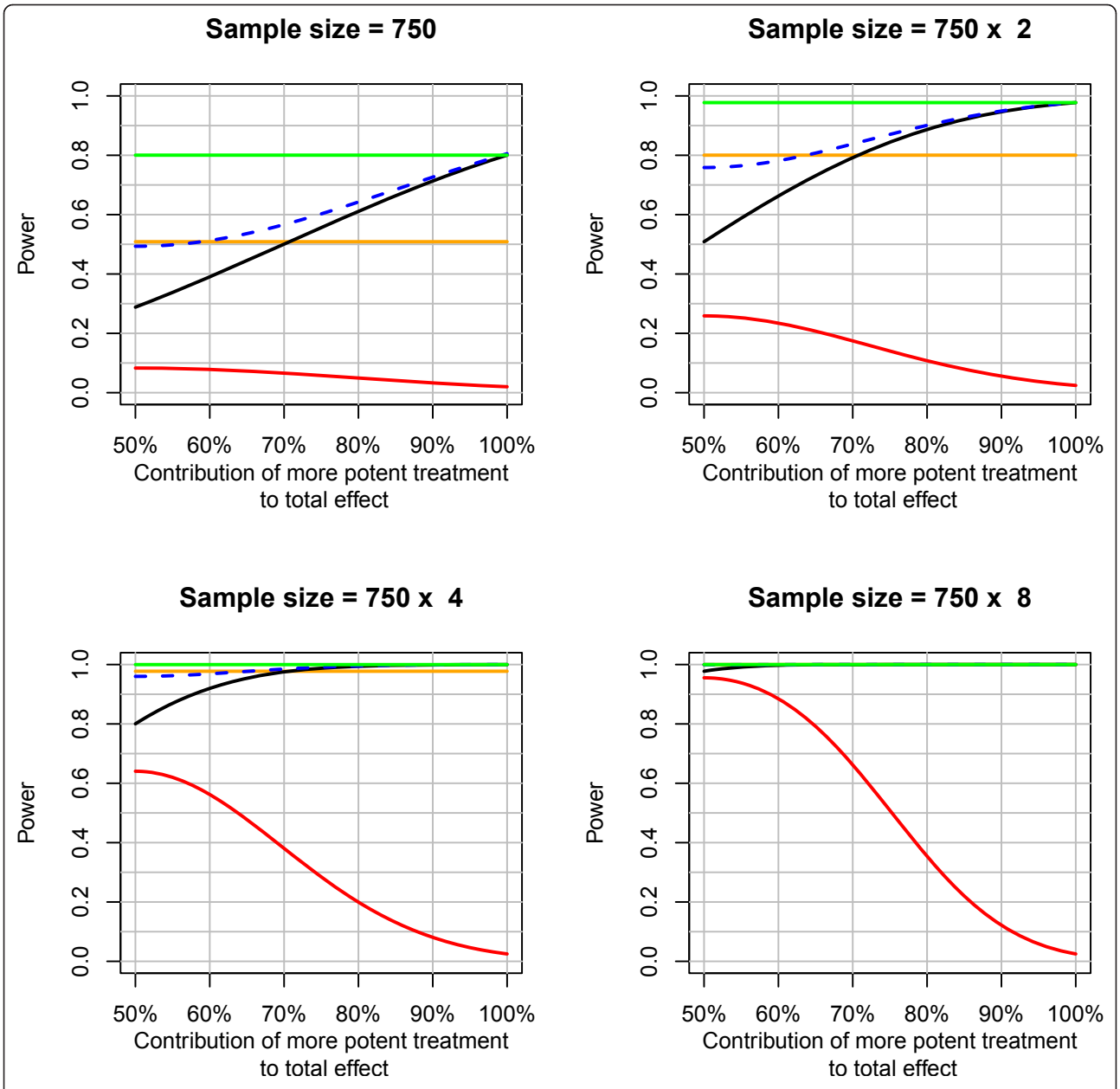

Figure 1 Power curves for 2 × 2 factorial designs without an interaction assuming a hazard ratio of combination treatment versus standard treatment of 0.7 and an overall mortality of 35%. Figure legend text: The black, dashed blue, and red lines correspond to the probability that the more potent individual treatment, at least one of the two treatments or both treatments jointly, respectively, reach statistical significance. The orange and green lines correspond to the probability of a significant difference between combination treatment and standard treatment in the factorial trial and in a 2-group trial of combination treatment versus standard treatment with equal sample size, respectively.

of combination treatment versus control, a factorial trial requires twice the sample size of the combination trial for equal power (ignoring multiple testing issues for the factorial design).

Power curves for the factorial design assuming equal individual treatment effects plus an interaction depending on the sample size and the strength of the interaction are displayed in Figure 2. The curves show that the power of the main effect of the trial is strongly reduced in the presence of a negative interaction. Further, in order to detect an interaction of -100%, i.e. that the effect of combination treatment is equal to either drug alone, with 80% power, one would need 16-fold the sample size of the two-group combination trial. With an 8-fold sample size and a liberal one-sided significance level of 10%, power to detect an interaction of this size would be 76%.

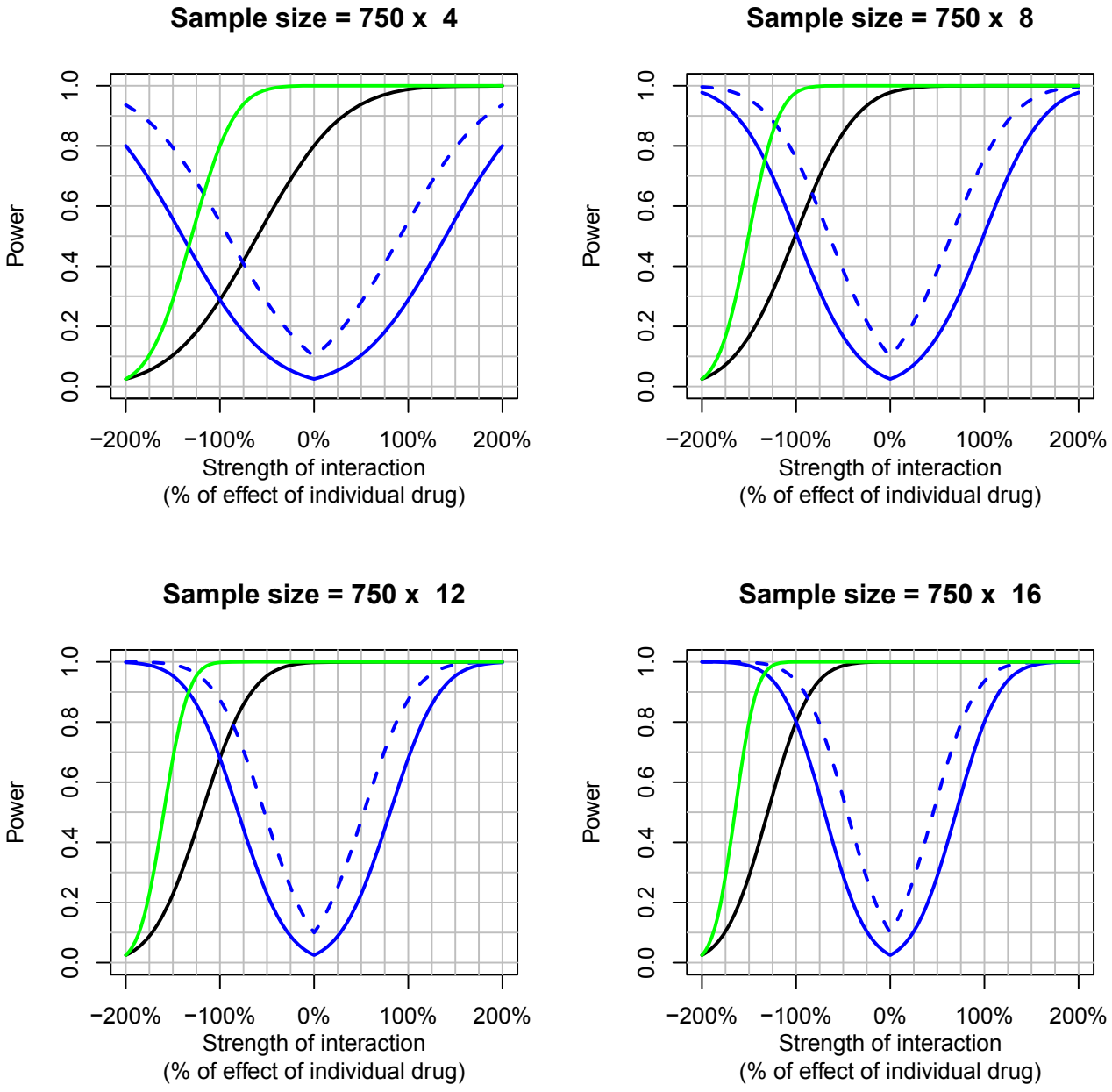

Figure 2 Power curves for 2 × 2 factorial designs with an interaction assuming equal individual contributions from both treatments corresponding to hazard ratios of 0.84 and an overall mortality of 35%. Figure legend text: The blue lines correspond to the power of the interaction test (solid line: one-sided 2.5% significance level, dashed line: one-sided 10% significance level), the black line to the power of the main effect for treatment A, and the green line to the power of a 2-group trial of combination treatment versus standard treatment with equal sample size.

Discussion

We found that a 2 × 2 factorial design powered to detect individual treatment effects would require at least 8-fold the sample size of a two-group combination treatment trial based on the same assumptions. This increase in sample size would guarantee sufficient power to detect both individual treatment effects assuming that the contribution of each individual treatment to the joint effect is at least 35% and that there are no interactions. Moreover, at least an 8-fold increased sample size is required to detect at least mild evidence for a qualitative interaction of -100%, i.e. that either treatment alone has the same effect as the combination. The reasons for this high price for separating out combination effects come from two main sources: First, if there is no interaction and both drugs have equal contributions to the

total effect, their individual effect would be half the combination effect which requires 4-fold the sample size to detect individually (and more than 5-fold the original sample size to detect both jointly with 80% power). A further increase in sample size is mandated to protect against the possibility of unequal contributions of the two drugs. Second, it is well-known that interaction tests lack power, i.e. in order to detect an interaction of the same size as a main effect with equal power, 4-fold the sample size is required [3]. Moreover, modest negative interactions can considerably diminish the power to detect treatment effects in the factorial design even in cases that have little power to detect this interaction [4].

An 8-fold sample size increase, i.e. 6'000 patients in total, would transform our study protocol from what will be the largest trial ever conducted in TBM to an impossible study. Thus, a factorial design would have to make much more aggressive assumptions regarding the individual treatment effects in order to arrive at a more realistic sample size. However, such assumptions would lead to an increased likelihood that the trial is underpowered. Even if either of the two intervention effects reaches significance in such an underpowered study, we have shown that chances are high that substantial interaction effects are not discovered and that only one of the treatments reaches statistical significance even if both are equally effective. An earlier publication showed that typical analysis strategies in factorial designs have a relatively low chance of finding the optimal treatment combination in many situations [5] and these chances are even more diminished if the trial does not even have sufficient power to detect the individual treatment effects.

Based on these findings, we believe that a pragmatic two arm trial of combination therapy versus standard treatment is the design of choice under the following assumptions: First, an adequately powered factorial design which allows separating out individual treatment effects is not feasible due to excessive sample size. Second, both interventions test the same broad study hypothesis and the combination is considered most promising. Third, it is considered likely that either both or neither of the two drugs are effective and at most moderate interactions between the two interventions are expected. As we have seen, in case only one of the two interventions is efficacious and there is no interaction, a factorial trial would be optimal and could indeed deliver two answers for the price of one. Factorial designs and, more generally, fractional factorial designs are very efficient designs to screen several potential interventions many of which are likely inefficacious [9]. Fourth, neither of the two interventions has substantially higher costs or is expected to be much more toxic than the other. Finally, a pragmatic combination trial is unlikely to be acceptable for regulatory drug approval which requires proof of efficacy for each individual component.

Our proposed trial in TBM fulfils all of the above conditions. TBM is a relatively rare disease with a limited number of patients globally, but devastating for those who are affected. Both interventions in our TBM trial test the same broad study hypothesis, i.e. that increasing levels of effective anti-mycobacterial drugs in the cerebrospinal fluid will improve treatment outcome. If this trial is successful, it will likely lead to follow-up trials which may further optimize the anti-tuberculosis treatment. This optimization may be based on the new drugs which are currently under development, e.g. TMC207 or PA824. One could in principle also revisit the question whether both components of the intensified treatment are necessary, perhaps based on a 3-arm non-inferiority trial of individual interventions versus combination therapy. However, such a series of simple (superiority and non-inferiority) trials would likely be less efficient than one large factorial trial. If the combination trial is not successful, follow-up trials may focus on new study hypotheses. For example, it could be that drugs which prevent or reduce the risk of infarction result in lower mortality and better outcome for TBM patients [10].

Our main interest in the factorial design is that it allows separating out individual treatment effects and investigation of interactions. An alternative would be to perform the same trial but analyze it as a 4-arm trial instead which focuses on the comparison of each intervention and combination treatment, respectively, to the standard treatment. As we saw, such a trial would only require 2-fold the sample size of the combination trial to detect a combination effect if no adjustment for multiplicity is performed (and 2.7-fold the sample size using a Bonferroni correction). In addition, such a trial could be adaptive, i.e. allow for intermediate dropping of inefficacious arms [11]. However, such an analysis would not exploit the factorial design and have very low power to detect individual treatment effects. Other alternative designs might also be considered. Options would include 3-arm trials of the standard treatment versus the two most promising combinations or to first initiate a pilot study to further assess the safety and pharmacological profile of both interventions and their interaction. However, none of these trials can avoid the fundamental problem that the cost of separating out combination treatment effects into their components may be prohibitively high.

Conclusions

Pragmatic combination trials of multiple interventions versus standard therapy are valuable in diseases with a limited patient pool if all interventions test the same treatment concept, if it is considered likely that a

combination effect is based on contributions from all individual interventions, and only moderate (negative or positive) treatment interactions are suspected. In the case of two interventions, a 2 × 2 factorial design which is adequately powered to detect individual treatment effects would require at least 8-fold the sample size of the combination trial.

Additional material

Additional file 1: Technical appendix. Power calculation for 2 × 2 factorial trials.

Abbreviations

TBM: tuberculous meningitis

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Authors' contributions

All authors contributed to the design of the TBM trial protocol and discussions regarding alternative designs which formed the basis for this manuscript. MW performed all statistical calculations and drafted the first version of the manuscript. All authors reviewed the manuscript critically and read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Intensified Antituberculosis Therapy in Adults with Tuberculous Meningitis

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ABSTRACT

BACKGROUND

Tuberculous meningitis is often lethal. Early antituberculosis treatment and adjunctive treatment with glucocorticoids improve survival, but nearly one third of patients with the condition still die. We hypothesized that intensified antituberculosis treatment would enhance the killing of intracerebral *Mycobacterium tuberculosis* organisms and decrease the rate of death among patients.

METHODS

We performed a randomized, double-blind, placebo-controlled trial involving human immunodeficiency virus (HIV)–infected adults and HIV-uninfected adults with a clinical diagnosis of tuberculous meningitis who were admitted to one of two Vietnamese hospitals. We compared a standard, 9-month antituberculosis regimen (which included 10 mg of rifampin per kilogram of body weight per day) with an intensified regimen that included higher-dose rifampin (15 mg per kilogram per day) and levofloxacin (20 mg per kilogram per day) for the first 8 weeks of treatment. The primary outcome was death by 9 months after randomization.

RESULTS

A total of 817 patients (349 of whom were HIV-infected) were enrolled; 409 were randomly assigned to receive the standard regimen, and 408 were assigned to receive intensified treatment. During the 9 months of follow-up, 113 patients in the intensified-treatment group and 114 patients in the standard-treatment group died (hazard ratio, 0.94; 95% confidence interval, 0.73 to 1.22; P=0.66). There was no evidence of a significant differential effect of intensified treatment in the overall population or in any of the subgroups, with the possible exception of patients infected with isoniazid-resistant *M. tuberculosis*. There were also no significant differences in secondary outcomes between the treatment groups. The overall number of adverse events leading to treatment interruption did not differ significantly between the treatment groups (64 events in the standard-treatment group and 95 events in the intensified-treatment group, P=0.08).

CONCLUSIONS

Intensified antituberculosis treatment was not associated with a higher rate of survival among patients with tuberculous meningitis than standard treatment. (Funded by the Wellcome Trust and the Li Ka Shing Foundation; Current Controlled Trials number, ISRCTN61649292.)

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Early treatment with antituberculosis chemotherapy and adjunctive treatment with glucocorticoids reduce the rate of death and disability from tuberculous meningitis, but the disease still kills or disables almost half the patients with the condition.[1,2] The current guidelines recommend treatment with four antituberculosis drugs for at least the first 2 months of therapy, followed by treatment with two drugs (rifampin and isoniazid) for an additional 7 to 10 months.[3,4] However, these recommendations are based on data from pulmonary tuberculosis and do not take into account the differential ability of antituberculosis drugs to penetrate the brain.

Rifampin is considered to be a critical drug in tuberculosis treatment, but concentrations of the drug in cerebrospinal fluid (CSF) are less than 30% of the concentration in plasma.[5-7] In pulmonary tuberculosis, an increase in the oral dose of rifampin from 10 to 13 mg per kilogram of body weight had an acceptable side effect profile and led to a 65% increase in plasma concentrations of the drug.[8] A recent randomized comparison of higher-dose intravenous rifampin (approximately 13 mg per kilogram per day) versus a standard oral dose (10 mg per kilogram per day) in 60 Indonesian adults with tuberculous meningitis showed that mortality among patients who received the higher intravenous dose was 50% lower than that among patients who received the standard dose.[9]

Fluoroquinolones are active antituberculosis agents with good penetration of the blood–brain barrier.[10] For example, the concentration of levofloxacin in CSF reaches 70% of the concentration in plasma, and the drug has early bactericidal activity approaching that of isoniazid.[10-12] A randomized study involving Vietnamese adults with tuberculous meningitis suggested that the initial addition of levofloxacin to a standard four-drug antituberculosis regimen improved the survival rate, especially among patients who were treated before the onset of coma.[10] We therefore sought to test the hypothesis that intensified antituberculosis treatment — with higher-dose rifampin (15 mg per kilogram per day) and the addition of levofloxacin (20 mg per kilogram per day) for the first 8 weeks of treatment — would result in lower rates of death and disability from tuberculous meningitis than the rates with the currently recommended regimen.

Methods

Study Population and Setting

We recruited study participants from two centers in Ho Chi Minh City, Vietnam: Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease and the Hospital for Tropical Diseases. These 500-bed hospitals serve the local community and act as tertiary referral centers for patients with severe tuberculosis (Pham Ngoc Thach Hospital) or infectious diseases (Hospital for Tropical Diseases) in southern Vietnam.

A Quick Take is available at NEJM.org

A full description of the methods has been published elsewhere[13] and is provided in the protocol, available with the full text of this article at NEJM.org. Adults (≥18 years of age) with a clinical diagnosis of tuberculous meningitis (at least 5 days of meningitis symptoms, nuchal rigidity, and CSF abnormalities) were eligible to enter the trial. Patients were subsequently classified as having definite, probable, or possible tuberculous meningitis or an alternative condition, in accordance with published diagnostic criteria[14] (Table S1 in the Supplementary Appendix, available at NEJM.org). Patients could not enter the trial if they had received more than 7 days of antituberculosis drugs for the current infection; if they were known or suspected to be pregnant; if they had known or suspected hypersensitivity to or unacceptable side effects from fluoroquinolones or rifampin; if multidrug-resistant tuberculosis was known (on the basis of previous sputum drug susceptibility test results or Xpert MTB/RIF assay [Cepheid]) or suspected to be present; or if the plasma creatinine concentration was more than three times the upper limit of the normal range (for males, >360 μmol per liter [4.07 mg per deciliter], and for females, >300 μmol per liter [3.39 mg per deciliter]), if the plasma bilirubin concentration was more than 2.5 times the upper limit of the normal range (total bilirubin >42.5 mmol per liter), or if the plasma aspartate or alanine aminotransferase level was more than five times the upper limit of the normal range (>185 U per liter or >200 U per liter, respectively).

Study Oversight

Written informed consent to participate in the study was obtained from all patients or from their relatives if the patient could not provide consent. The trial was approved by the Oxford

Tropical Research Ethics Committee, the institutional review board at the Hospital for Tropical Diseases and at Pham Ngoc Thach Hospital, and the ethics committee of the Ministry of Health, Vietnam. An independent data and safety monitoring board reviewed the data after 6 months, 1 year, 2 years, and 3 years. The Xpert MTB/RIF assays used in the study were purchased. The rifampin and its matching placebo, as well as some of the levofloxacin, was purchased from Mekophar and Sanofi, respectively. Some of the levofloxacin and all of the levofloxacin matching placebo were donated by Sanofi. Neither Mekophar nor Sanofi played a part in the design, implementation, or analysis of the study, including manuscript preparation, or in the decision to submit the results for publication. All the authors vouch for the accuracy and completeness of the data and for the fidelity of this report to the study protocol.

LABORATORY INVESTIGATIONS

CSF specimens were stained and cultured with the use of standard methods for pyogenic bacteria, fungi, and mycobacteria and were tested with an Xpert MTB/RIF assay. Isolates of *Mycobacterium tuberculosis* were tested for susceptibility to isoniazid, rifampin, ethambutol, and streptomycin by means of the mycobacterial growth indicator tube method.[15] All patients were tested for antibodies to human immunodeficiency virus (HIV) and hepatitis C and for the presence of hepatitis B surface antigen. CD4 cell counts were measured for all HIV-infected adults as soon as possible after randomization.

STUDY TREATMENT

All patients received standard oral antituberculosis treatment, which consisted of isoniazid (5 mg per kilogram per day; maximum, 300 mg per day), rifampin (10 mg per kilogram per day), pyrazinamide (25 mg per kilogram per day; maximum, 2 g per day), and ethambutol (20 mg per kilogram per day; maximum, 1.2 g per day) for 3 months, followed by rifampin and isoniazid at the same doses for an additional 6 months. Patients who had previously received treatment for tuberculosis also received streptomycin (20 mg per kilogram per day; maximum, 1 g per day) for the first 3 months. All patients received adjunctive treatment with dexamethasone for the first 6 to 8 weeks of treatment, as described previously.[16] Intensified treatment consisted of the standard 9-month regimen with the addition for the first 8 weeks of treatment of a weight-based dose of rifampin (5 mg per kilogram per day, to achieve a total dose of 15 mg per kilogram per day) and of levofloxacin (20 mg per kilogram per day) (Table S2 in the Supplementary Appendix). Adherence to treatment was ensured with the use of supervised drug intake for inpatients, encouraged by detailed instructions at discharge, and measured by pill counts at the monthly follow-up visits. For patients infected with *M. tuberculosis* that was resistant to rifampin, isoniazid, or both, treatment was adjusted in accordance with local practices and the susceptibility of the organism.

HIV-infected patients received antiretroviral therapy in accordance with Vietnamese guidelines. Antiretroviral therapy that was started before enrollment was continued unless it was contraindicated for use with rifampin. If the antiretroviral therapy regimen that the patient was receiving at the time of enrollment included nevirapine, that drug was switched to efavirenz. For patients who had not previously received antiretroviral therapy, the therapy was started after 8 weeks of antituberculosis therapy.[17] Cotrimoxazole prophylaxis (960 mg per day) was given to all patients who had CD4 cell counts below 200 per cubic millimeter.

RANDOMIZATION AND CONCEALMENT OF STUDY-GROUP ASSIGNMENTS

Patients were stratified at study entry according to site, HIV infection status, and the modified British Medical Research Council criteria (MRC grade).[18] MRC grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or a score of 15 with focal neurologic signs), and grade 3 a score of 10 or lower. Patients were randomly assigned in a 1:1 ratio to receive either standard or intensive antituberculosis treatment according to a computer-generated randomization list, with randomization in variable block sizes of 4 and 6.

The study pharmacist prepared visually matched pills in identical, sequentially numbered treatment packs according to the randomization list for dispensation in sequential order as patients were recruited. All the participants,

enrolling physicians, and investigators remained unaware of the treatment assignments until the last patient completed follow-up. The attending physicians were responsible for enrolling the participants and for ensuring that the study drug was given from the correct treatment pack. Daily monitoring of all inpatients by one of the investigators ensured uniform management between the study sites and accurate recording of clinical data in individual study notes.

OUTCOME ASSESSMENTS

The condition of the patients was reviewed daily until discharge from the hospital for assessment of clinical progress and neurologic and drug-related adverse events. After discharge, monthly visits were scheduled for clinical evaluation and laboratory monitoring until the completion of treatment at 9 months.

The primary outcome was death by 9 months after randomization. The secondary outcomes included neurologic disability at 9 months, time to the first new neurologic event or death, and serious adverse events. The disability outcome was assessed with the use of the "simple questions" score (based on the answers to two yes-or-no questions regarding the patient's dependency on others in daily activities and whether the illness has left the patient with any other problems) and the modified Rankin score (a disability score that ranges from 0 [no symptoms] to 5 [totally dependent on others]) and was classified as "good outcome," "intermediate outcome," "severe disability," or "death," as described previously.[16,17,19] Patients were assessed at 2, 6, and 9 months after randomization; the worst score from either questionnaire was taken as the outcome. If the 9-month disability assessment was missing, the previous assessment was used instead. New neurologic events were defined as the occurrence of any of the following: cerebellar symptoms; monoplegia, hemiplegia, paraplegia, or tetraplegia; seizures; cranial nerve palsy; or a decrease in Glasgow coma score of 2 or more points for 2 or more days from the highest previously recorded score.

STATISTICAL ANALYSIS

We calculated that with a sample size of at least 750 patients, including a minimum of 350 HIV-infected patients, the trial would have 80% power to detect a 10-percentage-point lower 9-month risk of death among patients receiving the intensified treatment than among those receiving the standard treatment (30% vs. 40%, corresponding to a target hazard ratio of 0.7) in the overall population and a 15-percentage-point lower risk of death in the subgroup of HIV-infected patients (50% vs. 65%), at a two-sided 5% significance level.

The statistical analysis followed the protocol[13] and the statistical analysis plan (see the Supplementary Appendix). The primary outcome was analyzed in all patients and in prespecified subgroups, with the analysis based on the Cox proportional-hazards model with stratification according to HIV infection status and MRC grade. The ordinal disability score was compared between the two study groups with a proportional-odds logistic-regression model with adjustment for HIV infection status and MRC grade. Secondary time-to-event outcomes were analyzed in the same way as the primary outcome. Additional prespecified multivariable Cox regression analyses and analyses of the disability score were based on multiple imputation of missing covariates and disability outcomes, as detailed in the statistical analysis plan.

The primary analysis population was the intention-to-treat population, which included all patients who underwent randomization. The analysis of the primary outcome was repeated in the per-protocol population, which did not include patients with unlikely tuberculous meningitis or an alternative diagnosis according to the diagnostic criteria,[14] patients with multidrug-resistant infections, or patients who received less than 50 days of treatment with the study drug for reasons other than death. All statistical analyses were performed with the statistical software R, version 3.1.2.[20]

RESULTS

STUDY POPULATION

From April 18, 2011, through June 18, 2014, a total of 817 adult patients were randomly assigned to receive standard antituberculosis treatment plus either placebo (409 patients; standard-treatment group) or additional rifampin and levofloxacin (408 patients; intensified-treatment group). A total of 53 patients (28 in the standard-treatment group and 25 in the intensified-treatment group) did not complete follow-up for reasons other than death.

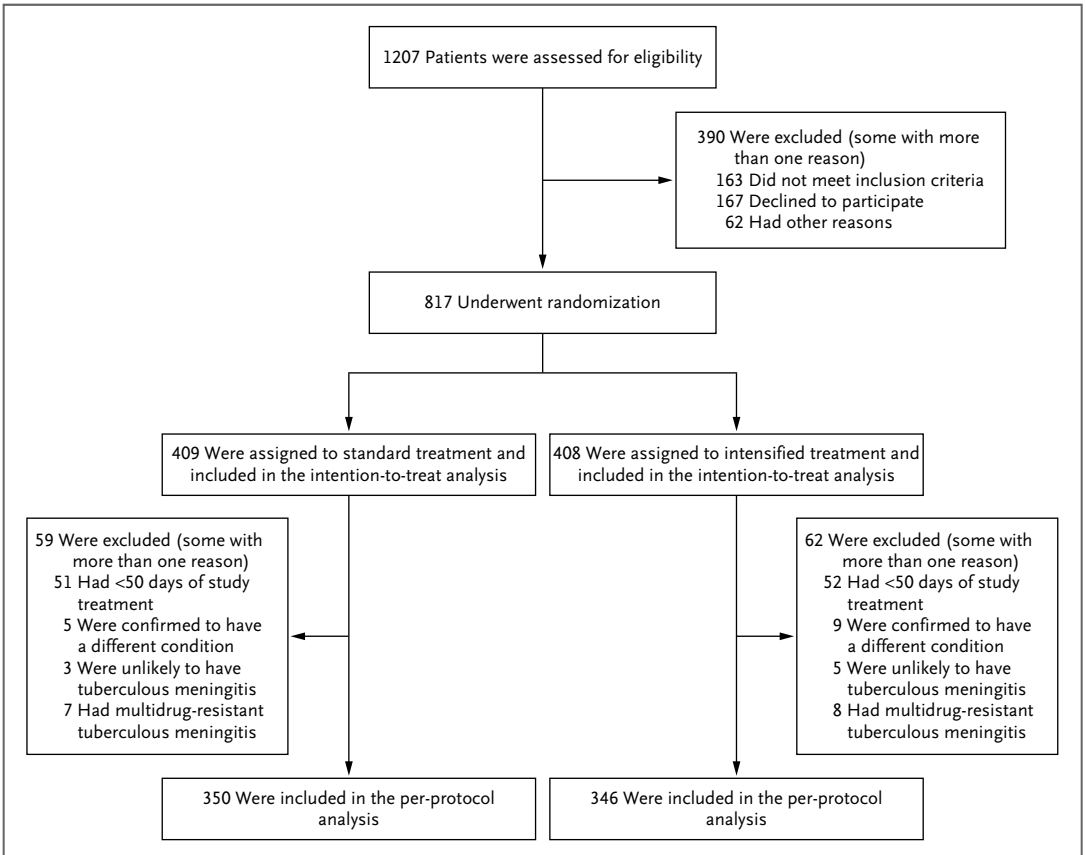


Figure 1. Screening and Randomization.

Among the patients in the intention-to-treat population, in the standard-treatment group, 22 were lost to follow-up, 5 withdrew from the study, 1 went home to die but could not be contacted, 1 died before the study treatment was given, and 6 had their treatment assignment revealed; in the intensified-treatment group, 21 were lost to follow-up, 2 withdrew from the study, 2 withdrew before the study treatment was given, and 3 had their treatment assignment revealed. Among the patients who were confirmed to have a condition other than tuberculous meningitis, in the standard-treatment group, four had cryptococcal meningitis and one had an intracranial tumor; in the intensified-treatment group, seven had cryptococcal meningitis, one had encephalitis caused by herpes simplex virus, and one had eosinophilic meningitis.

A total of 121 patients (59 in the standard-treatment group and 62 in the intensified-treatment group) were not included in the per-protocol population. A condition other than tuberculous meningitis was diagnosed in 14 patients (5 in the standard-treatment group and 9 in the intensified-treatment group), and 8 patients (3 in the standard-treatment group and 5 in the intensified-treatment group) were deemed unlikely to have tuberculous meningitis. A total of 103 patients received less than 50 days of treatment with the study regimens for reasons other than death, and 15 of these patients were determined to have multidrug-resistant tuberculous meningitis (Fig. 1). We assessed adherence to the 8-week intervention, and 4.0% of the participants (33 of 817, 19 in the standard-treatment group and 14 in the intensified-treatment group) were judged to be nonadherent (<100% medication doses received).

BASELINE CHARACTERISTICS

The characteristics of the patients at baseline were balanced between the two treatment groups, with the exception of sodium concentrations in plasma (lower in the intensified-treatment group), the frequency of a previous episode of tuberculosis (higher in the intensified-treatment group),

Table 1. Characteristics of the Patients at Enrollment.*

Characteristic	Standard Regimen (N=409)	Intensified Regimen (N=408)	All Patients (N=817)
Male sex — no. (%)	278 (68.0)	282 (69.1)	560 (68.5)
Median age (IQR) — yr	35 (30–47)	35 (29–45)	35 (29–46)
MRC grade — no. (%)†			
1	160 (39.1)	158 (38.7)	318 (38.9)
2	178 (43.5)	179 (43.9)	357 (43.7)
3	71 (17.4)	71 (17.4)	142 (17.4)
HIV-infected — no. (%)	174 (42.5)	175 (42.9)	349 (42.7)
Median CD4 count (IQR) — cells/mm^3‡	38 (15–82)	38 (14–113)	38 (14–101)
Diagnostic category — no. (%)§			
Definite tuberculous meningitis	201 (49.1)	206 (50.5)	407 (49.8)
Probable tuberculous meningitis	109 (26.7)	105 (25.7)	214 (26.2)
Possible tuberculous meningitis	91 (22.2)	83 (20.3)	174 (21.3)
Unlikely to be tuberculous meningitis	3 (0.7)	5 (1.2)	8 (1.0)
Confirmed other condition	5 (1.2)	9 (2.2)	14 (1.7)
Resistance category			
Drug-susceptibility test results available — no.	156	166	322
No isoniazid or rifampin resistance — no. (%)¶	107 (68.6)	113 (68.1)	220 (68.3)
Isoniazid monoresistance — no. (%)	41 (26.3)	45 (27.1)	86 (26.7)
Rifampin monoresistance — no. (%)	1 (0.6)	0	1 (0.3)
Multidrug resistance — no. (%)	7 (4.5)	8 (4.8)	15 (4.7)

* No characteristic differed significantly between the study groups (P≤0.05 at baseline according to Fisher's exact test for categorical data or the Wilcoxon rank-sum test for continuous data), with the exception of sodium concentration (P=0.004), frequency of a previous episode of tuberculosis (P=0.045), total white-cell count in the cerebrospinal fluid (P=0.006), and lymphocyte percentage in the cerebrospinal fluid (P=0.01); the complete list of baseline characteristics is provided in Table S3 in the Supplementary Appendix. IQR denotes interquartile range.

† Medical Research Council (MRC) grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less.

‡ CD4 cell counts were assessed only in HIV-infected patients. Data were missing for 30 patients in the standard-treatment group and for 25 patients in the intensified-treatment group.

§ Diagnostic categories were assigned according to the consensus case definition.[14] Patients whose condition was unlikely to be tuberculous meningitis had a score of less than 6 on the scale based on the consensus case definition (maximum score, 20) (see Table S1 in the Supplementary Appendix). Confirmation of another condition was made only on the basis of microbiologic evidence.

¶ Isoniazid monoresistance is defined as resistance to isoniazid but not to rifampin. Multidrug resistance is defined as resistance to at least isoniazid and rifampin. In all categories, resistance to other drugs may be present.

total white-cell count in the CSF (higher in the intensified-treatment group), and lymphocyte percentage in the CSF (lower in the intensified-treatment group) (Table 1, and Table S3 in the Supplementary Appendix). A total of 68.5% of the patients were men, the median age of the patients was 35 years, and the median duration of illness was 15 days. A majority of the patients had mild-to-moderate illness; only 17.4% had MRC grade 3 illness at enrollment. A total of 42.7% of patients were infected with HIV. Using the published diagnostic criteria,[14] we defined 49.8% of the patients as having definite tuberculous meningitis, 26.2% as having probable tuberculous meningitis, and 21.3% as having possible tuberculous meningitis. Among the patients with culture-confirmed disease, 26.7% had isoniazid-resistant infection, and 4.7% had multi-

drug-resistant infection. The baseline clinical characteristics of the patients according to MRC illness severity grade are provided in Table S4 in the Supplementary Appendix.

PRIMARY OUTCOME

During 9 months of follow-up, 113 patients in the intensified-treatment group and 114 patients in the standard-treatment group died (hazard ratio, 0.94; 95% confidence interval [CI], 0.73 to 1.22; P=0.66) (Fig. 2). There was no evidence of a differential effect of intensified treatment in the overall population or in any of the prespecified subgroups, although there was a suggestion of benefit of intensified treatment for patients with isoniazid-resistant infections (P=0.06) (Table 2). The probability of overall survival according to treatment group in the per-protocol population and MRC-grade groups is shown in Figures S1 and S2 in the Supplementary Appendix.

A Cox regression analysis (Table S5 in the Supplementary Appendix) identified the following factors as predictors of poor survival: more severe neurologic compromise at treatment initiation, as indicated by a higher MRC grade (hazard ratio for grade 2 vs. grade 1, 2.41; 95% CI, 1.70 to 3.42; hazard ratio for grade 3 vs. grade 1, 6.31; 95% CI, 4.36 to 9.12); HIV infection (hazard ratio, 2.53; 95% CI, 1.90 to 3.36); and multidrug-resistant or rifampin-resistant infection (hazard ratio, 4.72; 95% CI 2.41 to 9.24) or infection with unknown drug resistance (hazard ratio as compared with no isoniazid or rifampin resistance, 1.76; 95% CI, 1.27 to 2.45). In HIV-infected patients, a higher CD4 cell count was associated with reduced mortality (hazard ratio per increase of 100 cells per cubic millimeter, 0.62; 95% CI, 0.44 to 0.87).

SECONDARY OUTCOMES AND ADVERSE EVENTS

There was no evidence of a differential effect of intensified treatment on any of the prespecified secondary outcomes (Table S6 in the Supplementary Appendix). Overall, there was no significant difference between the treatment groups with regard to clinical adverse events, apart from a higher frequency of seizures in the intensified-treatment group than in the standard-therapy group (23 vs. 11 patients, P=0.04), as well as a higher frequency of vision impairment in the intensified-treatment group (14 vs. 4, P=0.02) (Table 3). Signs of drug allergy were more frequent in the intensified-treatment group than in the standard-therapy group (occurring in 30 patients vs. 17 patients); however, this difference did not reach significance (P=0.052). The difference between the study groups in the number of adverse events leading to interruptions in antituberculosis treatment also did not reach significance (64 events in the standard-treatment group vs. 95 in the intensified-treatment group, P=0.08) (Table S7 in the Supplementary Appendix). There were more interruptions due to jaundice in the intensified-treatment group than in the standard-treatment group (in 19 vs. 7 patients, P=0.02). Additional laboratory abnormalities are listed in Table S5 in the Supplementary Appendix. There were significantly more patients with grade 3 or grade 4 increases in bilirubin level in the intensified-treatment group than in the standard-treatment group (49 vs. 31, P=0.04), as well as significantly more patients with grade 3 or 4 hyponatremia (112 vs. 81, P=0.01) (Table S8 in the Supplementary Appendix). The median duration of the initial hospitalization was 31 days in the intensified-treatment group and 30 days in the standard-treatment group. A total of 11 patients (4 in the standard-treatment group and 7 in the intensified-treatment group) had a prolongation of the corrected QT interval above the critical threshold of 500 msec (calculated with the use of the Framingham formula) at any time between baseline and 4 weeks of treatment.

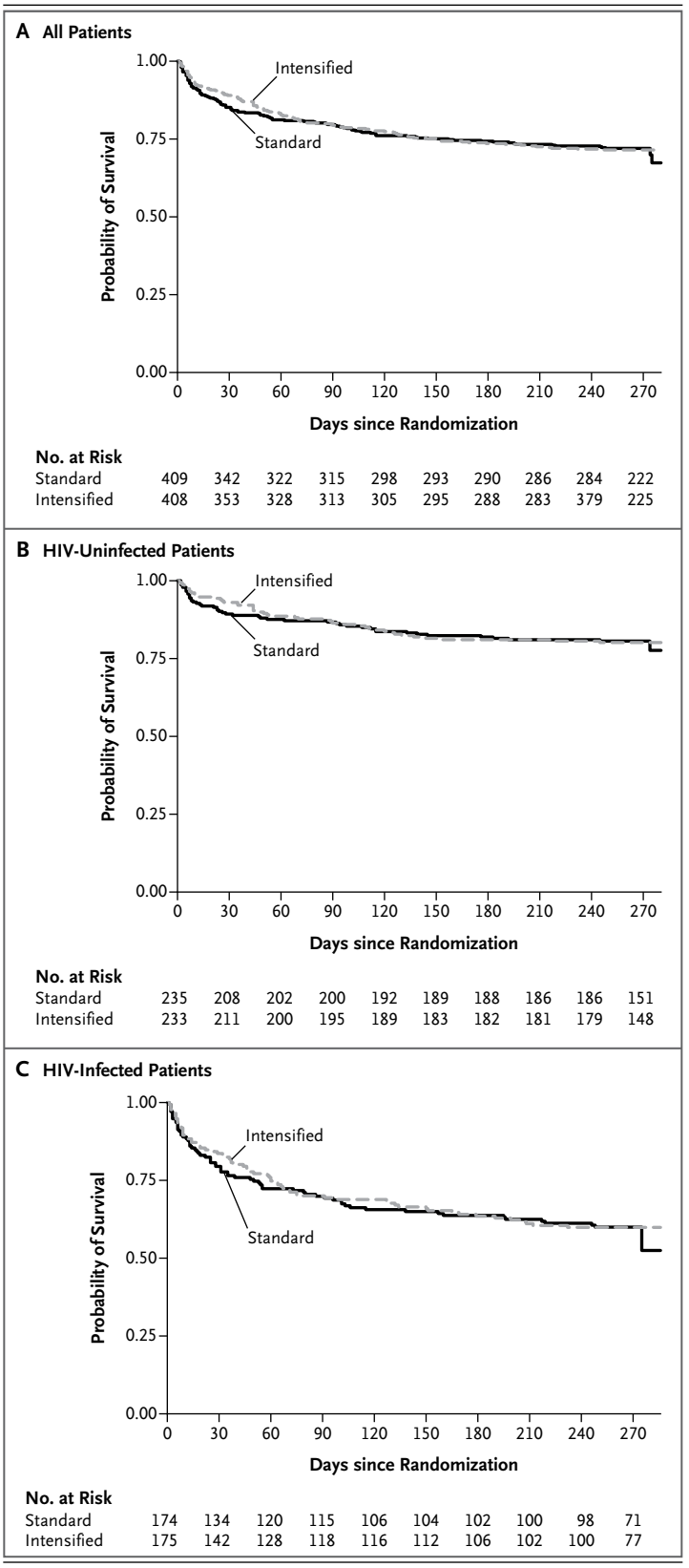
DISCUSSION

In this pragmatic, randomized, double-blind, placebo-controlled trial involving adults with tuberculous meningitis, intensified antituberculosis treatment was not associated with a higher rate of survival than the rate with standard treatment. The results contradict the findings of previous studies that suggested that an increase in rifampin dose[9] and the addition of a fluoroquinolone to the standard regimen[10] may improve the outcome in patients with tuberculous meningitis.

A limitation of our study was that we tested a regimen rather than the contribution of individual drugs. A factorial design may have enabled the latter but would have led to the need for a prohibitively large sample size.[21] However, our

Figure 2. Kaplan–Meier Curves for Overall Survival According to Treatment Group and HIV Infection Status.

In accordance with the statistical analysis plan, all deaths in the database were included in the final analysis; this included two deaths on days 274 and 275. Because the 9-month follow-up visit was on days 270 through 272 for most patients, the numbers of patients at risk on days 274 and 275 were low, which accounts for the sharp decrease in the Kaplan–Meier curves at the end of the study period.



negative findings suggest that neither a higher dose of rifampin nor a higher dose of levofloxacin improves tuberculous meningitis treatment.

There are a number of possible explanations for our results. It is possible that the oral rifampin dose used in our study (15 mg per kilogram per day) did not increase the intracerebral drug concentrations sufficiently to enhance bacterial killing. Recent data suggest that much higher doses of rifampin (up to 35 mg per kilogram per day) may have an acceptable side-effect profile and may be necessary to significantly increase the killing of *M. tuberculosis* in pulmonary tuberculosis.[22] Furthermore, oral administration probably results in substantially lower rifampin concentrations in plasma than does intravenous administration of equivalent doses.[23] Some reports have suggested that the relative benefit of rifampin in the treatment of tuberculous meningitis may be modest in the presence of effective mycobacterial killing by isoniazid.[24] The main role of rifampin in the treatment of pulmonary tuberculosis is probably to shorten the treatment duration rather than to enhance early mycobacterial killing.[25,26] In contrast, fluoroquinolones have enhanced the early sterilization of sputum but have not allowed the duration of therapy to be shortened, because of unacceptable increases in disease relapses.[27-29] Previous studies have shown that fluoroquinolones either have no effect on the outcome of tuberculous meningitis[9] or confer a possible benefit in patients with mild disease.[10] A pharmacokinetic and pharmacodynamic analysis involving the patients recruited for our trial may help address these possibilities.

An intensified antituberculosis regimen may, however, benefit patients infected with isoniazid-resistant *M. tuberculosis*. The way in which this finding should influence clinical practice is un-

Table 2. Survival during the 9-Month Study Period.

Subgroup	Standard Regimen (N=409)	Intensified Regimen (N=408)	Hazard Ratio (95% CI)	P Value	P Value for Heterogeneity*
	no. of deaths/total no. of patients				
Intention-to-treat population	114/409	113/408	0.94 (0.73–1.22)	0.66	
Per-protocol population	94/350	93/346	0.91 (0.68–1.21)	0.52	
MRC grade					0.69
1	25/160	21/158	0.82 (0.46–1.46)	0.49	
2	50/178	52/179	1.07 (0.72–1.57)	0.74	
3	39/71	40/71	0.87 (0.56–1.36)	0.55	
HIV infection status					0.74
Uninfected	46/235	45/233	1.00 (0.66–1.51)	1.00	
Infected†	68/174	68/175	0.91 (0.65–1.27)	0.57	
Previous tuberculosis					0.82
No	89/347	78/324	0.91 (0.67–1.23)	0.53	
Yes	25/62	35/84	0.99 (0.59–1.67)	0.97	
Receiving antituberculosis treatment at enrollment‡					0.02
No	36/150	49/149	1.38 (0.89–2.12)	0.15	
Yes	78/259	64/259	0.74 (0.53–1.03)	0.07	
Diagnostic category§					0.93
Definite tuberculous meningitis	56/201	55/206	0.90 (0.62–1.31)	0.57	
Probable tuberculous meningitis	30/109	33/105	1.10 (0.66–1.83)	0.71	
Possible tuberculous meningitis	28/91	25/83	0.94 (0.54–1.63)	0.82	
Resistance category					0.04
No or other resistance	22/107	30/113	1.54 (0.88–2.71)	0.13	
Isoniazid resistance	16/41	11/45	0.45 (0.20–1.02)	0.06	
Rifampin or multidrug resistance	6/8	5/8	0.63 (0.15–2.69)	0.53	

* Heterogeneity was tested with a likelihood ratio test for an interaction term between the subgroup variable and the randomly assigned treatment group. As prespecified, the Cox regression was stratified according to MRC grade and HIV infection status.
† Among the patients who were not receiving antiretroviral therapy at enrollment, 49 of 114 patients (43%) in the intensified-treatment group died (30 of them in the first 8 weeks), and 44 of 115 patients (38%) in the standard-treatment group died (29 of them in the first 8 weeks). Among the patients who were receiving antiretroviral therapy at enrollment, 19 of 61 patients (31%) in the intensified-treatment group died (9 of them in the first 8 weeks), and 24 of 59 patients (41%) in the standard-treatment group died (18 of them in the first 8 weeks).
‡ Patients were not eligible to enter the trial if they had received more than 7 days of antituberculosis treatment before enrollment. The median duration of antituberculosis treatment was 4 days (interquartile range, 2 to 5).
§ A total of 22 patients with unlikely tuberculous meningitis or a confirmed other condition were not included.

certain, given that the detection of isoniazid resistance usually requires bacterial culture and often takes many weeks. The development of rapid molecular tests that can reliably detect isoniazid resistance in cerebrospinal fluid may aid in early diagnosis and treatment adjustments. However, empirical intensification of treatment regimens may be warranted in patients who are at high risk for isoniazid-resistant infection or in settings with a high prevalence of isoniazid-resistant bacteria.

The overall mortality in our population was lower than that anticipated on the basis of previous reports. This may be due to a combination of earlier diagnosis (38.9% of patients had MRC grade 1 disease at randomization), increased availability of second-line drugs for drug-resistant infections, and improved management of

Table 3. Clinical Grade 3 and 4 Adverse Events.*

Adverse Event	Standard Regimen (N=409)	Intensified Regimen (N=408)	P Value†
	no. of patients (%)		
Any event	229 (56.0)	240 (58.8)	0.44
Neurologic event	155 (37.9)	173 (42.4)	0.20
Deterioration of consciousness	89 (21.8)	90 (22.1)	0.93
Headache	30 (7.3)	36 (8.8)	0.44
Hemiplegia	21 (5.1)	31 (7.6)	0.16
Paraplegia	9 (2.2)	10 (2.5)	0.82
Urinary retention	12 (2.9)	10 (2.5)	0.83
Cranial-nerve palsies	11 (2.7)	13 (3.2)	0.69
Seizures	11 (2.7)	23 (5.6)	0.04
Vision impairment	4 (1.0)	14 (3.4)	0.02
Hepatotoxicity	28 (6.8)	17 (4.2)	0.12
Jaundice	17 (4.2)	29 (7.1)	0.07
Respiratory event	18 (4.4)	18 (4.4)	1.00
Event requiring mechanical ventilation	10 (2.4)	14 (3.4)	0.42
Signs of drug allergy	17 (4.2)	30 (7.4)	0.052
Cardiologic events	14 (3.4)	13 (3.2)	1.00
Diarrhea	7 (1.7)	5 (1.2)	0.77
Vomiting	13 (3.2)	18 (4.4)	0.37
Severe abdominal pain	12 (2.9)	5 (1.2)	0.14
Fever	8 (2.0)	9 (2.2)	0.81
Hemorrhage or anemia	16 (3.9)	17 (4.2)	0.86
Other hematologic event	4 (1.0)	3 (0.7)	1.00
New AIDS-defining illness	6 (1.5)	10 (2.5)	0.33
Gastrointestinal bleeding	3 (0.7)	1 (0.2)	0.62
Other gastrointestinal symptoms	3 (0.7)	9 (2.2)	0.09
Renal event	2 (0.5)	3 (0.7)	0.69
Urinary symptoms	0	1 (0.2)	0.50
Dermatologic symptoms	5 (1.2)	6 (1.5)	0.77
Peripheral edema	0	4 (1.0)	0.06
Musculoskeletal symptoms	3 (0.7)	3 (0.7)	1.00
Exhaustion	2 (0.5)	4 (1.0)	0.45
Other	14 (3.4)	12 (2.9)	0.84

* In total, 446 adverse events occurred in the standard-treatment group and 534 adverse events occurred in the intensified-treatment group (P=0.09, by the Wilcoxon rank-sum test). AIDS denotes acquired immunodeficiency syndrome.
† P values were calculated with the use of Fisher's exact test.

HIV infection. Although the results of our study do not support a change in the currently recommended treatment regimens for tuberculous meningitis, enhanced antituberculosis treatment with higher doses of first-line antituberculosis drugs, including intravenous rifampin, or the newer antituberculosis drugs bedaquiline and delamanid, still require investigation. In the

meantime, the key determinants of survival from this dangerous infection are earlier diagnosis and treatment.

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Therapy for Tuberculous Meningitis

TO THE EDITOR: Heemskerk et al. (Jan. 14 issue)[1] report no survival benefit for intensified antituberculosis treatment for patients with tuberculous meningitis in Vietnam, a finding that was contrary to the results of our study in Indonesia.[2] Our unblinded study was smaller and had a higher proportion of patients with advanced tuberculous meningitis. However, we think that the contrasting results are explained by the dose of rifampin that was administered. The Vietnam study compared 10 mg of rifampin per kilogram of body weight per day with an increased dose of 15 mg of rifampin per kilogram, whereas we evaluated 13 mg of rifampin per kilogram administered intravenously. The high dose used in our study resulted in an increase in rifampin exposure in blood and cerebrospinal fluid by a factor of 3, with a relationship between the drug level and the therapeutic response.[3] In our study, 38% of patients who were receiving 13 mg of rifampin per kilogram had drug exposure that was lower than the target exposure, which suggests that the dose was not high enough.

Recent trial data for pulmonary tuberculosis also support higher doses of rifampin than that used in the Vietnam study. Regimens consisting of doses of less than 35 mg of rifampin per kilogram did not reduce the time to culture conversion.[4] We are currently evaluating similarly increased rifampin doses for the treatment of tuberculous meningitis to prepare for a phase 3 trial. Pharmacokinetic and pharmacodynamic analyses may show how many patients in the Vietnam trial with its modest dose increase reached target exposures of rifampin.[3]

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No potential conflict of interest relevant to this letter was reported.

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TO THE EDITOR: Heemskerk et al. investigated an "intensified" treatment for tuberculous meningitis and did not find a higher survival rate than that with standard treatment. We think that the dose of rifampin was too low. We have shown that doses up to 35 mg per kilogram are tolerated.[1,2] The authors suggest that the benefits of rifampin may be modest. Our data show that higher doses of rifampin were associated with a faster decline in the bacterial load, which suggests that enhanced efficacy can occur with higher doses. Thus, it is difficult to draw conclusions about the failure of the experimental regimen when the trial regimen was possibly suboptimal.

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No potential conflict of interest relevant to this letter was reported.

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TO THE EDITOR: The study by Heemskerk et al. raises important questions about the approach to the treatment of tuberculous meningitis. The Kaplan–Meier curve for mortality in the subgroup with grade 3 disease according to British Medical Research Council criteria appeared to favor the intensified-treatment regimen until it began to converge with that of the standard-treatment regimen around day 150. A brief, but less substantial, divergence of the curves was seen early on in other subgroups as well. This raises the question as to whether a more prolonged intensive-treatment strategy might be beneficial. This approach is not without precedent, as described by Donald et al. in the editorial accompanying the article[1] and in a study of intensive therapy.[2] Although most new regimens that are evaluated have focused on reducing the duration of treatment or modifying the intensive phase in patients with pulmonary tuberculosis,[3] perhaps the use of multiple drugs for longer periods may have an effect on mortality in patients with tuberculous meningitis.

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THE AUTHORS REPLY: We agree with all three groups of correspondents that higher doses of rifampin, perhaps given throughout treatment after initial intravenous administration, may yet improve outcomes for patients with tuberculous meningitis. This effect, however, is predicated on the hypothesis that enhanced intracerebral killing of *Mycobacterium tuberculosis* by "intensified" antituberculosis treatment will increase survival from tuberculous meningitis.

There are good reasons to question this hypothesis. First, our trial tested the addition of higher-dose rifampin and levofloxacin to standard four-drug treatment for the first 2 months of therapy. Leaving aside the chosen rifampin dose, levofloxacin is highly active against *M. tuberculosis*, and oral administration of the dose used in the trial achieves high concentrations in the cerebrospinal fluid.[1] Yet the addition of levofloxacin to treatment had no discernible effect on survival from tuberculous meningitis.

Unlike pulmonary tuberculosis, tuberculous meningitis is caused by a small number of bacteria. This fact hampers both the microbiologic diagnosis and investigations linking drug exposures with destruction of bacteria and clinical outcomes, but neither the bacterial load in cerebrospinal fluid nor the time to its sterility has ever been clearly linked with survival among patients with tuberculous meningitis.[2] Instead, the clinical outcome has been much more closely associated with the intracerebral inflammatory response, which may explain why the use of adjunctive glucocorticoids improves survival[3] and why intensifying antituberculosis treatment has so far met with limited success.

In addition, increased doses of antituberculosis drugs increase the risk of drug-induced hepatic toxicity. In patients with pulmonary tuberculosis, this complication can usually be managed safely by stopping the drugs and reintroducing them slowly. But in patients with tuberculous meningitis, any interruption in treatment with antituberculosis drugs is an independent risk factor for death.[3] This suggests that any potential survival benefit of intensified regimens may be offset by even moderately increased toxicity and consequent reductions in dose or withdrawal of drugs.

In patients with tuberculous meningitis, survival depends on controlling inflammation and killing bacteria before the development of complications of advanced disease: hydrocephalus,

infarction, tuberculomas, and coma. Diagnostic delay and the failure to start antituberculosis drugs before the onset of coma is the strongest predictor of death from tuberculous meningitis.[4] The extent to which enhanced killing of bacteria through intensified antituberculosis regimens might improve outcomes remains a question worth asking, but it should not detract from the need to find better diagnostic tests and to better understand and control the immunopathology of the disease.

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Planned Out-of-Hospital Birth and Birth Outcomes

TO THE EDITOR: Snowden et al. (Dec. 31 issue)[1] report increased mortality among neonates in Oregon whose mothers had planned out-of-hospital births and therefore corroborate our previous findings of significantly increased risks of perinatal deaths and neonatal seizures among neonates whose mothers had out-of-hospital births in the United States.[2-4] Snowden et al. undeniably underestimate the absolute and relative neonatal risks associated with out-of-hospital births, specifically with home births. First, they lump together the outcomes for infants born in birthing centers (which are associated with better outcomes and have a better selection of patients) with the outcomes for those whose mothers had planned home births.[2] Second, they excluded deliveries associated with higher risk (twins, breech births, and anomalous births).[1] Both the absolute risk and the relative risk of death for infants whose mothers have home births are therefore likely to be even greater than the authors report. Reducing the risks of childbirth is an ethical imperative for professionals in perinatal care who are committed to increasing the safety and improving the quality of childbirth.[5] Women planning an out-of-hospital birth should be informed of the modern perinatal interventions available in the hospital that are often lifesaving and can prevent the increased neonatal risks associated with planned out-of-hospital births.

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TO THE EDITOR: Snowden et al. report higher rates of perinatal death, depressed 5-minute Apgar scores, neonatal seizures, and maternal blood transfusions among births that occur out

Clinical Infectious Diseases

MAJOR ARTICLE

Clinical Outcomes of Patients With Drug-Resistant Tuberculous Meningitis Treated With an Intensified Antituberculosis Regimen

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Background. Drug-resistant tuberculous meningitis (TBM) is difficult to diagnose and treat. Mortality is high and optimal treatment is unknown. We compared clinical outcomes of drug-resistant and -susceptible TBM treated with either standard or intensified antituberculosis treatment.

Methods. We analyzed the influence of *Mycobacterium tuberculosis* drug resistance on the outcomes of patients with TBM enrolled into a randomized controlled trial comparing a standard, 9-month antituberculosis regimen (containing rifampicin 10 mg/kg/day) with an intensified regimen with higher-dose rifampicin (15 mg/kg/day) and levofloxacin (20 mg/kg/day) for the first 8 weeks. The primary endpoint of the trial was 9-month survival. In this subgroup analysis, resistance categories were predefined as multidrug resistant (MDR), isoniazid resistant, rifampicin susceptible (INH-R), and susceptible to rifampicin and isoniazid (INH-S + RIF-S). Outcome by resistance categories and response to intensified treatment were compared and estimated by Cox regression.

Results. Of 817 randomized patients, 322 had a known drug resistance profile. INH-R was found in 86 (26.7%) patients, MDR in 15 (4.7%) patients, rifampicin monoresistance in 1 patient (0.3%), and INH-S + RIF-S in 220 (68.3%) patients. Multivariable regression showed that MDR (hazard ratio [HR], 5.91 [95% confidence interval {CI}, 3.00–11.6]), $P < .001$), was an independent predictor of death. INH-R had a significant association with the combined outcome of new neurological events or death (HR, 1.58 [95% CI, 1.11–2.23]). Adjusted Cox regression, corrected for treatment adjustments, showed that intensified treatment was significantly associated with improved survival (HR, 0.34 [95% CI, .15–.76], $P = .01$) in INH-R TBM.

Conclusions. Early intensified treatment improved survival in patients with INH-R TBM. Targeted regimens for drug-resistant TBM should be further explored.

Keywords. tuberculous meningitis; tuberculosis; drug-resistance; isoniazid; levofloxacin.

Tuberculous meningitis (TBM) is the most fatal form of tuberculosis (TB), killing approximately one-third of patients despite antituberculosis treatment [1–4]. Reports on drug-resistant TBM are limited due to the rarity of the disease and the difficulty of isolating mycobacteria from the cerebrospinal fluid (CSF), but resistance prevalence is broadly consistent with background *Mycobacterium tuberculosis* resistance rates [5–8]. The optimal treatment of drug-resistant TBM is unknown.

Isoniazid and rifampicin are the principal drugs in TBM treatment, given throughout the regimen. Isoniazid penetrates the blood–brain barrier effectively and has the highest early bactericidal activity (EBA) of all first-line drugs, killing approximately 95% of mycobacteria in the first 2 days [9]. Despite low penetration into the CSF, rifampicin is thought to be highly active in the days thereafter, against both dividing and persistent mycobacteria. The complementary role of isoniazid and rifampicin is illustrated by the high mortality of patients with confirmed multidrug-resistant (MDR) TBM, which approaches 100% when treated with standard first-line regimens and as low as 40% when second-line regimens are used [8, 10]. Isoniazid resistance has also been associated with mortality in TBM. In US patients, isoniazid-resistant TBM was associated with death (odds ratio, 2.07 [95% confidence interval {CI}, 1.30–3.29]) regardless of human immunodeficiency virus (HIV) status [11]. In Vietnam, isoniazid-resistant TBM was associated with higher mortality (adjusted hazard ratio [HR], 1.78 [95% CI, 1.18–2.66]) in HIV-infected subjects, but not in HIV-uninfected subjects [8, 12].

In a 1:1 randomized double-blind trial, we investigated whether intensified treatment with higher-dose rifampicin and levofloxacin would benefit patients with TBM [13]. Although the trial did not show an overall benefit of intensified treatment,

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the results suggested reduced mortality in isoniazid-resistant infection (HR, 0.45 [95% CI, .20–1.02], $P = .06$) [14]. Higher doses of rifampicin are associated with higher EBA [15] and may lead to improved intracranial drug exposure [16, 17]. Fluoroquinolones have antituberculosis activity comparable to that of isoniazid [18]. Levofloxacin penetration in the CSF is good, with levels of 70% of plasma levels [19]. In isoniazid-resistant TBM, the addition of these agents to the first-line regimen may substitute for the loss of isoniazid early bacterial killing. In our trial, intensified treatment was initiated at the onset of treatment regardless of the drug resistance profile of the mycobacteria. This subgroup analysis describes the effect of intensified treatment on outcome in drug-resistant TBM.

METHODS

Study Design and Participants

Patients were recruited from 2 tertiary referral hospitals in Ho Chi Minh City, Vietnam. The full protocol and primary results are published elsewhere [13, 14]. In short, adults with a clinical diagnosis of TBM were eligible to enter. Exclusion criteria were a positive CSF Gram stain or India ink stain; known or suspected pregnancy; laboratory contraindications to antituberculosis therapy; MDR TBM diagnosed prior to enrollment; and lack of consent. Written informed consent to participate was obtained from all patients or their relatives. The trial was approved by the Oxford Tropical Research Ethics Committee and the institutional review boards of the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital and the Ministry of Health, Vietnam.

Treatment

Patients were randomized to receive standard treatment or intensified treatment. Standard treatment consisted of isoniazid (5 mg/kg/day), rifampicin (10 mg/kg/day), pyrazinamide (25 mg/kg/day) and ethambutol (20 mg/kg/day) or streptomycin (20 mg/kg/day) for 3 months, followed by rifampicin and isoniazid at the same doses for 6 months. Those previously treated for tuberculosis received additional streptomycin or ethambutol for the first 3 months. Intensified treatment consisted of the standard regimen with an additional, weight-based dose of rifampicin (5 mg/kg/day) to achieve a total dose of 15 mg/kg/day, and levofloxacin (20 mg/kg/day) for the first 8 weeks of treatment. For patients infected with *M. tuberculosis* resistant to rifampicin and/or isoniazid on drug susceptibility testing (DST), treatment was adjusted at the discretion of the treating clinician, guided by the organism susceptibility. In general, isoniazid resistance was treated with a fluoroquinolone (levofloxacin 750 mg and, in 1 case, moxifloxacin 400 mg until treatment end); occasionally an aminoglycoside (kanamycin 20 mg/kg/day for 3 months) was used early in treatment. However, treatment adjustment was not uniform across centers, as the optimal regimens are not known. Consequently, a subset received additional treatment with at least a fluoroquinolone, regardless of randomized allocation, but treatment was not adjusted in all patients. Patients found to have rifampicin-resistant infection were treated with second-line drugs (a fluoroquinolone and an injectable agent, plus at least 3 other active drugs). All patients received adjunctive dexamethasone for the first 6–8 weeks as previously described [1]. HIV-infected patients received antiretroviral treatment according to Vietnamese guidelines [20].

Assessment of Outcome

The primary outcome was death during 9-month follow-up. Patients were monitored for drug toxicity, neurological deterioration, and other clinical parameters. A secondary outcome was time to first new neurological event or death. New neurological events were defined as occurrence of any of the following adverse events after enrollment: cerebellar symptoms; coma/consciousness deterioration or a fall in Glasgow Coma Scale score by ≥2 points for ≥2 days from the highest previously recorded; cranial nerve palsy; hemiplegia, paraplegia, tetraplegia, or monoplegia; neurological deterioration requiring ventilation; seizures; or cerebral herniation. Disability was also assessed as a secondary outcome based on the 2 simple questions and the modified Rankin score and classified as good outcome, intermediate outcome, severe disability, or death, as previously described [1, 3, 20].

Investigations

Patients underwent lumbar puncture at baseline. CSF samples were centrifuged at 4000*g* for 15 minutes. Supernatant was removed to leave a 0.5-mL deposit, which was Ziehl-Neelsen stained, Xpert MTB/RIF (Cepheid) tested, and cultured by Bactec MGIT (Becton Dickinson) [21]. All positive cultures were tested for drug susceptibility to rifampicin, isoniazid, streptomycin, and ethambutol using a Bactec MGIT SIRE kit (Becton Dickinson) following manufacturer's instructions, which contained the following critical concentrations: streptomycin 1.0 μg/mL, isoniazid 0.1 μg/mL, rifampicin 1.0 μg/mL, and ethambutol 5.0 μg/mL.

Resistance Categories

Resistance was categorized based on the results of MGIT DST performed against rifampicin, isoniazid, on baseline samples (defined as sample taken any time in between 7 days prior to, or 3 days after enrollment) of CSF, but also sputum, gastric fluid, or blood. The following categories were defined:

- MDR: resistance against both isoniazid and rifampicin.
- Rifampicin resistant (RIF-R): resistance against rifampicin, susceptible to isoniazid.
- Isoniazid resistant (INH-R): resistance against isoniazid, susceptible to rifampicin.
- No isoniazid or rifampicin resistance (INH-S + RIF-S): no resistance to isoniazid or rifampicin.

In all categories, resistance to streptomycin and/or ethambutol may be present.

Statistical Analysis

Baseline characteristics for each of the resistance categories were summarized as median (interquartile range [IQR]) for continuous data and number (percentage) for categorical data. Clinical variables associated with specified resistance categories were assessed by univariate analysis. The Kruskal-Wallis test was used to compare continuous parameters and Fisher exact test for comparisons between categorical parameters. Kaplan-Meier estimates were used to display survival of patients in the defined resistance categories, by randomized treatment arm. Overall survival and the time to the first new neurological event or death were modeled using Cox regression with resistance categories as the main covariate and additional adjustment for the randomized treatment arm, TBM severity grade, and HIV status. The ordinal disability outcome was modeled using a proportional odds logistical regression model and the same covariates. The effect of intensified treatment on survival in the INH-R group was modeled with a Cox regression model stratified by TBM severity grade and HIV status in accordance with the primary trial publication [14]. To dissect the impact of later treatment alterations with fluoroquinolones in INH-R subjects, "fluoroquinolone use" was assigned as a time-dependent covariate (ie, the start day of fluoroquinolone use was defined as study day 1 (baseline) in subjects randomized to intensified treatment, or the study day corresponding to the start of fluoroquinolone administration if it was initiated in the control arm) in an additional Cox regression model, stratified by TBM severity grade and HIV status. This means that fluoroquinolone usage was modeled to only affect the hazard (ie, the rate) of death from the time-point when fluoroquinolone treatment was actually started onward.

All statistical analyses were performed using the statistical software R version 3.0.2.

RESULTS

Baseline Characteristics

A total of 817 patients were randomized from April 2011 to June 2014. Of those, 322 had DST results for baseline samples: 86 (26.7%) were classified as INH-R, 15 (4.7%) patients as MDR, 1 (0.3%) patient as RIF-R, and 220 (86.3%) patients as sensitive to both drugs. Only 176 (54.7%) patients had fully susceptible infection (Figure 1). Coinfection with HIV was found in 185 (57.5%) patients. Patients in the different resistance categories had a similar clinical presentation, although patients infected with INH-R or MDR were more likely to report a previous episode of TB infection ($P < .001$). In particular, British Medical Research Council (MRC) grade on presentation and chest radiographic findings were similar between groups, and CSF results were also broadly similar. Although not statistically significant,

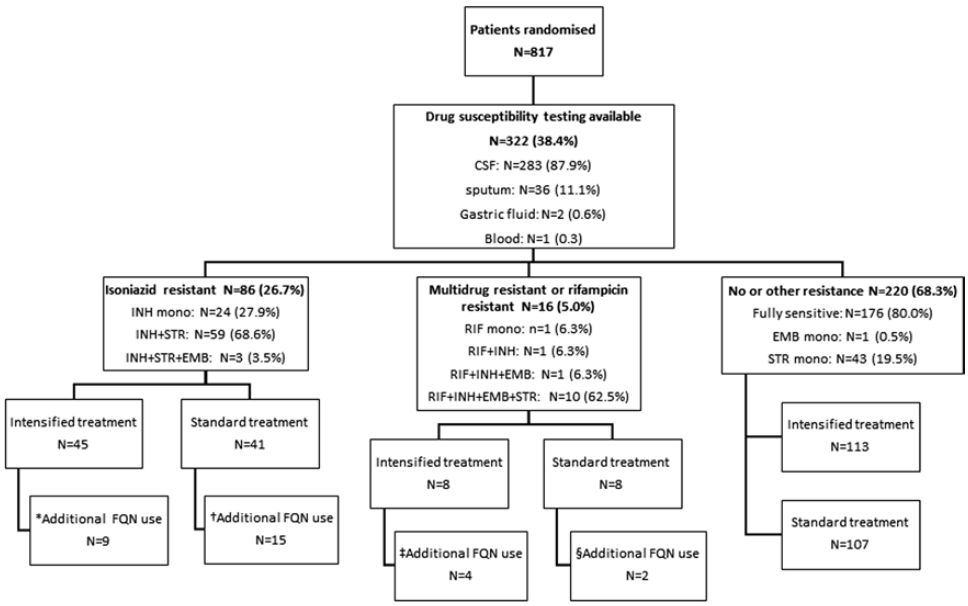

Figure 1. Study flow diagram. *Nine patients who had isoniazid resistance detected and were randomized to intensified treatment received treatment adjustment with the addition of at least a fluoroquinolone. †Fifteen patients who had isoniazid resistance detected and were randomized to standard treatment received treatment adjustment with the addition of at least a fluoroquinolone. ‡Four patients who had rifampicin resistance detected and were randomized to intensified treatment received treatment adjustment with at least a fluoroquinolone. For 3 patients, this was part of a second-line treatment schedule and 1 patient received levofloxacin and amikacin added to first-line drugs. §Two patients who had rifampicin resistance detected and were randomized to standard treatment received treatment adjustment with at least a fluoroquinolone. One patient with rifampicin monoresistance received this as part of second-line therapy. The other patient had multidrug resistance and received only additional levofloxacin and no other second-line agents. Abbreviations: CSF, cerebrospinal fluid; EMB, ethambutol; FQN, fluoroquinolone; INH, isoniazid; mono, monoresistance; RIF, rifampicin; STR, streptomycin.

a higher proportion of patients with MDR were HIV infected (13/16 [81.3%]) (Table 1).

Time to Diagnosis of Drug Resistance and Relevant Treatment Adjustment With Fluoroquinolones

The median time to diagnosis of INH-R in the 86 INH-resistant patients was 70 days (IQR, 48–83 days). When drug resistance was detected prior to 56 days, the study intervention was stopped (without unblinding of the allocation), if treatment adjustments were made. Among the 41 subjects in the standard-of-care arm, 15 subsequently had their treatment adjusted, by adding (at least) a fluoroquinolone (levofloxacin, n = 14; moxifloxacin, n = 1) after a median time to initiation of 76 days (IQR, 61–92 days). In the intensified treatment arm (n = 45), 9 INH-R patients received additional fluoroquinolone treatment (levofloxacin), with a median time to treatment adjustment of 105 days (IQR, 66–113 days) (Figure 1). Characteristics for patients receiving adjustment of treatment and those who did not are given in Supplementary Table 1.

Characteristics, outcome, and drug management of patients with MDR TBM are given in Supplementary Table 2. One patient had RIF-R (detected by Xpert MTB/RIF) and received

Table 1. Baseline Characteristics

Characteristic	No or Other Resistance (n = 220)		INH Resistant (n = 86)		RIF Resistant or MDR (n = 16)		All Patients (n = 322)		*P* Value[a]
No. of patients		220/322 (68.3)		86/322 (26.7)		16/322 (5.0)		322/322 (100)	
Age, y, median (IQR)	220	34 (29–41)	86	34 (29–41)	16	34 (31–41)	322	34 (29–41)	.84
Male sex	220	156 (70.9)	86	61 (70.9)	16	14 (87.5)	322	231 (71.7)	.42
Weight, kg, median (IQR)	220	47.7 (43.0–52.0)	86	48.0 (42.1–50.0)	16	50.0 (42.8–52.0)	322	48.0 (43.0–51.0)	.54
Duration of illness, d, median (IQR)	220	15.0 (10.0–30.0)	86	15.0 (10.0–29.5)	16	25.0 (10.3–30.0)	322	15.0 (10.0–30.0)	.68
Previous episode of TB	220	33 (15.0)	86	25 (29.1)	16	8 (50.0)	322	66 (20.5)	<.001
HIV infected	220	125 (56.8)	86	47 (54.7)	16	13 (81.3)	322	185 (57.5)	.13
Cranial nerve palsy	220	52 (23.6)	86	23 (26.7)	16	2 (12.5)	322	77 (23.9)	.51
Hemiplegia	220	31 (14.1)	86	19 (22.1)	16	3 (18.8)	322	53 (16.5)	.23
Paraplegia	220	7 (3.2)	86	9 (10.5)	16	0 (0)	322	16 (5.0)	.03
Quadriplegia	220	4 (1.8)	86	1 (1.2)	16	0 (0)	322	5 (1.6)	1.00
GCS score, median (IQR)	220	14 (11–15)	86	15 (11–15)	16	15 (13–15)	322	14 (11–15)	.40
MRC grade[b]	220		86		16		322		.41
1		87 (39.5)		29 (33.7)		8 (50.0)			
2		83 (37.7)		39 (45.3)		7 (43.8)			
3		50 (22.7)		18 (20.9)		1 (6.2)			
Serum sodium level, mmol/L, median (IQR)	205	126.0 (122.0–131.0)	81	125.0 (121.0–130.0)	16	125.5 (121.0–128.0)	302	126.0 (122.0–131)	.16
Chest radiograph	220		85		16		321		.75
Consistent with TB		124 (56.4)		45 (52.9)		9 (56.3)		178 (55.5)	
Miliary TB		51 (23.2)		16 (18.8)		3 (18.8)		70 (21.8)	
Abnormal other		21 (9.6)		12 (14.1)		1 (6.3)		34 (10.6)	
Normal		24 (10.9)		12 (14.1)		3 (18.8)		39 (12.2)	
CSF results									
WBC count, cells/μL, median (IQR)	216	199.5 (72.8–386)	86	108.5 (32.3–316)	16	253.5 (64.3–340.0)	318	180.0 (58.0–382)	.06
Lymphocytes, %, median (IQR)	210	74.5 (40.0–90.0)	84	86.0 (53.5–100.0)	15	80.0 (34.0–95.0)	309	78.0 (44.0–95.0)	.04
Protein level, g/L, median (IQR)	211	1.48 (1.02–2.38)	82	1.24 (0.81–1.87)	16	1.28 (0.74–2.38)	309	1.41 (0.90–2.30)	.15
Lactate, mmol/L, median (IQR)	199	5.93 (4.50–7.69)	78	5.20 (4.40–6.84)	15	5.50 (4.81–7.30)	292	5.80 (4.49–7.40)	.15
Glucose, mmol/L, median (IQR)	212	1.49 (0.98–2.00)	81	1.58 (1.04–2.11)	16	1.60 (0.95–2.28)	309	1.51 (1.00–2.10)	.52
CSF/blood glucose ratio, median (IQR)	177	0.25 (0.16–0.33)	72	0.25 (0.19–0.34)	15	0.26 (0.18–0.36)	264	0.25 (0.16–0.33)	.69
Ziehl-Neelsen smear positive	210	135 (64.3)	83	45 (54.2)	15	7 (46.7)	308	187 (60.7)	.14
Xpert result positive	202	166 (82.2)	79	62 (78.5)	14	7 (50.0)	295	235 (79.7)	.02
Duration of initial admission, d, median (IQR)	219	30 (23–38)w	86	31 (26–37)	16	30 (12–32)	321	30 (24–37)	.46

Abbreviations: CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; HIV, human immunodeficiency virus; INH, isoniazid; IQR, interquartile range; MDR, multidrug resistant; MRC, British Medical Research Council; RIF, rifampicin; TB, tuberculosis; WBC, white blood cell.

[a]Summary statistics are frequency (%) for categorical and median (IQR) for continuous variables. *P* values are based on Fisher exact test (categorical data) or the Kruskal-Wallis test (continuous data).

[b]MRC denotes modified British Medical Research Council criteria. Grade 1 indicates a GCS score of 15 with no neurologic signs (baseline); grade 2, a score of 11–14 (or 15 with focal neurologic signs); and grade 3, a score of ≤10.

treatment with levofloxacin as part of second-line treatment, 8 days after TBM treatment initiation. Three patients with MDR infection (detected by Xpert MTB/RIF) were treated with additional levofloxacin as part of second-line treatment with a median time to treatment of 10 days (IQR, 6–23 days). Two patients with MDR TBM received additional levofloxacin, but not second-line treatment, 1 in each arm (Figure 1).

Outcome

Overall, 90 of 322 (28.0%) patients died during follow-up: 27 of 86 (31.4%) in the INH-R category, 11 of 16 (68.8%) in the MDR/RIF-R group, and 52 of 220 (23.6%) in the INH-S + RIF-S category (Figure 2A). Multivariable Cox regression identified HIV infection (HR, 2.60 [95% CI, 1.62–4.17], $P < .001$), disease severity grade (HR, 1.07 [95% CI, .62–1.84] for grade 2 vs 1;

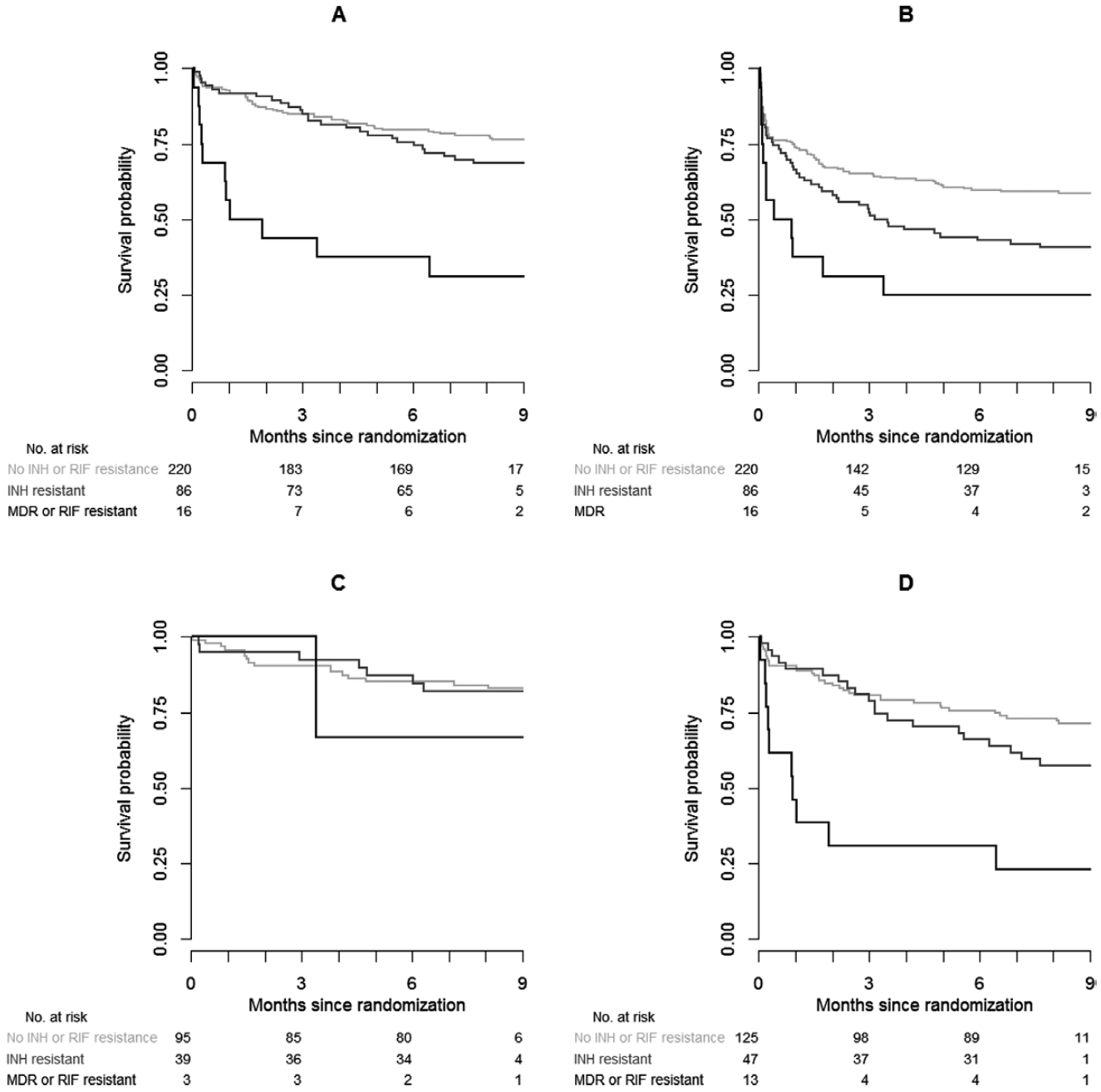

Figure 2. Time-to-event analysis by resistance category and human immunodeficiency virus (HIV) status. *A*, Kaplan-Meier (KM) estimates of survival during 9 months by resistance category. *B*, KM estimates of combined endpoint of time to new neurological event or death by resistance category. *C*, KM estimates of survival by resistance category of HIV-uninfected patients. *D*, KM estimates of survival by resistance category of HIV-infected patients. The light gray line represents patients with tuberculous meningitis (TBM) with no isoniazid (INH) or rifampicin (RIF) resistance. The darker gray line represents patients with TBM with INH resistance, but no RIF resistance. The black line represents patients with TBM with RIF resistance or multidrug resistance. Abbreviations: INH, isoniazid; MDR, multidrug resistance; RIF, rifampicin.

HR, 4.53 [95% CI, 2.71–7.59] for grade 3 vs 1; overall $P < .001$) and MDR infection (HR, 5.91 [95% CI, 3.00–11.64], $P < .001$) as independent predictors of death, but not intensified treatment (HR, 0.92 [95% CI, .60–1.40], $P = .70$) or INH-R (HR, 1.30 [95% CI, .81–2.07], $P = .28$), consistent with previous predictors [14].

Of 322 patients, 154 (47.8%) patients met the combined endpoint of new neurological event and death: 64 (19.9%) neurological events in survivors, 69 (21.4%) neurological events with subsequent death, and 21 (6.5%) deaths in patients without a prior recorded neurological event. Adjusted Cox regression showed a significant effect of isoniazid resistance on the occurrence of any new neurological event or death combined (HR, 1.58 [95% CI, 1.11–2.23], $P = .01$) (Figure 2B).

Mortality in HIV-infected patients was increased (Figure 2C and D). Although mortality appeared higher in INH-R compared to those without resistance in this subgroup, the comparison did not reach statistical significance (adjusted HR, 1.48 [95% CI, .86–2.57], $P = .16$) (Figure 2D).

There was a nonsignificant trend toward worse disability at 9 months in the INH-R category (cumulative OR, 1.44 [95% CI, .90–2.32], $P = .13$). Three of 5 survivors in the MDR/RIF-R group were left severely disabled (Table 2).

Seventy-eight INH-R isolates were available for molecular analysis: 70.5% had mutations in the KatG region (associated with high-level resistance), 19.2% had mutations in the inhA promoter region (associated with low-level resistance), and 12.8% had no mutation identified. Survival by resistance mutation is shown in Supplementary Figure 1. Resistance mutations were not associated with mortality by Cox regression (adjusted HR, 0.80 [95% CI, .31–2.06], $P = .66$).

Intensified Treatment

The overall effect of randomized intensified treatment on survival in INH-R and MDR/RIF-R is shown in Figure 3. In INH-R TBM, when stratified by disease severity and HIV status, the HR of death of intensified treatment vs standard treatment was 0.45 (95% CI, .20–1.02, $P = .06$). For the combined outcome of new neurological event or death in INH-R patients, the stratified HR of intensified treatment was 0.60 (95% CI, .34–1.08, $P = .09$) (Figure 3C). Intensified treatment appeared to be predominantly beneficial in HIV-uninfected patients with isoniazid-resistant infection (Figure 3D and 3E). Of the HIV-uninfected patients, 6 of 17 (35.3%) died in the standard treatment arm, compared with 1 of 22 (4.6%) in the intensified treatment arm (HR, 0.11 [95% CI, .01–.93], $P = .04$) (Figure 3D). In contrast, 10 of 24 (41.7%) of HIV-infected patients with isoniazid resistance died in the standard treatment arm, compared with 10 of 23 (43.5%) in the intensified treatment arm (HR, 0.91 [95% CI, .38–2.18], $P = .83$) (Figure 3E).

Effect of Treatment Adjustment for INH-R

To extricate the effect of treatment adjustments on the over- or underestimation of the effect of early intensified treatment in INH-R TBM, we performed explorative analyses accounting for these late adjustments. Two different Cox regression models were created, including "fluoroquinolone use" as a time-dependent covariate, as initiation of treatment adjustment is dependent on survival up to drug resistance detection. Within the INH-R group, 60 patients received treatment with at least a fluoroquinolone either as part of intensified treatment (n = 45, early) or as targeted INH-R treatment (n = 15, late), while 26 patients did not receive any intensified or adjusted treatment throughout 9 months of treatment. Of those receiving intensified treatment or adjustment with a fluoroquinolone, 15 of 60 (25.0%) died. In the standard treatment group, with no treatment adjustment, 12 of 26 (46.1%) patients died. Cox regression showed a significant benefit of overall "fluoroquinolone use" on survival (HR, 0.38 [95% CI, .18–.80], $P = .01$), stratified by disease severity grade and HIV infection. A significant benefit of later adjustment only could not be established in this population (HR, 0.59 [95% CI, .18–1.91], $P = .38$) (Table 3). Furthermore, the second model showed a more pronounced effect on the rate of death for immediate treatment (HR, 0.34, affecting survival from the time of randomization onward) than of subsequent initiation (HR, 0.59, affecting survival only from the time of fluoroquinolone treatment initiation onward), suggesting that earlier fluoroquinolone treatment is better.

Table 2. Disability Status at 9 Months by Resistance Category

Disability	No or Other Resistance (n = 220)	INH Resistant (n = 86)	RIF Resistant or MDR (n = 16)	Cumulative Odds Ratio[a] (95% CI)	*P* Value
Good	91 (41.4)	31 (36.0)	2 (12.5)	INH: 1.44	
Intermediate	53 (24.1)	17 (19.8)	0 (0)	(.90–2.32)	.13
Severe	20 (9.1)	11 (12.8)	3 (18.8)	RIF MDR: 10.31	
Death	52 (23.6)	27 (31.4)	11 (68.8)	(3.39–31.36)	<.001

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CI, confidence interval; INH, isoniazid; MDR, multidrug resistance; RIF, rifampicin monoresistance.

[a]Cumulative odds ratios for INH and RIF MDR (compared to no or other resistance) were obtained from a proportional odds logistical regression model with the resistance category as the main covariate and adjustment for human immunodeficiency virus status, tuberculous meningitis severity grade, and randomized treatment arm.

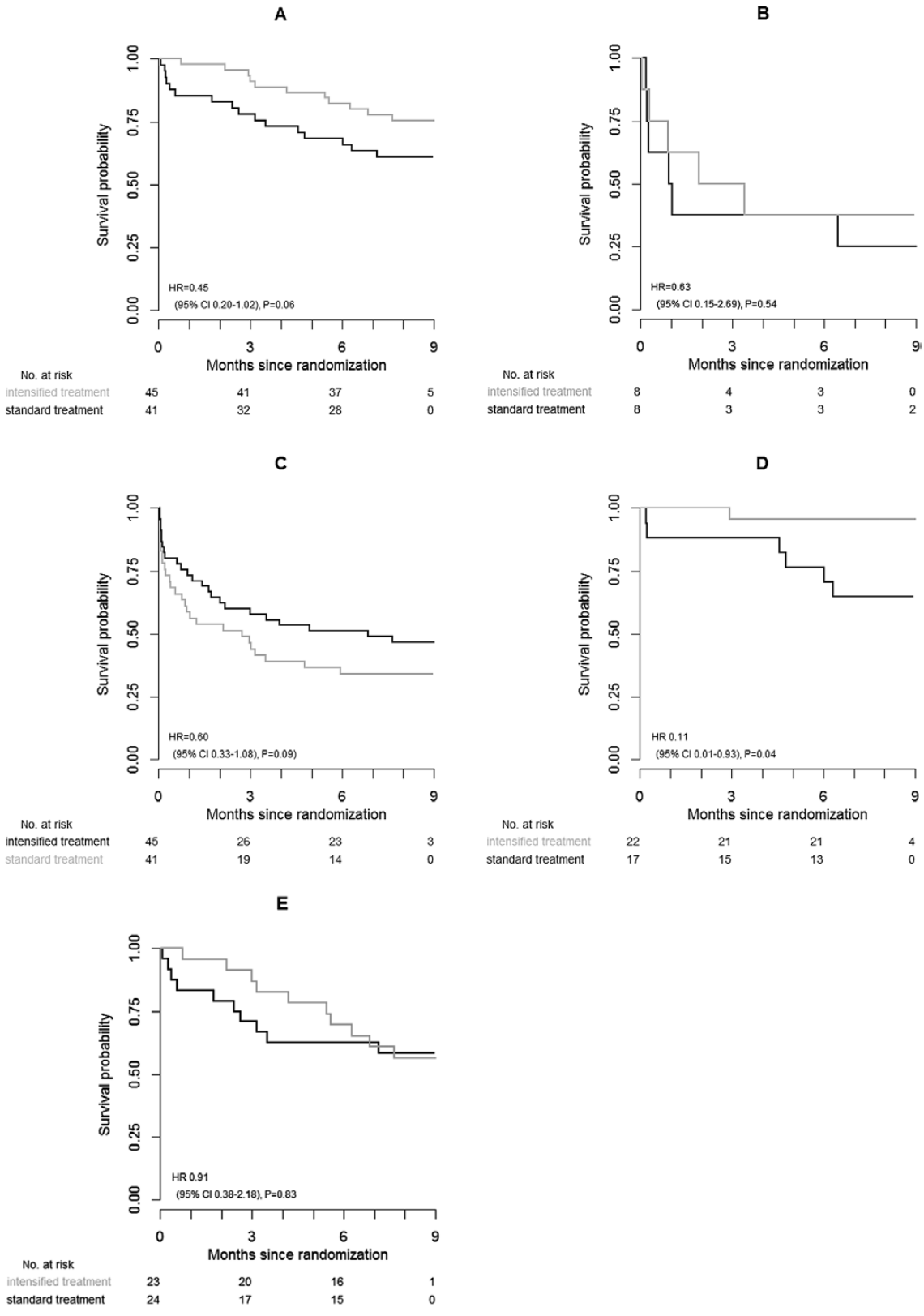

Figure 3. Time-to-event analysis by randomized treatment. *A*, Overall 9-month survival by randomized arm of 86 patients in the isoniazid-resistant, rifampicin-susceptible (INH-R) category. *B*, Overall 9-month survival by randomized treatment arm of 16 patients in the rifampicin multidrug resistance category. *C*, Time to new neurological event or death by randomized treatment of 86 patients in the INH-R category. *D* and *E*, Overall 9-month survival by randomized treatment of 39 human immunodeficiency virus (HIV)–uninfected (*D*) and 47 HIV-infected (*E*) patients in the INH-R category. Cox regression was stratified by tuberculous meningitis severity grade and HIV status. Abbreviations: CI, confidence interval; HR, hazard ratio.

Table 3. Effect of Intensified and Adjusted Treatment in Isoniazid-Resistant, Rifampicin-Susceptible Tuberculous Meningitis

Treatment	Hazard Ratio (95% CI)	*P* Value
Fluoroquinolone use at any time point[a]		
Intensified or adjusted treatment (Tdc)	0.38 (.18–.80)	.01
MRC grade		
1	1 (reference category)	
2	1.60 (.67–3.82)	.29
3	2.96 (1.11–7.89)	.03
HIV infection		
Uninfected	1 (reference category)	
Infected	2.93 (1.31–6.59)	.01
Effect of later adjustments[b]		
Late adjustment regimen (Tdc)	0.59 (.18–1.91)	.38
Randomized arm		
Standard treatment	1 (reference category)	
Intensified treatment	0.34 (.15–.76)	.01
MRC grade		
1	1 (reference category)	
2	1.57 (.66–3.75)	.31
3	3.03 (1.14–8.08)	.03
HIV infection		
Uninfected	1 (reference category)	
Infected	2.82 (1.25–6.39)	.01

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; MRC, Medical Research Council; Tdc, time-dependent covariate.

[a]Cox regression including treatment enrichment with a fluoroquinolone as a time-dependent covariate, adjusted for tuberculous meningitis (TBM) severity grade (MRC grade) and HIV status.

[b]Cox regression including only late treatment adjustment as a time-dependent covariate, while treatment allocation is treated as a baseline covariate, corrected for TBM severity grade and HIV status. Eighty-six patients in the isoniazid-resistant, rifampicin-susceptible category were included in the analysis.

Multidrug-Resistant TBM

In total, 15 patients had MDR and 1 patient had RIF-R TBM. The median time to death of MDR patients was 27 days (IQR, 7–45 days). In the MDR/RIF-R group combined, 4 patients received second-line treatment according to the Vietnamese TB program guidelines (including an injectable aminoglycoside, kanamycin 20 mg/kg/day; a fluoroquinolone, levofloxacin 750 mg/day; pyrazinamide 25 mg/kg/day; and at least 2 of ethionamide 15–20 g/kg/day, prothionamide 15–20 mg/kg/day, cycloserine 15–20 mg/kg/day, or para-aminosalicylic acid 8 g/day). Four patients receiving second-line treatment and 1 patient on standard treatment survived 9 months. All other patients died, with a median time to death of 27 days (IQR, 7–45 days). A summary of individual patient characteristics and treatment are given in Supplementary Table 2. The sample size was too small to detect potential efficacy of intensified treatment (HR, 0.63 [95% CI, .15–2.69], $P = .54$) (Figure 3B).

DISCUSSION

To date, no clinical trials have explored optimal treatment regimens for drug-resistant TBM. In our blinded randomized controlled trial of intensified antituberculosis treatment for TBM, intensified treatment led to reduction of mortality in INH-R infection. This finding is crucial, as isoniazid resistance is increasingly prevalent and associated with worse outcome. Improved treatment will impact mortality and morbidity of TBM. A considerable proportion of baseline isolates were INH-R (26.7%), reflecting the high incidence of isoniazid-resistant TB in Vietnam.

We performed a time-dependent Cox regression, to correct for relevant treatment adjustments made for drug-resistant infection. This showed that treatment benefit in INH-R was mainly generated by the early randomized intervention. We could not establish whether fluoroquinolones improved outcomes from INH-R TBM when added later in treatment based on DST, but our findings underscore the necessity for improved early drug resistance detection. The use of rapid molecular testing of pulmonary TB by Xpert MTB/RIF has facilitated early diagnosis of rifampicin resistance and second-line treatment initiation [22]. Xpert MTB/RIF has been recommended by the World Health Organization for direct use on CSF as the initial diagnostic test [21, 23]; however, isoniazid resistance cannot be detected by the current Xpert cartridge. The line-probe assays, such as Genotype MTBDR (Hain Life-Science, Nehren, Germany), can detect both rifampicin and isoniazid resistance. Although the bacillary load in CSF is likely too low for direct use, these assays may be used on bacteria cultured from CSF samples, which should reduce time to resistance detection.

Eleven of 16 (68.8%) of those with MDR/RIF-R TBM died by 9 months. Of the 5 survivors, 4 received early second-line treatment, based on the results of Xpert MTB/RIF resistance testing. Early diagnosis of MDR TBM allows the instigation of second-line regimens, but few data are available to guide regimen tailoring.

The limitations of this study include the potential for missing patients with drug-resistant disease, due to the poor sensitivity of CSF culture. Only 39.4% (322/817) had a known drug resistance profile; the impact of resistance on the remaining patients could not be assessed. Positive CSF culture may be linked to higher bacterial loads and worse outcomes, which may confound our findings. We had no minimum inhibitory concentrations or pharmacokinetic data for isoniazid. Higher-dose rifampicin and fluoroquinolone were given jointly as 1 intervention; hence, it is impossible to tease the effect of both drugs apart. Higher doses of rifampicin have been associated with improved outcome in an Indonesian study [24]. In addition, the impact of later (nonrandomized) fluoroquinolone treatment on outcome is hard to assess given the small numbers and the potential for differences in disease severity and postrandomization management to confound the analysis.

In conclusion, early intensified treatment increased survival in patients with INH-R TBM. Early detection of drug-resistant TBM is key to improving outcomes.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Disclaimer. The funders played no role in the design, interpretation, or publication of the study.

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Statistical analysis plan for the 05TB study (ISRCTN61649292)

"Intensified treatment with high dose rifampicin and levofloxacin compared to standard treatment for adult patients with tuberculous meningitis (TBM-IT)"

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Reviewed by Guy Thwaites, Jeremy Day, Maxine Caws
Version: 2.0, April 5, 2015 – final version prior to unblinding

Purpose

This document details the planned analyses and endpoint derivations for the ISRCTN61649292 trial as outlined in the published study protocol (Trials 2011, 12:25; doi:10.1186/1745-6215-12-25). It focuses on the analysis for the main clinical trial publication and does not include analysis for any subsidiary studies.

Statistical software

Data derivations will be performed with the statistical software SAS v9.2 (SAS Insitute, Cary, North Carolina, US). All statistical analyses will be performed with the statistical software R using the current R version at the time of the final analysis (R Foundation for Statistical Computing, Vienna, Austria).

Analysis populations

Intention-to treat population (ITT)

The main analysis population for all analyses is the full analysis set including all randomized patients and analysis is according to the randomized treatment arm.

Per-protocol population

The main comparison for the primary endpoint and Kaplan-Meier survival curves will also be done on the per-protocol population. The following patients from the ITT will be excluded from the per-protocol population:

- Patients without TBM according to the diagnostic score [both unlikely TBM and confirmed other diagnosis, see note in section "Baseline characteristics→ Neurological and TBM features" below]
- Patients with MDR-TBM
- Those receiving < 50 days of administration of study drug for other reasons than death.]. For early deaths, subject who stopped either study drug >7 days prior to death will also be excluded. [Study drugs are recorded as TBDR.TBDrug=1 ("Rifampicin/Placebo (Study Drug)") and TBDR.TBDrug=2 ("Levofloxacin/Placebo (Study Drug)")) and subjects might have multiple records for each drug in case of short treatment interruptions.]
  Note: Protocol wording for this is "<2 months of randomized study drug for reason other than death". At the time of the writing of this analysis plan it was decided that short interruptions were acceptable and hence the threshold of "<2 months" is interpreted as "<50 days".

Baseline characteristics

Baseline characteristics will be summarized as median (IQR) for continuous data and n(%) for categorical data. The amount of missing data for each baseline characteristic will also be displayed.

Formal comparisons of baseline characteristics between study arms are discouraged by most statisticians (see e.g. Senn SS (2008): Statistical Issues in Drug Development, 2nd Edition, Wiley [p. 98f]) but mandated by some journals. To satisfy all potential publishers, we will calculate p-values (based on the Wilcoxon rank sum test and Fisher's exact test for continuous and categorical data, respectively) but will only report them if mandated by the journal.

Baseline/date of randomization is defined as the date of the first dose of study treatment (first TBDR.DateStart for TBDR.TBDrug=1 ("Rifampicin/Placebo (Study Drug)") or 2 ("Levofloxacin/Placebo (Study Drug)")). In case a subject did not receive any study treatment at all, baseline will be defined as the date of the enrolment assessment (ASREC.StudyVisit=0 "Baseline").

The following baseline characteristics will be summarized by treatment arm [with derivation rules in brackets]:

Demographics and history

- Age [Enrolment year – SCREX.DateBirth_Y]
- Sex [ENREX.Sex; 1="male", 2="female"]
- (Admission) hospital ["PNT" if first digit of patient number is 1 or 2, "HTD" if first digit is 3 or 4]
- Weight, BMI [ENREX.Weight/(ENREX.Height/100)^2]
- Duration of illness [SCREX.DayIlless]
- TB symptoms: Persistent cough for more than 2 weeks [SCREX.Cough2W; 1="yes", 2="no"], weight loss [SCREX.WeightLoss], night sweats [SCREX.NightSweat]
- General examination: Temperature [SCREX.Temp], pulse [SCREX.Pulse], systolic and diastolic blood pressure [SCREX.SBP and SDP (recoded as "DBP")] Chronic medical illness [ENREX.ChronicIll; 1="yes", 2="no"]: diabetes (ENREX.Diabetes; 1="yes", 0="no"), liver disease [ENREX.Liver], renal disease [ENREX.Renal], other disease [ENREX.OtherIll]
- Contact with someone known to have TB within the past year [SCREX.ContactTB]
- Past history of TB [ENREX.PreviousTB]; pulmonary TB? [ENREX. PulTB; 1="yes", 2="no"]
- Currently on TB treatment? [Due to a CRF design flaw, the corresponding CRF question (ENREX.TBTreat) is non-informative because it was only answered if the patient had a past history of TB. Thus, rather than relying on this CRF question, the variable will be derived based on the recorded TB drug administration form as follows: The patient is considered currently on TB treatment if any Turbezid (TBDR.tbdrug=3) or Turbe (TBDR.tbdrug=7) is recorded with start date before enrolment and stop date after enrolment or missing. The duration of prior TB treatment will also be derived.]
- HIV status [HIVDR.HIVStat; 1="positive", 2="negative"]
- HIV status used in stratified randomization ["positive" if first digit of patient number is 1 or 3, "negative" if first digit is 2 or 4]
- Known HIV diagnosis prior (>1 month) to enrollment [ENREX.KnownHIV with ENREX.DateHIV at least 28 days before baseline]
- On ARV treatment at enrollment [any ARV recorded with start date prior to baseline and stop date missing or after baseline, i.e. HIVDR_ARV.DateStart<baseline date and (HIVDR_ARV.DateStop>= baseline date or missing)]

- Ever used intravenous drugs [ENREX.Intravenous]

Neurological and TBM features
- Cranial nerve palsy any ["no"= "none ticked", i.e. SCREX.CNPnone=1, "yes" if . SCREX.CNPnone=0]
- Cranial nerve palsy details ["nerve 6" if SCREX.CNPLeft6=1 or SCREX.CNPRight6=1, "other nerve(s)" otherwise]
- Hemiplegia [SCREX.Hemi]
- Paraplegia [SCREX.Para]
- Quadriplegia [SCREX.Quadri]
- Urinary retention present at baseline [NEREVE.NeuDesc=12, NeuSymp=2]
- Severe headache present at baseline [NEREVE.NeuDesc=11, NeuSymp=2]
- GCS at screening [SCREX.Gcs)
- GCS at enrolment [GCS.gcs for week 0, enrolment (GCS.WeekMonth=1)]
- GCS at baseline [GCS at enrolment [if non-missing], otherwise GCS at screening]
- TBM grade at baseline [ENREX.TBMGrade]
- TBM grade used in stratified randomization [second digit of patient number]
- TBM diagnosis according to the diagnostic score

Note on the diagnostic score: The TBM diagnostic score published in (Lancet Infect Dis 2010; 10: 803–12) will be used and subjects will be categorized as "definite"/"probable"/"possible"/"no TBM" with "no TBM" further divided into "unlikely" (by score) or "confirmed other diagnosis". Details regarding the derivation rules are in a separate document.

Baseline investigations
- Chest X-Ray at screening [SCREX.XrayResult; 1="Normal" ,2="Abnormal Miliary TB", 3="Abnormal Consistent with TB", 4="Abnormal Other"]
- Haematology [all measurements in HAE] – closest to baseline within -7/+3 days
- Biochemistry [all measurements in BIO] – closest to baseline within -7/+3 days
- CD4 cell count (HIV-positives only) [IMMU.CD4] - closest to baseline within +/- 14 days
- Hep B/C co-infection (VIRO.HBsAg, VIRO.HCV) – positive, if at least one positive result recorded (at any date, i.e. regardless of timing); negative, if at least one result recorded and all recorded results are negative
- CSF appearance and color at screening [SCREX.CsfApp and CsfColor]
- CSF [all measurements recorded in CSF_HAE, CSF_BIO]- closest to baseline within -7/+3 days [CSF to plasma glucose ratio will also be calculated if there is a matching plasma glucose measurement within +/-1 hour of the corresponding CSF measurement.]

Note: For lab measurements for which a measurement a few days after randomization is still considered as "baseline", preference will be given to measurements prior to or at baseline (if available).

TB drug resistance at baseline
- Resistance based on conventional DST against the following antibiotics: STR, INH, RIF, and EMB ["yes" if one or more CSF (lab dataset CSF), sputum (SPUTUM), gastric fluid (GASTRICFLUID), or other sample (OTHERSAMPLEs) is positive at baseline; "no" if at least one baseline sample with non-missing resistance is documented and it is negative).
  Note: PZA resistance will not be reported as the assay is unreliable.
- RIF resistance according to GeneXpert (CSF.csfGeneXpertDe=1 "Rifampicin resistant detected").

- Drug resistance category (MDR-TB / rifampicin (mono-)resistant / isoniazid resistant / no or other resistance) – derivation based on conventional DST only (not GeneXpert resistance result):
  - MDR-TB: resistance against both INH and RIF according to the above definition
  - Rifampicin resistant: resistance against RIF according to the above definition but not MDR-TB
  - Isoniazid resistant: resistance against INH according to the above definition but not MDR-TB
  - No or other resistance: no resistance to INH or RIF documented

Note: Baseline for the derivation of resistance is defined as any sample within -7/+3 days of baseline.

Planned analyses
Baseline table for all variables as detailed above for the ITT population.

We plan that the following variables will be included in the baseline table of the corresponding publication and a separate table with these variables only will be created for both the ITT and the per protocol population:

- Age, gender, weight, diagnosis at baseline (definite, probable, possible, unlikely TBM according to consensus definition), previous TB episode, duration of symptoms, GCS, cranial nerve palsy, hemiparesis, paraparesis, quadri-paresis, pulmonary/ miliary TB on chest Xray, baseline sodium level, MRC grade, HIV status, CD4 cell count, drug resistance category.

Primary endpoint – overall survival during the 9 month follow-up period

Derivation
Definition time to death: [date of death or censoring]-[date of randomization]+1
Definition event indicator: =1 if patient died =0 otherwise

[Date of randomization]: as defined at the beginning of the "Baseline characteristics" section.

[Date of death]:OUTC.DateDeath (if OUTC.Reason9FU=1 "(1) Patient died")
[Date of censoring] – last date patient is known to be alive:
- If OUTC.Completed9FU=1 "yes, completed 9 months of follow-up": OUTC.DateAssess
- If OUTC. Completed9FU=2 "did not complete 9 months of follow-up" and patient not dead: OUTC.DateAlive ("last date patient was known to be alive")
- If outcome form OUTC is missing, the last date alive will be defined as the latest of: last visit data, last neurological or clinical adverse event start or stop date, last hematology, chemistry in blood or csf date. [Note: This last part is required only while the study is ongoing. At the end of the study, there must be an outcome form OUTC for every subject.]

The date of the actual 9-month follow-up visit will be used in the calculation. However, for subjects for whom the 9 month visit was delayed by >2 weeks or those who died >2 weeks after month 9 will be treated as censored on month 9 plus 2 weeks (day 289) instead.

Planned analyses

Primary analysis

Cox regression with treatment as the only covariate and stratification by HIV status (positive/negative) and TBM disease severity (modified MRC grade I,II or III) at baseline.
Stratified Cox regression as implemented in the R function survival::coxph will be used with default arguments (e.g. tie handling according to the Efron approximation). Of note, the protocol pre-specified a log-rank test rather than Cox regression but the two approaches are essentially equivalent and Cox regression has the benefit of providing a treatment effect estimate (HR) and associated 95% confidence interval in addition to the p-value. In case the actual recorded TBM grade differs from the TBM grade used in the stratified randomization, the actual TBM grade will be used.

The proportional hazards assumption will be formally tested based on scaled Schoenfeld residuals and visually assessed by a plot of the scaled Schoenfeld residuals versus transformed time (as implemented in R function survival::cox.zph). In case of a significant test, a formal comparison of 9 month survival probabilities between the two groups will also be performed (using Kaplan-Meier estimation and Greenwood's formula to approximate variance).

Kaplan-Meier estimates of the survival curve by treatment arm

- Plots for all patients and subgroups defined by HIV status and TBM grade
- Explicit numeric estimates (with 95% CI) at 3, 6, and 9 months for all patients and by subgroups

Cox regression

- Include the following baseline covariates (in addition to the treatment group): TBM severity (grade I, II, III), HIV status (positive/negative), participating (admission) hospital (PNT/HTD), previous TB episode (yes/no), drug resistance category (MDR-TB / rifampicin (mono-)resistant / isoniazid resistant / no or other resistance; subjects without a resistance result will be treated as a separate category "unknown resistance" for the purpose of the Cox regression).
  Note: The protocol specified "previous TB treatment" rather than "previous TB" episode but only the latter was collected on the CRF.
- A separate analysis for HIV positive patients only including the above covariates and additionally prior ARV therapy (yes/no) and CD4 cell count at baseline.

Note: Derivation rules for the baseline covariates are as per the "Baseline characteristics" section above.

Pre-defined subgroup analyses

The following subgroups are pre-defined:

- Per protocol analysis
- TBM grade (I, II, or III)
- HIV status (positive/negative)
- Previous TB episode (yes/no)*
- On TB treatment at enrolment (yes/no)*
- TBM diagnostic category (definite / probable / possible) – Note: this is not mentioned in the protocol but formally added as a pre-defined subgroup analysis in this analysis plan
- drug resistance (MDR-TB (including rifampicin (mono-)resistant, if any) / isoniazid resistant / no or other resistance) – only culture-confirmed patients (in CSF) will be included in this analysis

* replacing the pre-defined subgroup "previous TB treatment" which was not collected on the CRF.

A Cox regression (stratified by HIV status and disease severity) will be calculated for all subgroups and Kaplan-Meier curves displayed. Potential heterogeneity of the treatment effect across sub-groups will be tested using likelihood ratio tests for an interaction term between treatment and the grouping variable.

Additional pre-defined analyses

- Plot of estimated hazard function in both arms assuming a piecewise constant (exponential) hazard for each monthly interval of follow-up (time dependent treatment effect, for patients on intensified treatment, the hazard may only be lowered in the first 2 months of treatment).

Other exploratory analysis

Will be performed as appropriate.

Treatment of missing values (multiple imputation)

Multiple imputation by chained equations as implemented in the R package mice will be used to deal with missing covariate values for the Cox regression analysis. As some baseline characteristics (CD4 cells count, prior ARV therapy) and outcomes (time to new new or recurrent AIDS defining illness or death) were only assessed in HIV-positives, separate imputations will be performed in HIV-negatives and HIV-positives, respectively. Twenty imputed sets will be generated and the dataset for multiple imputation will include the following variables:

- Randomization assignment and the following baseline variables: TBM severity (grade I, II, III), participating (admission) hospital (PNT/HTD), previous TB episode (yes/no), resistance category (MDR-TB or rifampicin (mono-)resistant / isoniazid resistant / no or other resistance / unknown resistance, i.e. no positive culture result on which resistance testing was performed), prior ARV therapy (yes/no)* and CD4 cell count*.
- Outcomes: Overall survival, neurological disability at month 2 (+/-14 days), 6 (+/-28 days), and 9 (+/-28 days), time to new neurological event or death, time to new or recurrent AIDS defining illness or death*.

  Time-to-event outcomes will be included as the cumulative baseline hazard at the observed event or censoring time and an event indicator as recommended by White and Royston (Statist. Med. 2009; 28:1982–1998).

*: Only used in HIV-positives.

Secondary endpoint – neurological disability

Derivation

The disability score was assessed at 2 (+/-14 days), 6 (+/-28 days) and 9 (+/-28 days) month follow-up. Of primary interest is the 9-month score. The score is composed of two sub-scores:

The “two simple questions” score:
if answer to the first question= yes; outcome is classified as ‘severe disability’ (DISAB.NeedHelp)
if answer to the second question = yes; outcome is classified as ‘intermediate’ (DISAB.IllnessProblem)
if answer to both questions = no; outcome is classified as ‘good’ (DISAB.NeedHelp/IllnessProblem)

The Rankin score: (DISAB.CurrCondition)
0 is the best score (DISAB.CurrCondition=1), 5 is the worst score (DISAB.CurrCondition=6)
If Rankin score=0 (DISAB.CurrCondition=1); outcome will be classified as ‘good’
If Rankin score =1 or=2 (DISAB.CurrCondition=2 or 3); outcome will be classified as ‘intermediate’
If Rankin score =3, =4 or=5 (DISAB.CurrCondition=4 to 6); outcome will be classified as ‘severe disability’

The worst disability outcome from either questionnaire ("2 simple questions" or Rankin score) will be used for analysis. Disability will be defined as "death" if the patient died before the scheduled time point.

The 9-month score is defined as the patients' last disability assessment if this last disability assessment was performed at 9 months (274 days +/-28 days) or the patient died before month 9.
Patients lost to follow-up will be analyzed according to their last recorded disability status (month 2 or 6) and if no disability assessment at a scheduled visit time is available at all, the 9-month score will be treated as missing.

Planned analysis
The ordinal 9-month score ("good">"intermediate">"severe">"death") will be compared between the two arms with a proportional odds logistic regression model depending on treatment arm, HIV status, and TBM severity grade. The result will be summarized as a cumulative odds ratio with corresponding 95% confidence interval and p-value. This analysis is preferred to the pre-defined analysis in the protocol because it also provides an estimate of the intervention effect in addition to the p-value and because it is straightforward to apply the same analysis technique to a complete dataset based on multiple imputation (see below).
In addition, the 9-month score will be compared between the two arms with a linear-by-linear association test with stratification by HIV status and TBM severity grade at baseline as implemented in R function coin::lbl_test will be used. This is a variant of the Cochran-Mantel-Haenszel test (pre-defined in the protocol) which takes into account that the disability score is ordinal.
The outcome will also be analyzed based on multiple imputation (see section "Treatment of missing values (multiple imputation)" above). As it is unclear how to pool results from the stratified linear-by-linear association test described above across multiple imputed sets, the analysis based on multiple imputation will be based on proportional odds logistic regression only.
The analysis will be performed in all patients and for HIV-positives and HIV-negatives separately.

Secondary endpoint – Time to new neurological event or death

Derivation
[date of first neurological event or death or censoring]-[date of randomization]+1

A new neurological event is defined any of the following:

- Any of the following neurological adverse events (with onset after enrollment, i.e. NEREVE.NeuSymp=1):
  - cerebellar symptoms (NEREVE.NeuDesc=2)
  - coma/consciousness deterioration (NEREVE.NeuDesc=1)
  - hemiplegia (NEREVE.NeuDesc=5), paraplegia (NEREVE.NeuDesc=7), tetraplegia (NEREV.NEUDesc=21), or monoplegia (NEREV.NEUDesc=20)
  - neurological detoriation requiring ventilation (NEREVE.NeuDesc==24)
  - seizures (NEREVE.NeuDesc==3)
  - cerebral herniation (NEREVE.NeuDesc==6)
  - cranial nerve palsy (NEREVE.NeuDesc==4)
- or a fall in Glasgow coma score (GCS) by ≥ 2 points for ≥ 2 days from the highest previously recorded GCS (including baseline); i.e. any GCS decrease by ≥ 2 points which is followed by a GCS which also

shows a decrease by ≥ 2 points at the next assessment or by death within 7 days and before any further GCS measurements are recorded.

Planned analysis

Stratified Cox regression, Kaplan-Meier curves, and subgroup analyses by TBM grade and HIV status as detailed for the primary endpoint analysis.

Secondary endpoint – new or recurrent AIDS defining illness or death (HIV-positive patients only)

Derivation

[date of first new or recurrent AIDS defining illness]-[date of randomization]+1

All new AIDS defining illnesses are recorded as new clinical events (CLINEVE.Occurrence=1) with CLINEVE.SYMP=10 ("New AIDS defining illness")

Planned analysis

Stratified Cox regression (by TBM grade) and Kaplan-Meier curves as detailed for the primary endpoint analysis.

Secondary endpoint – adverse events and TB treatment interruptions

Derivation

Adverse events (AE) are all events recorded on the CLINEVE (clinical adverse events) or NEREVE (neurological events) forms except for signs already present at baseline which were also recorded on these forms. A worsening in AE grade or seriousness was recorded as a new adverse event on the database but for the purpose of reporting, such AE with overlapping stop and start dates will be pooled to a single AE episode and the worst reported AE grade and seriousness of its components will be assigned to the episode.
Only grade 3 or 4 AE were systematically collected in this study and every recorded AE with grade 3 or 4 or missing grade will be designated a *grade 3 or 4 AE*. *Serious AE* are indentified in the database as those AE with SAE=1 "Yes".

AE leading to TB treatment interruption or stopping are those whose event numbers (CLINEVE.RECNO or NEREVE.RECONO) were also recorded as reasons for stopping drugs on the TB drug administration page (TBDR.AENo which refers to neurological events if TBDR.CLINER=1 "NEREVE" and clinical events if TBDR.CLINER=2 "CLINEVE").
If the stop date of TB treatment (TBDR.DateStop) coincides with the date of death (OUTC.DateDeath), the reason for the interruption of drugs is death rather than the event itself. In these cases, the event will not be recorded as an AE leading to drug interruption.

New laboratory abnormalities are defined as any worsening of a lab value to grade 3 or 4 (including changes from grade 3 to 4) compared to the subject's previous lab value. In addition, to be conservative, if a subject's baseline lab value was missing, the first post-enrolment lab value was also considered to be a new lab abnormality if it was of grade 3 or 4. A grading table for laboratory abnormalities is provided in the Appendix.

Planned analysis

- Summary of grade 3 and 4 AE – overall and by HIV-status (positive/negative)
  The number of patients with any such event and specific events, respectively, will be summarized and compared between the two treatment arms. The total number of AE episodes (including repeated events of the same type within patients) will also be summarized but no statistical comparison will be performed.
  Comparison between the two arms will be based on Fisher's exact test. Of note, the protocol specified a generalized Cochran-Mantel-Haenzsel test, stratified by HIV status, and TBM disease severity rather than Fisher's exact test. We prefer Fisher's exact test because it is directly reproducible based on the presented summary data and because the low numbers of some events requires an exact test which is unaffected by sparsity. Moreover, due to the large number of events compared and the resulting multiplicity issue, these tests should be interpreted as descriptive statistisc only.
- Summary of serious AE (same summary as for grade 3 and 4 AE)
- Summary of AE leading to treatment interruption (same summary as for grade 3 and 4 AE), other than drug interruptions for death
- Summary of laboratory abnormalities (same summary as for grade 3 and 4 AE)

Additional auxiliary analyses

- Summary of CD4 cell count at 9 months (+/-28 days) and change in CD4 cell count from baseline to 9 months in HIV-positive survivors
- Summary of time to ARV initiation in HIV-positives not on ARV at enrolment
- ECG were scheduled at baseline, week 1, and week 4. The QT interval will be corrected according to the following formulas:
  - Bazzett correction: QTcB =ECG.QtInterval/sqrt(RR) where
    RR= 60/heart rate=60/ECG.rate
  - Framingham correction: QTcF= 1000*(ECG.QtInterval/1000+ 0.154 (1 – RR))
    [Note that in the database, QtInterval is given in ms but the Framhingham formula requires seconds which is achieved by the formula above.]

  Amongst these two, the Framingham correction is usually considered more reliable. The QTc will further be classified as "normal" (<450 for males, <460 for females), ">=450 (m) or >=460 (f) but <=500", ">500".
  QTc according to both corrections will be summarized for all recorded ECG by treatment arm with stratification by time (baseline, day 2-6,day 7-9, day 10-25, day 26-30, day >30).

Appendix: Grading of laboratory abnormalities

	Grade 3	Grade 4
Haematological		
Haemoglobin	6.5 –7.9g/dl	<6.5 g/dl
White cell count	1.0 - 1.9 K/µl or g/L	<1.0 K/µl or g/L
Neutrophils	NEU % xWBC=NEU K/µl :0.5 – 0.74 K/µl	NEU % xWBC=NEU K/µl <0.5 K/µl
Platelets	20 - 49 K/µl or g/L	<20 K/µl or g/L
Biochemical		
Sodium	116-122 mmol/l	<116 mmol/l
Sodium	158 – 165 mmol/l	>165 mmol/l
Potassium	2.0 – 2.4 mmol/l	<2.0 mmol/l
Potassium	6.6 – 7.0 mmol/l	>7.0 mmol/l
Blood glucose	1.7 – 2.1 mmol/l or 30-39 mg/dl 14.1-28 mmol/l or 256-506 mg/dl	<1.7 mmol/l or < 30 mg/dl >28 mmol/l or >506 mg/dl
Creatinine	Male >360 – 720 µmol/L Female >300-600 µmol/L	Male >720 µmol/L Female >600 µmol/L
Bilirubin	Total Bilirubin > 42.5 – 85 µmol/L	Total Bilirubin > 85 µmol/L
AST	>185 – 370 U/L	>370 U/L
ALT	>200 – 400 U/L	>400 U/L