UNIVERSITY OF CAPE TOWN

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Investigations into HIV-associated tuberculous meningitis

Thesis presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Medicine
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Supervisors:
Professor Robert J Wilkinson
Associate Professor Graeme Meintjes
Associate Professor Katalin Wilkinson

“Train up a child in the way he should go: and when he is old, he will not depart from it.”

Proverbs 22:6

To my beloved parents, Hannes and Christa, who continue to teach me by example.

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Declaration

I, Suzaan Marais, do hereby declare that this thesis includes six journal manuscripts. Five of these manuscripts (Chapters 2- 5) have been published and one (Chapter 6) has been submitted for publication in an international journal. The contents of these manuscripts remain unchanged from that which have been published or submitted for publication. The manuscripts are listed below with a description of my contribution and the contributions of each author to the study.

Chapter 2

Marais S, Pepper DJ, Marais BJ, Török ME. **HIV-associated tuberculous meningitis-diagnostic and therapeutic challenges.** *Tuberculosis (Edinb)* 2010;**90**:367-74.

Suzaan Marais was the lead author on this review paper. She was responsible for reviewing the current literature, writing the first draft of the manuscript and finalizing the manuscript for submission following input from co-authors. Her supervisor, Robert J Wilkinson was the Commissioning Editor of *Tuberculosis* who invited this manuscript submission. He was therefore involved in the overall review of the quality and the content of the paper in addition to peer-review by independent reviewers.

Dominique Pepper, and Ben Marais critically reviewed the manuscript.

Estee Török supervised writing of, and critically reviewed, the manuscript.

Chapter 3

Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, Donald PR, Wilkinson RJ, Marais BJ. **Tuberculous meningitis: a uniform case definition for use in clinical research.** *Lancet Infect Dis* 2010;**10**:803-12.

This manuscript arose from an international tuberculous meningitis workshop that took place in Cape Town, South Africa, in 2009. The ultimate aim of this workshop was to establish a consensus case definition for tuberculous

meningitis to allow for standardization in future clinical research reports. Suzaan Marais was responsible for leading the organizing of the meeting hosted by the University of Cape Town. Fourty-one leading tuberculous meningitis researchers and clinicians with experience in managing patients with tuberculous meningitis from seven countries attended the workshop. Over the course of three days, several groups presented their clinical experience with tuberculous meningitis, which was followed by a discussion of the criteria to be included in a clinical definition that would be applicable to all tuberculous meningitis patients, regardless of HIV status or age. Following the meeting, Suzaan Marais was given the role of heading the writing committee for the consensus case definition. Over the following year she combined factors suggested during the meeting and those found relevant from her literature review to produce a draft manuscript that presented this case definition. The case definition was further developed through incorporating several rounds of comments from all co-authors via email correspondence by Suzaan Marais, who then finalized the manuscript for publication.

Guy Thwaites G, Johan F Schoeman, Estee Török, Usha K Misra, Kameshwar Prasad, Peter R Donald, Robert J Wilkinson and Ben J Marais were the other members of the writing committee who provided input by review of the manuscript.

Chapter 4

Part 1

Marais S, Pepper DJ, Schutz C, Wilkinson RJ, Meintjes G. **Presentation and outcome of tuberculous meningitis in a high HIV prevalence setting.** *PLoS One* 2011;**6**:e20077

Suzaan Marais was the lead investigator on this study. She designed the study, and collected, entered and analysed clinical data. She wrote the final manuscript, which was critically reviewed by all authors.

Dominique J Pepper was involved with study design, data collection and data analysis.

Charlotte Schutz assisted with data collection.

Robert J Wilkinson contributed to data analysis.

Graeme Meintjes supervised writing of the manuscript and was involved with study design and data analysis.

Part 2

Asselman V, Thienemann F, Pepper DJ, Boulle A, Wilkinson RJ, Meintjes G, Marais S. **Central nervous system disorders after starting antiretroviral therapy in South Africa.** *AIDS* 2010;**24**:2871-6.

Suzaan Marais and Valerie Asselman were co-investigators of this study and contributed equally to study design, assessment of study participants, clinical data extraction and data analysis. They further contributed equally to writing the final manuscript, which was revised critically by all other authors.

Friedrich Thienemann was involved in study design, assessing participants and collecting clinical data and data analysis.

Dominique J Pepper contributed to study design.

Andrew Boulle assisted with statistical analysis.

Graeme Meintjes conceived and designed the study. He was responsible for supervision of the study and he further contributed to patient assessment and clinical data collection.

Chapter 5

Marais S, Meintjes G, Pepper DJ, Dodd LE, Schutz C, Ismail Z, Wilkinson KA, Wilkinson RJ. **Frequency, severity, and prediction of tuberculous meningitis immune reconstitution inflammatory syndrome.** *Clin Infect Dis* 2013;**56**:450-60.

Suzaan Marais was lead investigator of this prospective observational study. She designed the study. She was involved in patient assessment and clinical data collection and data entry. She performed cytokine analysis in the laboratory at the Institute of Infectious Disease and Molecular Medicine. She performed both clinical and laboratory data analysis and wrote the final manuscript which was reviewed critically by all authors.

Graeme Meintjes supervised the clinical study conduct and writing of the manuscript. He was involved in study design, patient assessment and clinical data analysis.

Dominique J Pepper was involved with study design, patient assessment and clinical data collection and analysis.

Charlotte Schutz and Zahiera Ismail worked on the study at the clinical site and were involved in patient assessment and clinical data collection.

Lori E Dodd assisted with statistical analysis.

Katalin A Wilkinson performed and supervised laboratory assays and was involved in this data analysis.

Robert J Wilkinson was involved in conception and design of the study. He contributed to clinical and laboratory analysis and was responsible for overall study supervision.

Chapter 6

Marais S, Wilkinson KA, Lesosky M, Coussens A, Deffur A, Pepper DJ, Schutz C, Ismail Z, Meintjes G, Wilkinson RJ. **Neutrophil-associated central nervous system inflammation in tuberculous meningitis immune reconstitution inflammatory syndrome: A prospective observational study.** *Submitted for publication to The Lancet Neurology*.

This immunology study was conducted on samples collected from patients during the observational study of TBM-IRIS (Chapter 5). The role of Suzaan Marais and others in the clinical aspects of this study have been described

above (Chapter 5).

The immunology study was co-designed and supervised by Robert J Wilkinson and Katalin A Wilkinson. The assays (Luminex multiplex and ELISA) were performed by Suzaan Marais and Katalin A Wilkinson.

Maia Lesosky, Anna Coussens and Armin Deffur assisted with statistical analysis.

Graeme Meintjes contributed to data analysis and interpretation.

Suzaan Marais was involved in study design and was responsible for laboratory data entry and analysis. She wrote the first draft of the manuscript, which was critically reviewed by all co-authors prior to submission.

I confirm that no part of this thesis has been, is or will be submitted for a degree to this or any other institute. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purposes of research or teaching.

This thesis is presented for examination in fulfillment of the requirements for the degree of Doctor of Philosophy in Medicine.

Signed,

Signed by candidate

Suzaan Marais
Date: February 2014

Abstract

PhD candidate: Suzaan Marais

Title: Investigations into HIV-associated tuberculous meningitis

Date: February 2014

Background

Tuberculous meningitis (TBM) is a common form of tuberculosis in high TB incidence settings. However, the burden of disease and outcome in affected adults is unknown in Cape Town. The diagnosis of TBM is often challenging, particularly in HIV co-infected patients and no standardized clinical case definition exists. An emerging complication that contributes to poor outcome in HIV-associated TBM is neurological TB immune reconstitution inflammatory syndrome (TB-IRIS).

Methods

A consensus clinical TBM case definition was developed following a TBM meeting that I co-ordinated. I led two observational studies that determined the burden of HIV-associated TBM and neurological TB-IRIS at a district-level hospital in Cape Town. Patients with HIV-associated TBM were prospectively enrolled in a third cohort study to determine the clinical and immunological characteristics of paradoxical TBM-IRIS.

Results

TBM accounted for 57% of meningitis cases over a 6-months period; 88% of these patients were HIV-infected. At six months follow-up, mortality in HIV-associated TBM patients was 48%. Neurological TB-IRIS accounted for 21% of patients who presented with central nervous system (CNS) deterioration during the first year of antiretroviral therapy (ART) over a one-year period. TBM-IRIS developed in 47% of HIV-associated TBM patients and associated with extensive cerebrospinal fluid (CSF) inflammation both at TBM diagnosis and at TBM-IRIS presentation. Patients who did not develop TBM-IRIS, but who were culture-positive for *Mycobacterium tuberculosis* from CSF at TBM diagnosis, showed an immunological phenotype similar to TBM-IRIS patients; however neutrophils were increased in TBM-IRIS patients compared to culture-positive TBM-non-IRIS patients, both at TBM

diagnosis and two weeks after ART initiation.

Conclusions

HIV-associated TBM is a common cause of meningitis with a poor outcome in Cape Town. TBM-IRIS is a frequent complication of ART in HIV-associated TBM patients. CSF *Mycobacterium tuberculosis* culture positivity drives an inflammatory response that manifests as TBM-IRIS in most, but not all TBM patients. Neutrophils associate closely with the CNS inflammation that characterizes TBM-IRIS. An intensified TB treatment regimen with increased CSF penetration early during TB treatment may lead to improved mycobacterial clearance from the CNS, which may result in improved outcome during TBM treatment and a reduced frequency of TBM-IRIS. We aim to test this hypothesis in future studies.

Acknowledgements

The research reported in this thesis was supported through PEPFAR (Presidents Emergency Plan For AIDS Relief) through the Perinatal HIV Research Unit (Witwatersrand), who funded my salary from January 2008 through September 2010, as well as a Carnegie Corporation Training Award (2011-2012), a Discovery Foundation Academic Fellowship Award (2011-2012) and a Wellcome Trust Training Fellowship (097254, 2012-2015) awarded to myself. Additional funding for the research came through the Clinical Infectious Diseases Research Initiative from the Wellcome Trust.

I firstly want to thank my primary supervisor, Professor Robert J Wilkinson without whom this thesis would not have happened. His vision, enthusiasm, wise advice and guidance have played a crucial role in my career and this thesis. I also sincerely thank my co-supervisors Associate Professor Graeme Meintjes and Associate Professor Katalin Wilkinson for their insightful guidance and patient support since before and throughout the research period.

I thank the patients who participated in these studies and the staff at GF Jooste Hospital for the care they provided for study participants. I thank Ms Monica Magwayi, the study counselor and translator, who is loved by patients and colleagues alike due to her caring demeanor. I thank all colleagues who participated in the clinical studies at GF Jooste Hospital, including Dominique Pepper, Charlotte Schutz, Friedrich Thienemann and Zahiera Ismail. I am grateful to radiologists Ashmitha Rajkumar and Marisa Mezzabotta for their detailed reporting of brain imaging findings. I thank Kathryn Wood, Reyhana Solomon, Vanessa January and Rene Goliath for administrative support at the IDM; Ronnett Seldon for laboratory training and help with specimen collection and processing; Lori Dodd, Andrew Boulle, Chris Muller, Anna Coussens, and Maia Lesosky who assisted with statistical analysis; and Armin Deffur who, in addition to assisting with statistical analysis, was always willing to provide technical help with all computer-related issues. I further acknowledge Estee Torok and Ben Marais who critically reviewed the manuscript presented in Chapter 2, and all the international collaborators who contributed to the Tuberculous Meningitis Consensus Clinical Case Definition manuscript.

I would also like to give my sincerest thanks to colleagues in the Department of Neurology for their support, encouragement and teaching during my preparation for the FC Neurology examinations during the period 2010-2012.

Lastly, but not least, my most heartfelt thanks goes to my best friend Marinus Cloete, whose encouragement during all of my academic endeavors never wavers and who never tires of being my sounding board.

Suzaan Marais
Date: February 2014

List of Abbreviations

3TC	lamivudine
95%CI	95 percent confidence interval
ADA	adenosine deaminase activity
AFB	acid-fast bacilli
aHR	adjusted hazard ratio
AIDS	acquired immune deficiency syndrome
AOR	adjusted odds ratio
ART	antiretroviral therapy
AUC	area under the receiver operating characteristic curve
AZT	zidovudine
BCG	Bacille Calmette-Guerin.
BMRC	British Medical Research Council
CCR	chemokine receptor
CD4	CD4 T-lymphocyte
CLAT	cryptococcal latex agglutination test
CM	cryptococcal meningitis
CM-IRIS	cryptococcal meningitis immune reconstitution inflammatory syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
CXR	chest radiograph
D4T	stavudine
DOTS	Directly Observed Therapy Short-course
EFV	efavirenz
ELISA	enzyme-linked immunosorbent assay
ETV	endoscopic third ventriculostomy
GCS	Glasgow coma scale
Hb	haemoglobin
HIV	human immune deficiency virus
IDM	Institute of Infectious Disease and Molecular Medicine
IFN-γ	interferon gamma

IgG	immunoglobulin G
IgM	immunoglobulin M
IGRA	interferon-gamma release assay
IL	interleukin
INH	isoniazid
INSHI	International Network for the Study of HIV-associated IRIS
IQR	interquartile range
IRIS	immune reconstitution inflammatory syndrome
IV	intravenous
LAM	lipoarabinomannan
LED microscopy	light-emitting diode auramine fluorescent microscopy
LP	lumbar puncture
LTA4H	leukotriene A4 hydrolase
M. tuberculosis	*Mycobacterium tuberculosis*
M.tb	*Mycobacterium tuberculosis*
MDR	multidrug-resistant
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
NAAT	nucleic acid amplification test
non-TBM-IRIS	HIV-infected TBM patients who did not develop TBM-IRIS after ART initiation
OD	optical density
OR	odds ratio
PCR	polymerase chain reaction
PEPFAR	Presidents Emergency Plan For AIDS Relief
qPCR	quantitative PCR
RCT	randomized controlled trials
RR	relative risk
SA DoH	South African Department of Health
SNP	single nucleotide polymorphism
SOL	space occupying lesions
TB	tuberculosis
TB-IRIS	tuberculosis-associated immune reconstitution inflammatory syndrome

TBM	tuberculous meningitis
TBM-IRIS	tuberculous meningitis immune reconstitution inflammatory syndrome
TBM-non-IRIS	HIV-infected TBM patients who did not develop TBM-IRIS after ART initiation
TDF	tenofovir
TIMP	tissue inhibitor of matrix metalloproteinases
TNF	tumor necrosis factor
TST	tuberculin skin test
UCT	University of Cape Town
USD	United States Dollar
VP	ventriculo-peritoneal
WCC	white cell count
WHO	World Health Organization
Xpert	Xpert MTB/RIF test
ZDV	zidovudine
ZN	Ziehl-Neelsen

Chapter 1

Context

Tuberculosis (TB) remains a leading cause of mortality and morbidity worldwide; in 2012, 1.3 million deaths were attributed to TB with almost a quarter of these occurring in HIV-infected persons [1]. South Africa bears the brunt of the TB/HIV co-infection burden accounting for 20-30% of affected persons globally. Tuberculous meningitis (TBM) is the most devastating form of TB with an associated mortality during treatment of 28% of HIV-uninfected [2], and 58% of HIV-infected [3], patients in TBM randomized controlled trials. This is in stark contrast to results from randomized controlled trials performed in other forms of HIV-associated TB such as pulmonary [4], and pulmonary plus extra-pulmonary (other than TBM) cases [5], which report mortality during TB treatment of <3%, and <18%, respectively. HIV-infected patients, particularly those with severe immunosuppression, are at increased risk of disseminated forms of TB, such as meningitis that accounts for up to 19% of all hospitalized HIV-associated TB cases [6]. TBM is a common cause of meningitis in high HIV/TB co-infection settings, and accounted for 28% of microbiologically confirmed cases at GF Jooste Hospital between 2006 and 2008 [7]. HIV-infected TBM patients frequently present with atypical cerebrospinal fluid (CSF) findings, which may delay diagnosis and treatment initiation [8-10]. Due to limited epidemiological data the burden of TBM disease is uncertain in high HIV/TB co-infection settings such as South Africa and the outcome in affected adults has not been determined in Cape Town.

Clinicians face considerable challenges in the diagnosis and management of TBM [11]: disease pathogenesis remains poorly understood; rapid, sensitive and affordable diagnostic tests are lacking; and optimal management has

not been established by randomized controlled trials. Further research is urgently required, but the low frequency with which a definitive diagnosis is established presents a major obstacle. Detection of acid-fast bacilli and/or culture isolation of *Mycobacterium tuberculosis* from CSF has been reported with high frequency in some studies [12, 13], but remains the exception in most [14-19]. The diagnosis usually relies on clinical evidence which combines supportive clinical, laboratory and radiological findings, but standardised diagnostic criteria for TBM applicable to HIV-infected and uninfected patients did not exist prior to 2010.

It is estimated that more than 2.1 million people were receiving ART in 2012 in South Africa, compared to ~1.8 million in 2011 and ~50 000 in 2004 [20, 21]. Despite the benefit of ART in improving clinical outcome in TB/HIV co-infected patients [22], patients may deteriorate clinically after commencing ART due to the immune reconstitution inflammatory syndrome (IRIS) [23, 24]. IRIS occurs as a result of an exuberant inflammatory response towards previously diagnosed or occult opportunistic pathogens [25]; it has been described in relation to many pathogens, with mycobacteria being most frequently implicated [23]. TB-IRIS may present in one of two recognised ways; firstly, as paradoxical worsening of symptoms after commencement of ART and secondly, as an unmasking syndrome [25]. Paradoxical TB-IRIS occurs in 8-54% of TB/HIV co-infected patients after starting ART [25, 26]. Common manifestations include lymph node enlargement and pulmonary involvement [23-25, 27, 28]. Neurological TB-IRIS, although accounting for over 10% of paradoxical TB-IRIS cases at GF Jooste Hospital [29], is less well documented; by 2010 only one clinical case series [29, 30] and a handful of case reports existed [27, 28, 31-37]. Neurological TB-IRIS may present as meningitis [28, 29, 33, 35, 37], brain tuberculomas [27-29, 31, 32, 36, 37], brain abscess [32, 34

and radiculomyelitis [29, 35]. Outcome in neurological TB-IRIS is poor; in one case series published from Cape Town, mortality at 6 months follow-up was 13% and 17% of patients were lost to follow-up [29].

Although recent studies have advanced understanding of TB-IRIS through the identification of several cellular, immunological and genetic factors that associate with the syndrome, the immunopathogenesis remains unclear and no diagnostic test exists [38]. Thus far explorations of immune biomarkers implicated in TB-IRIS pathogenesis have focused mainly on those measured in the blood compartment, which is likely an incomplete representation of the immune response in affected tissues, e.g. lungs or CNS.

Key research aims

The key research objectives addressed in this thesis were developed to address gaps in knowledge of HIV-associated TBM. They include:

1) To establish a consensus clinical case definition for TBM for use in clinical research.
2) To describe the presentation and outcome of patients with TBM in a high burden HIV setting and investigate the predictors of mortality in these patients.
3) To describe the causes of CNS deterioration in HIV-infected patients during the first year of ART and to determine the proportion of these cases who presents with neurological TB-IRIS.
4) To determine the proportion of patients with HIV-associated TBM who develop paradoxical TBM-IRIS.

5) To describe the clinical and laboratory findings and outcomes of paradoxical TBM-IRIS.

6) To investigate the immunopathogenesis of TBM-IRIS, with a focus on inflammatory markers in the CSF of affected patients.

Setting

The clinical studies were conducted at GF Jooste Hospital, a public sector referral hospital, in Cape Town in the Western Cape Province of South Africa. The hospital is located in Manenberg on the Cape Flats and at the time of the studies served adults from a catchment population of 1.3 million people. The high-density low-income communities served by GF Jooste Hospital included Khayelitsha, Gugulethu, Mitchells Plain and Nyanga. At the start of the studies (2009-2010) the reported TB case notification rate exceeded 1500 cases per 100 000 people per year and 70 percent of TB cases were HIV-infected in Khayelitsha, where approximately 430 patients were enrolled into ART care monthly [39]. Eleven primary-level ART clinics and 16 TB clinics were situated in the GF Jooste Hospital referral area. Most patients who attended these clinics did not have health insurance. Therefore, most patients who presented with significant clinical deterioration at these facilities including all meningitis suspects, were referred to GF Jooste Hospital for assessment and management.

The immunological studies were performed in the laboratory of Robert J Wilkinson at the Institute of Infectious Disease and Molecular Medicine at the Faculty of Health Sciences of the University of Cape Town.

Chronology of studies

The prospective observational study to describe causes of CNS deterioration during the first year of ART was designed in 2007. Seventy-five participants were enrolled from November 2007 through October 2008. Data analysis and publication of the manuscript (presented in Part 2 of Chapter 4) occurred in 2010.

The prospective observational study to investigate the frequency and characteristics of TBM-IRIS was designed in 2008. The study recruited patients from March 2009 through October 2010 and patient follow-up was completed by July 2011. The study screened 67 patients with meningitis of whom 34 patients with TBM were included in the final analysis. The clinical characteristics of these patients were published in 2013 and are presented in Chapter 5. In addition, 18 HIV-infected patients without meningitis who were not receiving ART were enrolled as control participants. A manuscript describing the immunological findings of TBM and control participants has been submitted for publication and is presented in Chapter 6.

The consensus clinical case definition for TBM presented in Chapter 3 arose from a TBM workshop held in May 2009 in Cape Town. I was responsible for leading the organization of the meeting held by the University of Cape Town. Fourty-one participants from seven countries (South Africa, the United Kingdom, Vietnam, India, the United States of America, Malawi, and Indonesia) attended the meeting. The aim of this meeting was to develop a clinical case definition for TBM that would be applicable to all TBM cases, regardless of HIV status or age, for use in research. Following the meeting, I was given the role of heading the writing committee for the consensus

case definition manuscript. Over the following year I combined criteria suggested during the meeting and those found relevant from my literature review to produce a draft manuscript that presented this case definition. After members of the writing committee provided input, I finalized the manuscript that was published in 2010.

The retrospective study describing the frequency, presentation and outcome of TBM patients was designed in 2010. Laboratory records of all 698 patients who underwent a lumbar puncture from March 2009 through October 2009 were reviewed. Medical records of 182 patients with suspected TBM were reviewed and the presentation and outcome of 120 patients with TBM were described in a manuscript published in 2011. This manuscript is presented in Part 1 of Chapter 4.

Outline of the thesis

In **Chapter 2** (background and literature review) a detailed literature review of the clinical and laboratory features and outcome of HIV-infected TBM compared to findings in HIV-uninfected patients is presented. Diagnostic and therapeutic challenges are also discussed with specific reference to the treatment of HIV and paradoxical TB-IRIS. This review was published in 2010. Recent advances in knowledge of HIV-associated TBM and neurological TB-IRIS published since 2010 and a section on TB-IRIS pathogenesis have been included as an addendum to this chapter.

In **Chapter 3** the consensus clinical case definition for TBM is presented. This case definition (for use in clinical research) was developed to be

uniformly applicable, irrespective of the patient's age or HIV infection status or the resources available in the research setting.

In **Chapter 4** two descriptive studies are presented that aimed to determine the burden of HIV-associated TBM and neurological TB-IRIS at a district-level hospital in Cape Town. Firstly, in a retrospective study that describes the frequency, presentation and outcome of TBM, we also determined risk factors for inhospital and 6-month mortality in HIV-associated TBM. Secondly, in a prospective study we determined the incidence of referral for CNS deterioration during the first year of starting ART and the contribution of neurological TB-IRIS to this deterioration.

In **Chapter 5** the prospective clinical study of patients with HIV-associated TBM is presented. The aim of this study was to describe the clinical and laboratory features, and to determine the outcome, of patients who develop TBM-IRIS.

In **Chapter 6** the immunological findings in patients who developed TBM-IRIS is described and compared to those in patients who did not develop TBM-IRIS. The aim of this study was to investigate immunopathogenic mechanisms in TB-IRIS by determining inflammatory mediators at the site-of-disease (i.e. CSF).

Chapter 7 provides an integrated narrative of the thesis that synthesises the major findings from all chapters. Conclusions of the thesis as a whole are drawn, the implications of the research for the TBM field in general are discussed and priorities for future HIV-associated TBM research are identified.

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Chapter 2

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REVIEW

HIV-associated tuberculous meningitis – diagnostic and therapeutic challenges

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SUMMARY

HIV-associated tuberculous meningitis (TBM) poses significant diagnostic and therapeutic challenges and carries a dismal prognosis. In this review, we present the clinical features and management of HIV-associated TBM, and compare this to disease in HIV-uninfected individuals. Although the clinical presentation, laboratory findings and radiological features of TBM are similar in HIV-infected and HIV-uninfected patients, some important differences exist. HIV-infected patients present more frequently with extra-meningeal tuberculosis and systemic features of HIV infection. In HIV-associated TBM, clinical course and outcome are influenced by profound immunosuppression at presentation, emphasising the need for earlier diagnosis of HIV infection and initiation of antiretroviral treatment.

Introduction

Tuberculosis (TB) remains the most important infectious cause of death worldwide, with an estimated 1.82 million deaths in 2008, including 0.52 million deaths in persons co-infected with the human immunodeficiency virus (HIV).[1] HIV-infected persons may present with unusual clinical features of TB,[2] and are at risk of developing disseminated forms of TB, such as tuberculous meningitis (TBM).[3,4] HIV-associated TBM poses significant diagnostic and therapeutic challenges and carries a dismal prognosis.[5–10] In this review, we present the clinical features and laboratory findings of HIV-associated TBM. We also discuss the management of HIV-associated TBM, compared to TBM in HIV-uninfected individuals.

Clinical presentation

Several studies have investigated the effect of HIV infection on the clinical presentation of TBM in adults and children (Table 1).[3–21] The differences between the clinical, laboratory and radiological features of TBM in HIV-infected and HIV-uninfected persons are presented in Table 2.

TBM occurs most commonly in children under the age of five years,[22] although delayed exposure to *Mycobacterium tuberculosis* (*M.tb*) and/or selection bias may influence the age distribution in different settings. A second peak occurs during the third and fourth decades of life. The mean/median age at presentation is 23–67 months in HIV-infected children, versus 35–39 months in HIV-uninfected children.[5,8,17] With the exception of two studies,[7,11] most of the evidence suggests that HIV co-infection does not significantly influence the age of clinical presentation in adults (mean/median age 26–37 years in HIV-infected persons versus 28–46 years in HIV-uninfected persons).[4,6,9,12,14,18,19]

The symptoms, the interval from symptoms to clinical presentation, and the neurological findings at clinical presentation are similar in HIV-infected and HIV-uninfected patients (Table 2).[3–9,11,13–15,17,19] One study found a higher prevalence of cognitive dysfunction in HIV-infected adults not receiving antiretroviral therapy (ART) compared with HIV-uninfected adults (32% versus 0%, $p = 0.001$),[6] whereas another study found a lower prevalence of impaired consciousness in HIV-infected children not receiving ART compared with HIV-uninfected children (73% versus 50%, odds ratio [OR] 0.37, 95% confidence interval [CI] 0.15–0.91).[8] Regardless of HIV status, patients commonly present with severe disease, defined as modified British Medical Research Council (BMRC) grade II or III TBM; this is strongly associated with poor outcome.[7,9,18,20]

Abnormal clinical findings outside the central nervous system (CNS) occur more frequently in HIV-infected patients. Children are more likely to be malnourished,[8,17] while both adults and children

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Table 1
Overview of studies conducted in HIV-infected patients with tuberculous meningitis.

Authors	Location	HIV patients (*n*)	Children or adults	Study design	TBM diagnosis confirmed (%)*	Duration of TB treatment (months)	Corticosteroids (%)	Antiretroviral therapy (*n*)	Mortality measures
Berenguer et al.[3]	Spain	37	Adults	Retrospective, observational	100	Not stated	38%	Not stated	In-hospital
Dube et al.[11]	USA	15	Adults	Retrospective, observational	100	At least 6	Not stated	Not stated	In-hospital
Yechoor et al.[12]	USA	20	Adults	Retrospective, observational	75	9	32%	Not stated	9 months
Porkert et al.[13]	USA	16	Adults	Retrospective, observational	100	Not stated	Yes, number not stated	Not stated	6 months
Karstaedt et al.[14]	South Africa	39	Adults	Retrospective, observational	100	Not stated	Yes, number not stated	Not stated	In-hospital
Topley et al.[5]	South Africa	10	Children	Retrospective, observational	10	9–12	100%	Not stated	6–12 months
Katrak et al.[6]	India	22	Adults	Prospective, observational	32†	Not stated	Not stated	0	6 months
Schutte et al.[15]	South Africa	20	Adults	Prospective, observational	Not stated	Not stated	Not stated	Not stated	Treatment completion
Thwaites et al.[16]	Vietnam	11	Adults	Prospective, observational	100	9	No	0	In-hospital
Thwaites et al.[7]	Vietnam	96	Adults	Randomized, placebo-controlled	42	9	50%	0	9 months
Karande et al.[17]	India	8	Children	Prospective, observational	Not stated	Not stated	100%	Not stated	In-hospital
van der Weert et al.[8]	South Africa	34	Children	Retrospective, observational	36 ¶	6	100%	0	6 months
Azuaje et al.[4]	Spain	39	Adults	Retrospective, observational	80	9–12	Yes, number not stated	39 **	Treatment completion
Cecchini et al.[18]	Argentina	101	Adults	Retrospective, observational	100	Not stated	Not stated	Not stated	In-hospital
Torok et al.[9]	Vietnam	58	Adults	Prospective, observational	93	9	100%	4 ††	9 months
Torok et al.[20]	Vietnam	253	Adults	Randomized, placebo-controlled	62	9	100%	253 ¶¶	9 months
Croda et al.[19]	Brazil	108	Adults and children	Retrospective, observational	100	9	100%	36 ***	In-hospital, and 9 months
Cecchini et al.[10]	Argentina	101	Adults	Retrospective, observational	100	Not stated	Not stated	Not stated	In-hospital
Pepper et al.[21]	South Africa	13	Adults	Prospective, Observational †††	15	At least 9	100%	13	6 months post TBM-IRIS

n, number of patients; %, percentage of patients; TBM, tuberculous meningitis; TB, tuberculosis; *M.tb, Mycobacterium tuberculosis;* ART, antiretroviral therapy.
* Including patients with cerebrospinal fluid smear positive for acid-fast bacilli and/or culture positive for *M.tb*, unless otherwise stated.
† Including patients with brain autopsy evidence of *M.tb* infection.
¶ Including patients who cultured *M.tb* from gastric aspirate.
** 15 patients were receiving one or more antiretroviral drug at time of TBM diagnosis; ART was started during TBM treatment in 24 patients.
†† 4 patients were started on ART during TBM treatment; 3 patients had a history of ART exposure prior to TBM diagnosis.
¶¶ Patients were started on ART during TBM treatment.
*** 36 patients were receiving ART at time of TBM diagnosis; 63 patients had a history of ART exposure prior to TBM diagnosis.
††† Study conducted in patients who developed paradoxical tuberculous meningitis immune reconstitution inflammatory syndrome (TBM-IRIS).

exhibit other clinical features of HIV disease.[3,8,9] HIV-infected patients are also more likely to have disseminated TB or extra-meningeal involvement (i.e. involvement of organs other than the CNS), including pulmonary, nodal, genitourinary, spinal, pleural and abdominal disease.[4,7,14] Tuberculin skin test positivity is variable in HIV-infected patients.[3–5,8,12,17]

Most studies report similar frequencies of chest radiograph (CXR) abnormalities in HIV-infected and HIV-uninfected patients (53–67% versus 47–82%, respectively)[3,11,12,15] e.g. active TB, (46% versus 38%, respectively);[7] adenopathy (3–60% versus 0–37%, respectively);[3–5,8] and miliary TB (10–33% versus 20–44%, respectively).[5,7,8,11,12,15] One study in children (not receiving ART),[8] and one in adults (40% of whom were receiving ART),[4] found more CXR abnormalities in HIV-infected than HIV-uninfected cases (85% versus 65%, and 62% versus 33% ($p = 0.04$), respectively). Abdominal ultrasound may assist TB diagnosis; one study identified abdominal lymphadenopathy in more than half of HIV-infected patients.[3] *M.tb* may be cultured from the blood,[18,19] or urine[3] of HIV-infected patients, especially from those with disseminated disease. Lipoarabinomannan, a mycobacterial cell wall component, may be detected in the urine.[23]

Cerebrospinal fluid features

The cerebrospinal fluid (CSF) typically has a clear appearance, a leucocytosis (10–1000 $\times$ 10^6 cells/l; mostly lymphocytes), a raised protein concentration (>0.5 g/l), and a decreased CSF to blood glucose ratio (<0.5).[24] Most studies report similar CSF findings in HIV-infected and HIV-uninfected patients (Table 2).[3–5,7,8,11–15,25] In contrast, Thwaites et al. reported a reduced CSF white cell count (WCC) in HIV-infected adults not receiving ART compared to HIV-uninfected adults (152 $\times$ 10^6 cells/l versus 356 $\times$ 10^6 cells/l, $p = 0.007$).[16] Two studies reported similar findings, as well as significantly decreased CSF protein concentrations in HIV-infected adults.[6,10]

Atypical CSF findings occur in a substantial proportion of HIV-infected TBM patients, potentially causing diagnostic uncertainty and delayed treatment. Croda et al. reported the combination of pleocytosis (>5 $\times$ 10^6 cells/l), elevated CSF protein levels (>0.45 g/l) and reduced CSF glucose levels (<2.5 mmol/l) in only 64% of culture-confirmed HIV-infected TBM patients, a third of whom were known to be receiving ART.[19] A normal CSF WCC,[10,11,14,19] protein concentration,[3,4,11,12,14,19] glucose concentration,[3,11,12,14,15,19] and even a completely normal CSF,[3,14,19,25] have all been reported in HIV-associated TBM. In one study, 33% of HIV-infected TBM patients with a $CD4^+$ T-lymphocyte count less than 50 cells/μl had a normal CSF WCC.[10] Lymphocytes are the dominant CSF cell type in HIV-uninfected TBM patients[3,7,11,14,25] whereas neutrophils frequently predominate, and account for up to 70% of the CSF cell population, in HIV-infected TBM patients.[9,14,25]

The diagnostic gold standard for TBM is microbiological confirmation, either by visualization of acid-fast bacilli (AFB) in, or culture of *M.tb* from, the CSF. Some studies have achieved extremely high

Table 2
Comparison of clinical, laboratory and radiological features in HIV-infected and HIV-uninfected patients with tuberculous meningitis*.

	HIV-infected	HIV-uninfected
Age		
Children: months, mean/median[5,8,17]	23–67	35–39
Adults: years, mean/median[4,6,7,9,11,12,14,18,19]	26–37	28–46
History/symptoms		
TB contact positive, %[5,17]	70–75	37–57
Previous TB, %[4,7–9,11,13,18,19]	8–58	0–25
Duration of illness: days, mean/median[3–7,9,15,17]	9–42	11–22
Cough[3,8,11]	22–47	16–36
† Fever, % [3,4,6,11,13–15,17,19]	68–100	45–97
Headache/irritability, %[3,4,6,8,11,13,15,19]	41–100	22–100
Malaise, %[3,13]	27–44	22–32
Weight loss, %[6,8]	18–47	3–51
Nausea/vomiting, %[3,4,6,8,13,19]	26–50	22–54
Seizures, %[3–5,8,14,17,19]	3–50	0–64
Examination findings		
¶Mantoux test positive %		
Children[5,8,17]	13–45	18–77
Adults[3,4,12]	33–50	42–80
Weight for age (Z-score), children, mean[5,8]	−1.77 to −2.58	−1.2–1.77
Impaired consciousness, %[3–6,8,11,14,19]	30–74	23–73
GCS, mean/median[7,9,15]	12–13	13–14
BMRC grade at presentation, %[3,5,7–9,12–14,17–19]		
Grade 1	0–50	0–34
Grade 2	35–80	42–82
Grade 3	0–63	0–88
Meningeal signs, %[3,4,6,8,11,14,15,17,19]	19–100	44–100
Cranial nerve palsies, %[6–8,17]	13–77	30–52
Hemiplegia, %[6–8]	19–36	15–34
**Focal neurological deficit, %[3–5,9,11,13,14,17,19]	16–80	11–80
Paraplegia, %[6,7]	4–23	8–16
Blood investigations		
Haemoglobin, g/l, mean/median[5,6,8,11,19]	85–119	99–115
††$CD4^+$ T-lymphocyte count, cells/μl, mean/median [3,4,7,9,11–15,18–20]	32–180	248
White cell count, $\times 10^9$ cells/l, mean/median[5,7–9,11,13,15,16]	6–12.6	8.2–17.7
Serum sodium concentration, mmol/l, mean/median[5,7,9,11,13]	126–129	128–132
Hematocrit,%, mean/median[7,9,13]	33.9–34.8	35.5–36.8
Cerebrospinal fluid (CSF) parameters		
White cell count, $\times 10^6$ cells/l, mean/median[3,4,6,7,9–14,16,19,25]	47–438	11–356
Lymphocyte count, $\times 10^6$ cells/l, mean/median[4,5,8,15]	89–219	13–179
Neutrophil count, cells $\times 10^6$/l, mean[5,8,15]	18–69	50–87
Protein, g/l, mean/median[3–15,19,25]	0.52–2.88	0.78–3.48
Glucose, mmol/l, mean/median[3–6,8,10–15,19,25]	1.3–2.7	1.1–2.8
Glucose, CSF: Blood ratio, median[7,9,19]	0.27–0.3	0.29
Adenosine deaminase, IU/l, mean/median[3,4,15]	10–12.6	13.5–16
¶¶AFB stain positive, % [3,4,6,8,9,11,13]	0–69	0–26
M.tb culture positive, %[4,6,7,9,12]	0–88	0–78
***Extrameningeal tuberculosis* [3,4,6,10,14,17,19]	48–100	9–75
Chest radiography		
Abnormal, %[3,4,8,11,12,15]	53–85	33–82
Miliary tuberculosis, %[5,7,8,11,12,15]	10–33	18–44
Adenopathy, %[3–5,8]	3–60	0–37
Parenchymal infiltrate, %[3,4,8,12,15]	22–49	7–55
Cerebral imaging		
Neuroimaging abnormalities, %[3–5,12,13,15,18,19]	55–100	50–90
Hydrocephalus, %[3,8,11–13,17]	20–72	30–98
Meningeal enhancement/basal exudates %[3,5,6,8,12,13,15,17]	16–63	6–82
Cerebral atrophy, %[5,6,8,12]	5–57	5–17
Infarcts/non-enhancing lesion, %[3,6,8,13,15]	13–50	6–44
Gyral enhancement/cerebritis, %[5,8,12]	0–60	0–17
Enhancing lesion/granuloma/mass lesion, %[3,5,6,8,11,13,15]	0–60	0–27
Outcome		
Fever clearance time, days, median[3,7]	8	9
Coma clearance time, days, median[7]	7	7
In-hospital mortality, %[3,10,11,14,17,19]	13–72	18–64
†††In-hospital survival without neurological deficit, % [3,15,17]	38–61	19–74
¶¶¶Mortality at 6-months follow-up, % [5,6,8,13]	24–50	0–22
†††Six-months survival without neurological deficit, % [6,8]	29–36	55–60
Mortality at 9-months follow-up %[7,9,12,19,20]	41–67	28–30
†††Nine-months survival without neurological deficit, %[7]	22	41

TB, tuberculosis; GCS, Glasgow Coma Scale; BMRC, British Medical Research Council; *M.tb, Mycobacterium tuberculosis;* BCG, Bacille Calmette-Guerin.

* Including studies conducted in HIV-infected ± HIV-uninfected adults and children.

† Including fever as symptom or sign.

¶ Positive Mantoux test criteria include: skin test reaction ≥ 5 mm of induration if child had close contact with infected adult or, ≥10 mm of induration if not[17]; skin test reaction ≥ 15 mm of induration if child is HIV-uninfected and received BCG, or ≥ 10 mm induration if no BCG received or child is HIV-infected[5]; skin test reaction ≥ 5 mm of induration if patient is HIV-infected or, ≥ 10 mm of induration if patient is HIV-uninfected[4,8,12].

** Including studies which exclude 6th cranial nerve palsies[3,14].

†† Including $CD4^+$ T-lymphocyte count for HIV-uninfected patients from one study[11].

¶¶ Including studies which only include patient who 1) cultured *M.tb* from CSF[3,11,13,25] or; 2) had positive CSF polymerase chain reaction for *M.tb* complex[25].

*** Diagnosed by clinical, radiological or microbiological findings.

††† Includes percentage of total number of patients.

¶¶¶ Including deaths attributed exclusively to tuberculous meningitis[13].

rates (>80%) of microbiological confirmation;[9,26,27] a number of factors (e.g. large CSF volumes, prolonged microscopic examination, and multiple CSF samples) have shown an improvement of diagnostic yield.[26,27] In the majority of studies, however, TBM diagnosis is presumptive and based on a combination of clinical, laboratory and radiological findings,[6,28–32] emphasising the need for development of a standardized case definition.[33] Although similar microbiological confirmation rates have been reported in HIV-infected and HIV-uninfected patients,[3,4,6,8,11–13] the numbers of culture-confirmed cases in most studies are low. Thwaites et al. isolated *M.tb* from a greater proportion of patients (42% of HIV-infected patients not on ART versus 30% of HIV-uninfected patients, $p = 0.029$)[7] and from significantly smaller volumes of CSF (1.5 ml in HIV-infected patients versus 4 ml in HIV-uninfected patients $p = 0.001$).[27] Torok et al. cultured *M.tb* from CSF in 88% of HIV-infected TBM patients most of whom were not on ART and observed that the quantity of bacilli seen in the CSF smear appeared to be higher.[9] This is supported by autopsy findings of AFB in the cerebral parenchyma and meninges of HIV-infected patients not on ART, which was not observed in HIV-uninfected patients.[6]

One of the major difficulties encountered in clinical practice is distinguishing TBM from other subacute meningoencephalitides in HIV-infected patients. Notably, cryptococcal meningitis (CM), which also occurs frequently in severely immunosuppressed patients, may present with similar clinical and laboratory features.[34–38] Other relatively common HIV-related CNS diseases, such as cytomegalovirus encephalitis, cerebral toxoplasmosis and primary CNS lymphoma, may also mimic TBM.[39] Diagnostic algorithms have been derived in order to distinguish TBM from other causes of meningitis, including bacterial meningitis.[31,32,40,41] One of these algorithms demonstrated high sensitivity (96–99%) and moderate specificity (72–82%) when evaluated prospectively in HIV-uninfected patients,[42,43] but sensitivity (78%) and specificity

(43%) declined markedly when it was used in a population with high HIV prevalence.[44] In addition to CSF Gram stain and bacterial culture (to exclude pyogenic bacterial meningitis), India ink microscopy, cryptococcal latex agglutination test and/or fungal culture (to exclude CM) are essential investigations in HIV-infected patients presenting with suspected TBM. Other laboratory tests that may assist in excluding alternative diagnoses include: 1) CSF viral nucleic acid amplification assays for viral meningitis; 2) CSF and serum syphilis antibody assays for neurosyphilis; and 3) serum *Toxoplasma gondii* antibody assays for toxoplasma encephalitis.

Laboratory features

A number of studies have compared the baseline laboratory features of TBM in HIV-infected and HIV-uninfected patients. Several studies have found haemoglobin (Hb) concentrations to be significantly reduced in HIV-infected compared with HIV-uninfected TBM patients.[6,8,17] Karande et al. reported that moderate to severe anaemia (Hb <80 g/l) was more frequent in HIV-infected compared with HIV-uninfected children with TBM (63% versus 12%, $p = 0.008$).[17] Thwaites et al. reported a significantly lower median haematocrit in HIV-infected adults not on ART compared with HIV-uninfected adults with TBM (33.9 versus 36.8, $p < 0.001$), a factor which was independently associated with death (OR 0.83, 95% CI 0.73–0.94, $p = 0.005$).[7] Some studies have reported significantly lower values of blood WCC in HIV-infected patients,[6,7,13,16] while others have indicated a trend.[5,8,11,15] One study has shown a significant reduction in platelet count in HIV-infected TBM children not on ART.[8]

While serum sodium levels are typically decreased in TBM patients, some studies suggest that serum sodium levels are lower in HIV-infected compared to HIV-uninfected patients.[5,7,11,13] Torok et al. found that severe hyponatremia in a cohort of HIV-infected TBM patients (most of whom were not on ART) was independently associated with an earlier time to death (adjusted hazard ratio [AHR], 0.93; 95% CI, 0.89 to 0.98; $p = 0.002$).[9] Finally Thwaites et al. showed that hepatic transaminases were significantly elevated in HIV-infected adults not on ART compared with HIV-uninfected adults with TBM, a finding which may be related to higher rates of hepatitis B virus co-infection in this group.[7]

Radiological features

Cerebral imaging is an invaluable tool in the diagnosis of TBM. Radiological features of TBM include one or more of the following findings: hydrocephalus; basal meningeal enhancement; infarction; and tuberculoma(ta).[45–47] The frequency of cerebral imaging abnormalities is similar in HIV-infected and HIV-uninfected TBM patients (55–100% versus 50–90%, respectively) (Table 2).[3,5,12,13,15,18,19] Only one study reports significantly more abnormalities on brain computed tomography (CT) (74% versus 37%, $p = 0.03$) and magnetic resonance imaging (100% versus 64%, $p = 0.04$) in HIV-infected compared with HIV-uninfected adults.[4]

Most studies report no difference in the presence of meningeal enhancement between HIV-infected and HIV-uninfected TBM patients.[3,5,12,13,15,17] Two studies report that basal meningeal exudates occur less frequently in HIV-infected patients not on ART compared to HIV-uninfected patients (33–38% versus 71–82%, respectively).[6,8] One study, conversely, showed a trend towards an increase in meningeal enhancement in HIV-infected patients, 40% of whom were receiving ART.[4]

Although the proportion of patients with hydrocephalus (obstructive and non-obstructive) is similar between the 2 groups,[3,4,8,11–13,17] obstructive hydrocephalus occurs less frequently in HIV-infected patients compared with HIV-uninfected patients (0–6% versus 20–64%, respectively).[6,8] Katrak et al. proposed that the reduced meningeal enhancement and obstructive hydrocephalus observed in HIV-infected patients not on ART was due to severe immune suppression causing a reduced inflammatory response.[6] This was supported by post-mortem findings. CT brain studies showed that cerebral atrophy was significantly more frequent in HIV-infected patients not on ART compared with HIV-uninfected adults (44% versus 5%),[6] and children (57% versus 17%).[8]

Contrast enhancing lesions occur with similar frequency in HIV-infected and HIV-uninfected TBM patients[3,6,13] although one small study reported more mass lesions in HIV-infected compared with HIV-uninfected patients (60% versus 14%, $p = 0.01$).[11] Whereas a contrast-enhancing lesion in a HIV-uninfected TBM patient intuitively represents a tuberculoma, the differential diagnosis is broader in HIV-infected patients and includes cerebral toxoplasmosis[6] and primary CNS lymphoma.[48,49] Indeed Katrak et al. diagnosed concomitant cerebral toxoplasmosis (by histology in five patients and by response to anti-toxoplasmosis treatment in one patient) in all HIV-infected TBM patients who presented with granulomata on CT brain.[6] Further reports of TBM/toxoplasmosis co-infection in HIV-infected individuals have been published.[3,20] Clinical algorithms may assist with presumptive diagnosis and initial management of HIV-infected patients presenting with focal brain lesions[50,51] but patients who deteriorate on empirical antimicrobial treatment may require a brain biopsy to confirm the diagnosis.

Medical management

The optimal treatment of TBM remains uncertain, as no randomized, controlled trials have determined the optimal drug combination, doses or duration of treatment.[24] Recommended first-line agents for the treatment of TBM include a backbone of rifampicin and isoniazid for nine to 12 months with pyrazinamide plus ethambutol or streptomycin or ethionamide during the initial phase (two months).[24] Isoniazid[52] and pyrazinamide[53] have excellent CSF penetration and are considered to be invaluable in the treatment of TBM. In contrast, the CSF penetration of rifampicin is poor, reaching concentrations only slightly in excess of the minimum inhibitory concentration against *M.tb*.[52] Although the role of rifampicin in the treatment of TBM has been challenged, evidence for its benefit is clear. While isoniazid resistance, with or without streptomycin resistance, has no significant effect on long-term outcome in adults with TBM, combined rifampicin and isoniazid resistance (multidrug resistance [MDR]) is associated with an increased mortality rate.[54] Plasma rifampicin concentrations may be reduced in HIV-infected patients compared to HIV-uninfected patients.[55] This raises the concern that low rifampicin concentrations in CSF may result in sub-optimal TBM treatment in HIV-infected patients.

Infection with drug-resistant *M.tb* further complicates the treatment of TBM in HIV-infected patients. HIV-infected patients with TBM frequently present with a history of previous TB (8%–58% of patients).[4,7–9,11,13,18,19] Isoniazid and streptomycin resistance have been associated with HIV infection[16] and prolonged clearance of *M.tb* from the CSF,[54] but have not been found to be associated with worse outcome.

By contrast, MDR-TBM has an extremely poor prognosis with a reported mortality of up to 100%.[9,19,54,56–58] This may partly be explained by delayed diagnosis and treatment resulting from the time taken to perform culture and drug susceptibility testing. Thwaites et al. found an independent association between MDR-TBM and HIV infection,[54] which occurred in 13% of HIV-infected and 4% of HIV-uninfected patients.[7] Other studies similarly found

a higher proportion of MDR-TB in HIV-infected compared with HIV-uninfected patients (42% versus 5% of isolates).[16,18] When treated appropriately, MDR-TBM in HIV-infected patients is not invariably fatal.[19,56,58] A high index of suspicion for the diagnosis should be maintained, especially in patients with a prior history of TB.[19]

Although corticosteroids improve the prognosis in HIV-uninfected persons with TBM,[59–62] its benefit in HIV-infected patients is uncertain. A Cochrane systematic review concluded that data regarding the benefit of adjuvant corticosteroids in HIV-infected patients with TBM was inconclusive.[62] It is common practice in many settings to use corticosteroids in HIV-infected patients with TBM, and some studies have suggested a benefit in these patients.[12,59] A randomized, placebo-controlled trial of dexamethasone in Vietnamese adults with TBM showed a non-significant reduction in mortality at nine months in HIV-infected patients not on ART.[59] Yechoor et al. found that not receiving corticosteroids was independently associated with a higher mortality at nine months in a predominantly HIV-infected patient population. However, corticosteroids may potentially cause harm in HIV-infected patients by exacerbating immune suppression and increasing the risk of infections or the development or exacerbation of Kaposi's sarcoma.[63–65]

Surgical management

Although medical therapy is the mainstay of treatment for TBM, surgical interventions may be required to assist in diagnosis, treatment of mass lesions or their complications, as well as treatment of hydrocephalus.[66] The presence of hydrocephalus in TBM patients is associated with a poor prognosis[67–70] but the management of hydrocephalus remains controversial as well-designed prospective randomized trials have not been conducted.[71] Surgical options include one or more of the following: external ventricular drainage, ventriculo-peritoneal (VP) shunting, and endoscopic third ventriculostomy (ETV).[71,72] Literature regarding the optimal surgical management of TBM-related hydrocephalus in HIV-infected patients is scarce. Nadvi et al. reported a dismal prognosis at one-month follow-up in patients who underwent VP shunting; 10/15 HIV-infected patients died compared to 4/15 HIV-uninfected patients.[73] The association between severe disease at presentation and poor outcome after VP shunting in TBM is well-recognised.[72,74] However, in the study by Nadvi et al., 80% of HIV-infected patients presented with milder disease severity (grade 1 and 2 of the Vellore Grading System[75]) compared to 47% of HIV-uninfected patients. The mean $CD4^+$ T-lymphocyte count in HIV-infected patients was 183 cells/μl and no patients received ART. These findings can not readily be generalized to patients with less severe immune suppression or those receiving ART. ETV is an emerging treatment option for TBM-associated hydrocephalus. Case series, using variable entry and outcome criteria, have shown success rates of 41–77% in TBM patients.[76–80] One of the major obstacles in the application and success of this procedure in TBM is the distortion of brain anatomy by basal inflammatory exudates.[72,76,77] Although the use of ETV in HIV-infected TBM patients has not been studied explicitly, it is considered to be of benefit in these patients, as: i) HIV-infected patients may have less basal exudate,[6] which allows permeability of the floor of the third ventricle and better visualization of basal brain structures and, ii) the potential hazard of shunt infection/blockage is avoided.[71]

Treatment of HIV infection

TBM is classified by the World Health Organisation as an HIV stage 4 disease requiring ART.[81] Starting ART during antituberculosis treatment improves outcome in HIV/TB co-infected patients,[82] but the optimal time to start ART in these patients is not known. Initiating ART during antituberculosis treatment may be associated with overlapping drug toxicities, drug–drug interactions and, paradoxical TB immune reconstitution inflammatory syndrome (TB-IRIS), all of which may be detrimental to the patient.[55,83] Conversely, delayed initiation of ART may result in progression of HIV disease and death. Two retrospective studies in HIV/TB co-infected patients have found a reduced mortality in patients who commenced ART earlier (within 2 months, compared to later[84]) and, in patients with $CD4^+$ T-lymphocyte counts <100 cells/μl, 2 weeks, compared to 8 weeks[85] after starting antituberculosis treatment. In the first randomized, double-blind, placebo-controlled trial comparing immediate ART (initiated ≤ 7 days after starting antituberculosis treatment) versus delayed ART (initiated 2 months after starting antituberculosis treatment) in adult patients showed similar mortalities (76/127 versus 70/126) at nine-month follow-up.[20] Grade 4 adverse events were significantly more frequent in the immediate ART group (80 vs. 69%; $p = 0.04$) during the 12 months of follow-up, suggesting that delayed treatment may be the preferred option.

One of the emerging complications of starting ART in patients on antituberculosis treatment is paradoxical TB-IRIS.[86,87] Paradoxical TB-IRIS is characterized by an exuberant inflammatory response against *M.tb* antigens in the context of a recovering immune system. The reported incidence is 8–43%.[86] Patients with paradoxical TB-IRIS typically present with clinical or radiological deterioration after starting ART, following an initial period of clinical improvement on antituberculosis treatment. Neurological involvement, documented in 12% of paradoxical TB-IRIS cases, is fatal in a significant proportion of patients[21] (see Figure 1 for illustrative case). Patients may present with new or worsening meningitis,[21,88,89] tuberculoma,[21,90–94] tuberculous brain abscess,[93,95] or radiculomyelitis.[21,89] Although paradoxical reactions have been described for HIV-uninfected and HIV-infected TBM patients not on ART,[96–101] paradoxical TB reactions (not restricted to the CNS) occur at much higher frequency in patients after initiating ART.[102] Diagnosing paradoxical neurological TB-IRIS is challenging; although case definitions have been published, no diagnostic test exists, and other causes for deterioration need to be excluded.[21,86] The differential diagnoses of paradoxical neurological TB-IRIS include: other CNS opportunistic infections; drug-resistant *M.tb* infection; non-compliance to antituberculosis treatment; and drug reactions and toxicities.[86] Sub-optimal antituberculosis drug doses should also be considered. The risk factors for paradoxical TB-IRIS include: disseminated TB; a shorter time interval from commencing antituberculosis treatment to initiating ART; severe immune suppression at TB diagnosis; and a rapid immune recovery following ART initiation.[55] The optimal management of patients with paradoxical neurological TB-IRIS is unknown. Support for the benefit of corticosteroids in these patients is derived from anecdotal case reports,[89,91] one case series,[21] and a randomized, placebo-controlled trial of prednisone in patients with mild to moderate paradoxical TB-IRIS.[103] The latter study showed significant symptomatic improvement and shorter duration of hospitalization in patients treated with prednisone (at a dosage of 1.5 mg/kg/day for 2 weeks followed by 0.75 mg/kg/day for 2 weeks) compared to placebo. However, patients with severe TB-IRIS manifestations (e.g. neurological IRIS) were not included in this study. Pepper et al. showed initial clinical improvement in 18/20 patients treated with prednisone for neurological TB-IRIS (at a starting dose of 1.5 mg/kg/day).[21] Six-month survival for the whole group ($n = 23$) was 70%. Alternative immunomodulatory therapies, such as thalidomide, pentoxifylline, montelukast (a leukotriene antagonist) and infliximab (a tumor necrosis factor–α inhibitor), have been used with success in selected cases of TB-IRIS involving extra-CNS sites or paradoxical CNS TB reactions

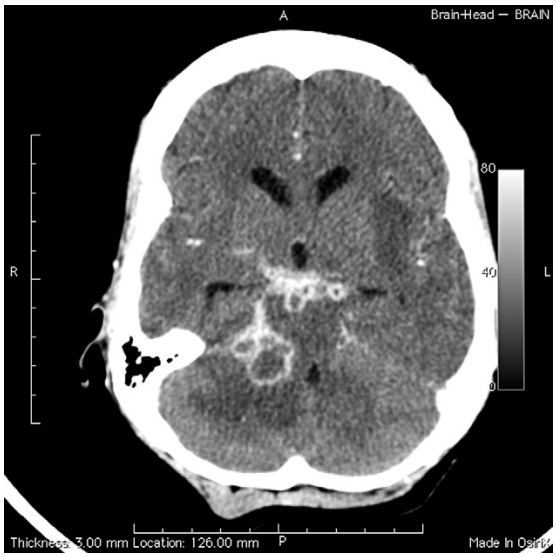

Figure 1. Contrast enhanced axial computed tomography (CT) image showing multiple tuberculomata, basal meningeal enhancement and hydrocephalus in a patient with tuberculous meningitis immune reconstitution inflammatory syndrome. A 41-year-old HIV-infected female (baseline $CD4^+$ T-lymphocyte count 123 cells/μl) was diagnosed with tuberculous meningitis; cerebrospinal fluid cultured *Mycobacterium tuberculosis* susceptible to rifampicin and isoniazid. She improved on antituberculosis treatment and was started on combination antiretroviral therapy (ART) nine weeks later. Seven days after ART initiation, she developed vomiting and difficulty walking. CT of the brain performed 18 days after symptom onset showed multiple ring-enhancing lesions with surrounding oedema, basal meningeal enhancement, and hydrocephalus with effacement of the fourth ventricle. Her $CD4^+$ T-lymphocyte count was 253 cells/μl and her HIV viral load was 290 copies/ml. She continued antituberculosis treatment and ART. Oral prednisone was started at a dose of 1.5 mg/kg/day and weaned over the next 3 months. Six weeks after initial presentation, her symptoms had resolved and her neurological examination was normal.

(in patients not on ART).[104] The potential application of these agents in TBM-IRIS still needs to be determined; their use in these patients may be limited due to potential serious adverse events.

Outcome

TBM is generally associated with a poor prognosis, even in HIV-uninfected patients.[28,30,59,61] Although one study found that the in-hospital mortality differed significantly between HIV-infected and HIV-uninfected patients (63% versus 18%, OR 7.4, 95% CI 3.0–18.5, $p = 0.0000$),[10] death during hospitalization appears to be similar between HIV-infected patients (13–72%) and HIV-uninfected patients (21–64%).[3,11,14,17,19] Cecchini et al. reported the following factors to be significantly associated with death during hospitalization in HIV-infected patients with TBM: BMRC grade II and III disease; infection with MDR-TB strains; and a $CD4^+$ T-lymphocyte count less than 50 cells/μl.[18] Berenguer et al. found a similar association between a low $CD4^+$ T-lymphocyte count (<200 cells/μl) and in-hospital mortality by univariate analysis[3] but this finding was not substantiated by other studies.[14,15] Other variables associated with reduced hospital survival in HIV-infected patients with TBM include duration of symptoms >14 days[3] and reduced Glasgow Coma Scale (GCS).[15]

Most studies report higher rates of death at six to nine months after initiation of antituberculosis treatment in HIV-infected patients (24–67%) compared to HIV-uninfected patients (0–30%).[5–8,12,28] Factors independently associated with death at six to nine months of follow-up in HIV-infected patients are: tachycardia,[19] increased TBM grade,[7,20] lower haematocrit,[7] not receiving corticosteroids,[12] and a history of ART prior to presentation.[19] Independent predictors of earlier time to death in HIV-infected patients predominantly not on ART include: increased TBM grade, decreased CSF lymphocyte percentage, and lower serum sodium.[9]

It is likely that severe immunosuppression and resultant HIV-related illnesses contribute to the poor outcomes observed in HIV-infected TBM patients. Most patients are severely immune suppressed at presentation (median/mean $CD4^+$ T-lymphocyte count 32–180 cells/μl),[3,4,7,9,11–15,18–20] and do not receive ART during TBM treatment.[6–9,16] A low $CD4^+$ T-lymphocyte count has been associated with a poor outcome by univariate, but not multivariate, analysis at nine months of follow-up.[7,19] This includes one study in which patients did not receive ART during antituberculosis treatment,[7] and another, in which a third of patients were known to be on ART at TBM diagnosis.[19] In the latter study, the authors did not comment on the number of patients who commenced ART during TBM treatment. In addition, patients who died were less likely to receive ART.[9] Unsurprisingly, a significant proportion of HIV-infected patients present with concomitant AIDS-defining illnesses at TBM diagnosis[3,4,6,18] or during the course of TBM treatment.[9] This was also observed in patients who commenced ART following TBM diagnosis.[20] Although rarely reported, AIDS-related illnesses account for up to 50% of patients with TBM (not on ART) who die.[8] Long-term mortality rates are also high for TBM patients receiving ART during antituberculosis treatment. In one study, the nine-month mortality in 253 patients started on ART within 2 months of antituberculosis treatment initiation (58%) was not markedly different compared to a historic control group predominantly not exposed to ART from the same site (67%).[9,20] In this study, the median $CD4^+$ T-lymphocyte count in patients started on ART was severely suppressed (40 cells/μl) at the time of TBM presentation, and a large proportion of patients died prior to peripheral blood HIV viral suppression. Croda et al. report a high mortality rate at nine months (41%) in a group of HIV-infected TBM patients, a third of which were known to be on ART at TBM diagnosis[19] Similar to the study by Torok et al. $CD4^+$ T-lymphocyte counts were severely suppressed at the start of antituberculosis treatment and did not differ between patients who were (76 cells/μl) and were not (70 cells/μl) receiving ART. The use of ART prior to TBM presentation was independently associated with nine-month mortality. The authors conclude that this unexpected finding probably relates to ART discontinuation or irregular use. It is conceivable that the timing of ART in patients enrolled into these two studies was 'a little too late' for many of them; improved outcomes might have been observed had ART been commenced prior to such extreme immunosuppression.

Conclusions

Although the clinical presentation, laboratory findings and radiological features of TBM are largely similar in HIV-infected and HIV-uninfected patients, some important differences exist. HIV-infected patients present more frequently with extra-meningeal tuberculosis and systemic features of HIV infection. The diagnosis poses significant challenges as the differential diagnosis is broader, and CSF parameters may differ, compared to HIV-uninfected patients. The management of HIV-infected TB patients is complicated by the need to treat both infections simultaneously, in the absence of a robust evidence base. In HIV-associated TBM, the clinical course and dismal outcome are undoubtedly influenced by profound immunosuppression at presentation, emphasising the need for earlier diagnosis of HIV infection and initiation of antiretroviral treatment. Strategies to improve early diagnosis and management of HIV-associated TBM are urgently required.

Conflicts of interest

We have none to declare.

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Addendum to Chapter 2

Burden of disease

Recent studies continue to emphasize the significant contribution of TBM to the health burden in high TB/HIV co-infection settings. Of HIV-infected patients admitted for CNS deterioration to a hospital in Ethiopia, TBM was the second most common cause, accounting for 22.5% of cases [1]. In a high HIV-prevalence setting in Malawi, *M. tuberculosis* was the cause of 18% of microbiologically confirmed meningitis cases [2]. In two studies from Cape Town each conducted over three years, TBM accounted for 28% (n=227) of microbiological confirmed meningitis cases in adults [3], and 53% (n=126) of bacterial meningitis (including bacterial and mycobacterial) cases in children [4], at a district-level hospital, and a tertiary referral hospital, respectively. Studies that define TBM based on laboratory methods are likely to underestimate TBM disease prevalence as, in contrast to cryptococcal meningitis and bacterial meningitis for which sensitive laboratory-based diagnostic methods exist, such tests are insensitive for TBM diagnosis [5].

Clinical and laboratory diagnosis

The most common differential diagnosis for TBM in HIV-infected patients is cryptococcal meningitis, which often presents with similar clinical and CSF features to that of TBM [1, 2, 6]. Although CSF India Ink, crytococcal latex agglutination test (CLAT) and/or fungal culture are necessary to rule out CM in HIV-infected TBM suspects, these tests are not always readily available in resource-poor settings. One study therefore investigated the utility of a diagnostic algorithm, based on clinical and routine laboratory findings, to distinguish TBM from CM [2]. In this study, higher lymphocyte counts (median [IQR]; 45 [0-280] versus 0 [0-18] cells/μL), lower CSF opening pressure (200

[145–275] versus 270 [160–340] mm H_2O), lower GCS (12.5 [8-15] versus 15 [14-15] /15), more frequent neck stiffness (78.3 versus 54.6 %) and higher temperature (mean ± SD; 39.8 ± 13.3 versus 37.2 ± 1.0 ^oC) distinguished CSF culture confirmed TBM (n=46, 89% HIV-infected) from CM (n=112, 98% HIV-infected) by multivariate analysis [2]. However, the diagnostic index that included these variables had insufficient sensitivity (83%) and specificity (79%) for its clinical application in the diagnostic approach to patients with HIV-associated meningitis.

A second prediction rule for the diagnosis of TBM in high HIV-prevalence settings developed during a study from Durban, South Africa, included the following factors that significantly associated with a definite diagnosis of TBM (i.e. either CSF culture or polymerase chain reaction [PCR] positive for *M. tuberculosis*) by multivariate analysis: CSF to serum glucose ratio ≤ 0.2 (score=2), CSF lymphocyte count >200 cells/μL (score=2), CD4 count <200 cells/μL (score=2) and a negative CLAT result (score=3) [7]. A combined score of ≥ 6 showed a sensitivity and specificity (95%CI) of 47% (31-64) and 98% (90-100), respectively, to diagnose definite TBM. The authors then included results of a lipoarabinomannan (LAM) antigen ELISA test performed on CSF in their diagnostic algorithm. Detection of LAM, a mycobacterial cell wall component, in urine is a useful, rapid diagnostic tool in HIV-associated TB patients with severe immunosuppression [8]. When CSF LAM (cut off OD ≥ 0.18) was included in the prediction-rule for definite TBM, the sensitivity increased to 63% (47-68) and specificity remained high at 93% (82-98) [7]. Although this algorithm shows promise as a good rule-in test for TBM in high HIV prevalence settings, its diagnostic application would need to be confirmed in larger studies.

A further laboratory method that is of potential value in the rapid diagnosis of TBM is the Xpert MTB/RIF test (Xpert, Cepheid, Sunnyvale, CA, USA), a real-time PCR assay for *M. tuberculosis* that simultaneously detects rifampicin resistance [9]. The Xpert assay performs better than microscopy in the diagnosis of pulmonary TB in *M. tuberculosis* culture positive sputum samples in both HIV-infected and uninfected patients [9]. Promising results have recently emerged for the application of Xpert in extra-pulmonary TB [10], including TBM [11-13]. A meta-analysis of the diagnostic accuracy of the test reported a pooled sensitivity (95% CI) of 80.4% (75.0-85.1), and a pooled specificity of 86.1% (83.5-88.4) in *M. tuberculosis* culture positive extra-pulmonary specimens [10]. In a study of TBM patients from Vietnam, the overall sensitivity of Xpert was 59.3% (n=108/182, 95% CI: 51.8-66.5) using clinical diagnosis as the reference standard and the specificity was 99.5% (97.2-100) [11]. In this study, the sensitivity of Xpert was significantly higher in HIV-infected compared to HIV-uninfected patients (sensitivity 78.8% versus 47.9%, OR: 4.01, 95% CI: 3.65-4.36, $p<0.001$). In a South African study that evaluated Xpert in the diagnosis of TBM in a predominantly HIV-infected co-hort (87% of the 204 participants were HIV-infected), the sensitivity and specificity of Xpert were 67% (n=36/54, 95%CI: 53-79) and 94% (n=61/65, 85-98), respectively; this analysis included patients with definite TBM and patients with other causes of meningitis [13]. Although the overall sensitivity decreased to 36% (n= 38/106, 95% CI: 27-46) when probable TBM was included in the reference standard, using a larger volume (3 ml) of centrifuged CSF compared to 1 ml of neat CSF increased the sensitivity markedly from 26% to 65%, in this analysis. In a study from India that compared the performance of light-emitting diode (LED) auramine fluorescent microscopy and Xpert for the diagnosis of TBM in HIV-infected TBM suspects, the number of positive results increased 12

fold with Xpert (n=35/142, 24.6%) compared to LED microscopy (n=3/142, 2.1%) [12]. Although results from these studies are encouraging for the utility of Xpert as a rapid rule-in test for TBM, the high costs associated with Xpert compared to LED microscopy (cost per test: 16.84 USD vs 0.2 USD), may restrict its use in resource-poor settings [12].

Outcome

Outcome in HIV-associated TBM remains poor in spite of increased availability of ART. A study from India reported a 12-month mortality of 51% in HIV-infected patients on standard TB treatment, 43% of these patients were receiving ART at time of TBM presentation [14]. In an Ethiopian study, 54% (42/78) of HIV-associated TBM cases, the majority of whom were not receiving ART at presentation, died during hospitalization [1].

Similar to previous studies, Alvarez-Uria *et al.* reported an association between lower CD4 count and mortality during TB treatment in HIV-associated TBM; adjusted hazard ratio (aHR [95% CI]) for CD4 ≤ 50 /μL = 1.95 (1.132–3.372) [14]. In this study, being on ART for less than six months compared to not receiving ART was associated with decreased mortality (aHR [95% CI] = 0.56 [0.311–0.995]) and low serum albumin concentration (≤ 3.8 g/dL) was an independent risk factor for death [14].

Medical management

The principles of treating TBM are still based on those used in pulmonary TB [5, 15], but there is increasing interest in exploring alternative evidence-based treatment regimens for TBM. Findings from two recent studies suggest a mortality benefit from 'intensified' treatment regimens early during TB treatment in TBM patients. Firstly, a randomized controlled

trial with a factorial design investigated the safety and pharmacokinetic profiles of higher than normal dose intravenous (IV) rifampicin as well as oral moxifloxacin in HIV-infected and -uninfected adults with TBM [16]. Although not powered to detect a mortality benefit, an improved outcome was observed in patients receiving IV rifampicin (~13mg/kg/day) compared to the oral standard dose (~10mg/kg/day) during the initial two weeks of TB treatment; patients who received IV rifampicin had a more rapid resolution of coma (median [IQR]; 4 [2-8] versus 5 [3-7] days) and reduced mortality at 6-month follow-up compared to those who received oral rifampicin (mortality, 34% versus 65% (aHR for death [95% CI]; 0.42 [0.20–0.91]) p=0.03). Secondly, an observational study in 203 HIV-infected TBM patients showed a mortality benefit in patients who received an initial intensified regimen of oral higher than normal doses isoniazid (~14mg/kg/day) and rifampicin (~21mg/kg/day) as well as pyrazinamide, ethionamide and levofloxacin for a median of seven days, compared to standard TB treatment (including rifampicin, isoniazid, ethambutol and pyrazinamide); the aHR (95%CI) for death with standard TB treatment was 2.05 (1.21-3.42) [14]. A further randomized controlled trial assessing the utility of higher doses of oral rifampicin (15mg/kg/day) and oral levofloxacin (20mg/kg/day) in improving TBM outcome is currently ongoing in Vietnam [17].

Corticosteroid treatment is associated with improved survival in HIV-uninfected patients with TBM and is recommended in all affected patients, regardless of HIV-status [15, 18]. In 2004 results from a randomized controlled trial of adjunctive dexamathosone in TBM were reported [18]; at 9-months follow-up, dexamethasone was associated with a reduced risk of death, compared to placebo which was significant in all patients (relative risk [RR] [95%CI]; 0.69 [0.52-0.92]; p=0.01). In subgroup analysis, the point estimate

of effect was similar in HIV-infected patients (n=98) but did not reach statistical significance (stratified RR of death [95 %CI]; 0.78 (0.59-1.04); p=0.08). Corticosteroid treatment was further associated with a decrease in severe adverse events, in particular hepatitis (0/274 versus 8/271 in the placebo group, p=0.004), in all patients. However, the outcome benefit seen with adjunctive corticosteroids was of limited duration. In a follow-up study, Torok *et al.* [19] determined outcomes of participants in Thwaites's trial [18] who received dexamethasone compared to placebo, two and five years after randomization. At 2-years follow-up, survival probability showed only a trend to being higher in the dexamethasone arm (0.63 [95%CI= 0.57-0.69] versus 0.55 [0.49-0.61]; p=0.07) and 5-year survival probability was similar (0.54 [0.48-0.60] versus 0.51 [0.45-0.57]; p=0.51). Furthermore, the dexamethasone group had a similar proportion of severely disabled patients among survivors at five years to the placebo group (17/128, 13.2% versus 17/116, 14.7%). As the authors suggested, it is likely that dexamethasone prevented death at nine months but left more patients disabled; these disabled patients then went on to die during the follow-up period.

Strokes occur in 15-58% of TBM patients and may occur prior to, or develop during, TB treatment [20]. The role of aspirin, an anti-platelet drug used widely for the prevention of ischemic stroke, have thus been explored in the management of TBM [21, 22]. In a randomized controlled trial of aspirin in 118 TBM patients (HIV status not reported), aspirin (150mg/day) was associated with a non-significant reduction in stroke (as evidenced by MRI findings) at 3-months follow-up, compared to the placebo group (24.2 versus 43.3 %; OR [95%CI]; 0.42 [0.12-1.39]) [21]. Importantly, aspirin was significantly associated with survival (OR [95% CI]; 3.17 [1.21-8.31]) at 3-months follow-up, and it was not withdrawn in any patients due to adverse

events. Conversely, a randomized controlled trial in 146 children with TBM (5 HIV-infected), found no significant benefit for either high-dose (100mg/kg/day) or low-dose (75mg/day) aspirin compared to placebo in relation to morbidity (hemiparesis and developmental outcome) and mortality during TB treatment [22]. In this study, one death was potentially related to aspirin and a second participant was withdrawn due to an aspirin-related adverse event. Further studies that include HIV-infected patients are required to confirm or refute the role of aspirin in the management of patients with TBM.

Treatment of HIV infection

The optimal time of starting ART in patients with HIV-associated TBM remains uncertain. Delaying ART in severely immune suppressed persons could result in HIV disease progression and increased vulnerability to other lethal opportunistic infections. Conversely, early ART initiation in TB patients increases the risk of developing TB-IRIS [23-25], which is frequently fatal when the CNS is involved [26, 27]. In a randomized controlled trial of patients with HIV-associated TBM reported in 2009, Torok *et al*. (referenced as an abstract in main text of this Chapter; reference 20) showed no difference in 9-months mortality between severely immunosuppressed TBM patients (median CD4 count = 41 cells/μL) who started ART immediately (within seven days of TB treatment), compared to later (two months after starting TB treatment) [28]. Since then, results from three randomized controlled trials that addressed the question of when to start ART in TB/HIV co-infected patients have been published [23-25]. These trials either showed a mortality benefit [23], or a decrease in the combined endpoint of death or AIDS-defining illness [24, 25], with starting ART 2-4 weeks, compared to 8-12 weeks, after TB treatment in patients with CD4 counts less than 50 cells /μL, in spite of an

increased risk of TB-IRIS in the early ART groups; a similar mortality benefit with starting ART early was not seen in patients with higher CD4 counts. The majority of patients in these studies had TB outside of the CNS and similarly, the majority of IRIS events did not involve the CNS [29-31]. Results may therefore not be applicable to patients with meningitis, who are at high risk of developing life-threatening intracranial TB-IRIS manifestations [32]. In view of this concern and the finding of an increased frequency of severe adverse events in TBM patients who commence ART early [28], current South African Department of Health guidelines recommend starting ART 4-6 weeks after TB treatment in TBM patients, regardless of CD4 count [33].

Paradoxical neurological TB-IRIS

Paradoxical TB-IRIS occurs in 8-54% of TB/HIV co-infected patients after starting ART [23-25, 34-38]; CNS involvement is reported in 0-31% of these cases [26, 27, 29, 35, 36, 38]. The risk of developing neurological TB-IRIS may be increased in patients who initially present with neurological involvement at TB diagnosis; in a study from Cape Town, 20% of neurological TB cases developed TBM-IRIS after ART initiation [36]. TBM-IRIS is the most life-threatening form of TB-IRIS with studies reporting associated mortality of 13-75% [26, 27, 38] compared to the 3.2 % estimate for all forms of paradoxical TB-IRIS combined [39]; however, in one small case series (n=6) no deaths were attributed to TBM-IRIS [36].

TB-IRIS pathogenesis

The pathogenesis of TB-IRIS is incompletely defined and no diagnostic test exists [40]. TB-IRIS typically occurs during the initial immune restoration phase following ART initiation that is characterized by a marked increase in CD4 count [34]. Earlier studies of TB-IRIS immunopathogenesis have

therefore focused on the adaptive immune system. Initial studies found that TB-IRIS was characterized by an expansion of *M. tuberculosis* antigen-specific CD4 cells after ART initiation compared to patients who did not develop TB-IRIS [41]. However, subsequent studies have shown that these expansions also occur in TB/HIV co-infected patients who do not go on to develop TB-IRIS, suggesting a contributing role for other factors to TB-IRIS pathogenesis [42]. Various recent lines of evidence are implicating the innate immune system as an important contributor to TB-IRIS pathogenesis [40]. Lung autopsy findings from a patient with unmasking pulmonary TB-IRIS reported in 2009 revealed the presence of CD68+ macrophages as the dominant inflammatory cell type [43]. Since then, higher natural killer cell activation states [44], monocytes [45], the complement system [46], pattern recognition receptors [47] and cytokines predominantly of myeloid origin such as IL-6 [38, 48], TNF [48] and IL-18 [49], have all been associated with TB-IRIS. Further reported associations between diverse inflammatory mediators and TB-IRIS *in vivo* at TB-IRIS presentation include elevated blood (serum or plasma) CXCL10 [49], IL-10 and IL-22 [50], IFN-γ [48] as well as matrix metalloproteinase (MMP)-7 [51]. Inflammatory mediators measured in plasma prior to ART initiation found to associate with subsequent TB-IRIS include the combination of decreased CCL2 and elevated CXCL10 and IL-18 (association was further strengthened in combination with increased IFN-γ responses to RD1 antigens and purified protein derivative; AUC 0.9) [49], and elevated IL-6 [38], IFN-γ (in patients not receiving corticosteroids) [52] and CXCL8 (in patients receiving corticosteroids) [52].

A potential mechanism by which corticosteroids result in symptomatic improvement in TB-IRIS [53] may be through its modulatory effects on cytokines and chemokines. Corticosteroids suppressed cytokine/

chemokine concentrations of IL-6, IL-10, IL-12p40, CXCL10 and TNF in blood of TB patients pre-ART [52] and during treatment for TB-IRIS [54]. Plasma TNF, IFN-γ, IL-6 and CXCL8 were further significantly higher in TB-IRIS patients not receiving corticosteroids, but not in those receiving corticosteroids, two weeks after ART initiation compared to patients who did not develop IRIS [52]. All TBM patients receive adjunctive corticosteroids for 6-8 weeks as part of routine care; this may reduce the frequency and/or severity of TB-IRIS in HIV co-infected TBM patients who initiate ART during this period.

No studies have thus far investigated immunological markers from the site-of-disease (e.g. lungs) in patients with TB-IRIS and no reports of immunopathogenesis in patients with neurological TB-IRIS exist. An excessive intracerebral inflammatory response is considered to be partly responsible for the neurological damage in TBM outside of the context of IRIS [55]; results from studies that investigated markers of inflammation in TBM patients could therefore direct research of TBM-IRIS immunopathogenesis. Studies in animals and *in vitro* models indicate that cytokines play an important part in the modulation of the immune response to infection with *M. tuberculosis* [56-58], and there is evidence for compartmentalization of various cytokines at the site of disease in humans [55, 59]. The following findings relate to CSF cytokines in TBM: 1) **Cytokine concentrations:** TBM is characterized by increased expression of pro-inflammatory cytokines (IFN-γ, TNF, IL-1β, IL-8, IL-6) and the regulatory cytokine IL-10 [55, 60-62]. Some of these inflammatory markers remain high for weeks to months after the initiation of TB treatment. 2) **Cytokine differences between HIV-infected and -uninfected patients with TBM:** In a study of 497 patients (89 HIV-infected), HIV co-infection was associated with six-fold lower IL-10

concentrations (log concentration [pg/ml] mean ± SD; 0.71 ±1.02 versus 1.47 ± 0.79, p<0.0001), and increased IFN-γ/IL-10 ratios (log ratio; 1.6 ± 0.70 versus 0.97 ± 0.99; p=0.0004) [61]; however these associations were not confirmed by others [60]. HIV-infected and -uninfected patients had similar concentrations of other cytokines, including IL-6, IL-8, IFN-γ and TNF [60, 61]. 3) **Correlation between cytokine concentrations and severity and outcome in TBM:** Simmons *et al*. [61] found that IL-6 concentration was independently associated with severe (British Medical Research Council [BMRC] grade III) disease (OR [95%CI]; 2.1 [1.1-3.8]; p=0.017) in HIV-uninfected patients and, death was independently associated with lower IFN-γ in HIV-infected patients, at presentation. He and others [62] did not find any correlation between TNF and disease severity, however Patel *et al.* [60] found a positive correlation between TNF (p=0.008) and IFN-γ (p=0.03) concentrations and disease severity (BMRC grade II and III versus I) in HIV-infected (n=17) and -uninfected (n=10) patients combined. 4) **Modulation of cytokine levels by corticosteroid therapy:** Only a marginal reduction in IFN-γ but no other cytokine concentration differences were found in patients treated with adjunctive dexamethasone (n=27) compared to patients on TB treatment alone (n=25) (mean ± SD; net reduction of IFN-γ during the first week of therapy: 2.2 ± 3.3 versus 2.0 ± 5.9 ng/ml; p = 0.06) [63].

Other inflammatory mediators including MMPs and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs), have also been reported as markers of disease severity in TBM [64-66]. MMPs are a family of zinc-dependant endopeptidases that degrade extracellular matrix components, including those found in the blood-brain-barrier [67]. To protect the host during inflammation, the activity of MMPs are inhibited by synchronously expressed TIMPs, which bind MMPs in a 1:1 ratio [68]. Elevated CSF MMP-

9 [64-66] and MMP-2 [64] concentrations and increased MMP-9:TIMP-1 ratios [65] have been associated with death [66] and neurological complications [64, 65] in patients with TBM. Cerebrospinal fluid concentrations of MMP-9 were previously shown to decrease in HIV-uninfected patients receiving dexamethasone, compared to those receiving placebo, during TBM treatment and it was postulated that this may represent a mechanism by which corticosteroids improve outcome in these patients [69].

Treatment and prevention of TB-IRIS

Corticosteroids continue to be the mainstay of paradoxical neurological TB-IRIS treatment and few recent studies have investigated alternative treatment options [70]. In a small case series, three children received thalidomide, in addition to high-dose corticosteroids during treatment for paradoxical neurological TB-IRIS; ART was interrupted in all cases [71]. Outcome in these patients was variable: one died; one recovered over months of treatment; and in the third, who had recurrent relapses after three attempts to re-introduce ART, the addition of mycophenylate mofetil eventually allowed the successful re-initiation of ART.

Results of the first randomized controlled trial for the prevention of IRIS have recently been reported [72]. Severely immunosuppressed (CD4 <100/μL) HIV-infected patients were randomized to receive maraviroc (600 mg twice daily) or placebo in addition to standard ART during the first 24 weeks of ART; the primary endpoint was the development of IRIS during this period. Maraviroc is an antiretroviral agent that blocks HIV entry into host cells through its antagonistic binding of chemokine receptor 5 (CCR5) [73]. It was therefore proposed that maraviroc could potentially down regulate inflammation by blocking the binding of natural CCR5 ligands to their receptors, such as

CCL3, CCL4, CCL5 and CCL8 [73, 74], resulting in a decreased incidence of IRIS. Disappointingly, maraviroc was not associated with a decrease in either the frequency (33/140, 23.6% versus 31/136, 22.8%), or the severity (severe IRIS: 5.5% versus 6.6%), of IRIS events compared to placebo.

Conclusions

HIV-associated TBM is a common devastating disease, particularly in high TB/HIV co-infection settings. In addition to challenges with regards to early diagnosis and optimal treatment strategies during the initial presenting event, neurological TB-IRIS further compromises survival in those patients who recover sufficiently to start ART. The pathogenesis of TB-IRIS is incompletely understood and no treatment is of known benefit in the prevention or treatment of neurological TB-IRIS. A better understanding of the immunopathogenesis of TBM-IRIS may direct future research of strategies for both the prevention and treatment of this frequently fatal phenomenon.

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Chapter 3

Tuberculous meningitis: a uniform case definition for use in clinical research

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Tuberculous meningitis causes substantial mortality and morbidity in children and adults. More research is urgently needed to better understand the pathogenesis of disease and to improve its clinical management and outcome. A major stumbling block is the absence of standardised diagnostic criteria. The different case definitions used in various studies makes comparison of research findings difficult, prevents the best use of existing data, and limits the management of disease. To address this problem, a 3-day tuberculous meningitis workshop took place in Cape Town, South Africa, and was attended by 41 international participants experienced in the research or management of tuberculous meningitis. During the meeting, diagnostic criteria were assessed and discussed, after which a writing committee was appointed to finalise a consensus case definition for tuberculous meningitis for use in future clinical research. We present the consensus case definition together with the rationale behind the recommendations. This case definition is applicable irrespective of the patient's age, HIV infection status, or the resources available in the research setting. Consistent use of the proposed case definition will aid comparison of studies, improve scientific communication, and ultimately improve care.

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Introduction

Tuberculous meningitis is the most severe form of tuberculosis and causes substantial morbidity and mortality in adults and children.[1–10] The outcome of this disease is especially grave in patients with HIV.[11–14] Clinicians face substantial challenges in the diagnosis and management of tuberculous meningitis:[15] disease pathogenesis is poorly understood; rapid, sensitive, and affordable diagnostic tests are not available; and the best management has not been established by randomised controlled trials. Further research is urgently needed, but the low frequency with which a definitive diagnosis is established and the absence of a uniform clinical case definition are major obstacles. Detection of acid-fast bacilli (AFB) and culture isolation of *Mycobacterium tuberculosis* from cerebrospinal fluid (CSF) have been reported with high frequency in some studies[16,17] but are the exception in most.[1,7,18,19] Diagnosis usually relies on clinical evidence, which combines supportive clinical, laboratory, and radiological findings. Standardised diagnostic criteria for tuberculous meningitis have not been established, and most reports have used different case definitions. This absence of standardisation makes comparison of research findings difficult, prevents the best use of the existing data, and limits progress in management. To address these issues, an international tuberculous meningitis workshop took place in Cape Town, South Africa, in May, 2009, to establish a consensus case definition for tuberculous meningitis for use in future clinical research. We present the consensus case definition, which should be uniformly applicable, irrespective of the patient's age or HIV infection status or the resources available in the research setting.

The organisers of the meeting invited leading tuberculous meningitis researchers and clinicians with experience managing patients with tuberculous meningitis. 41 participants from seven countries (South Africa, the UK, Vietnam, India, the USA, Malawi, and Indonesia) attended the meeting. Participants included paediatric and adult neurologists, neurosurgeons, infectious diseases specialists, microbiologists, immunologists, pharmacologists, and clinical trialists. 13 international tuberculous meningitis experts, all of whom have published on the disease in international peer-reviewed journals during the past 5 years, presented their research findings. On the final day of the meeting, the existing diagnostic criteria were assessed and a consensus case definition for tuberculous meningitis was developed. A writing committee of nine members was appointed to review the existing data and develop the final consensus statement.

Standardised clinical case definition

In the past 5 years, studies have used various case definitions for tuberculous meningitis (panel 1).[1,3,4,8,10,11,17,18,20–24] In most definitions, patients are given a definite, probable, or possible tuberculous meningitis status depending on clinical, laboratory, and radiological findings. Definite tuberculous meningitis cases usually include patients with AFB on CSF microscopy or *M tuberculosis* cultured from CSF or another CNS source. However, some studies also included patients with *M tuberculosis* identified from specimens such as gastric aspirates, urine, or sputum,[11,24] or with *M tuberculosis* identified by PCR[8,25–27] or IgM ELISA.[8] Criteria for probable or possible tuberculous meningitis cases differ greatly between studies, especially between studies done in children and adults. Some studies do not distinguish between patients with confirmed and suspected tuberculous meningitis.[4,7]

We propose that patients with suspected tuberculous meningitis should be allocated to one of four diagnostic categories depending on the strength of clinical, laboratory, or radiological findings. The proposed categories are definite, probable, possible, and not tuberculous meningitis (figure 1). A patient suspected to have tuberculous meningitis should be regarded as

Panel 1: **Examples of tuberculousis case definitions used for adults, children, or both in the past 5 years**

Adults only

Torok (2008)[17]

- Patients: HIV seropositive, ≥15 years of age
- Case definition includes definite and probable tuberculous meningitis cases
- Definite tuberculous meningitis: CSF smear positive for AFB and/or culture positive for *Mycobacterium tuberculosis*
- Probable tuberculous meningitis: clinically suspected tuberculous meningitis plus one or more of the following four criteria:
  - i) CXR consistent with pulmonary tuberculosis
  - ii) Other specimens (eg, sputum, lymph node, gastric washings) positive for AFB
  - iii) Evidence of extrapulmonary tuberculosis
  - iv) CT or MRI evidence of tuberculous meningitis
- Patients were excluded if there was microbiological evidence of another CNS infection.

Kalita (2007)[8]

- Patients: HIV seronegative, ≥13 years of age
- Case definition includes definite and suggestive tuberculous meningitis cases.
  - A) Clinical criteria: meningitic symptoms including fever, headache, and vomiting for 2 or more weeks
  - B) Supportive criteria:
    - i) CSF cells ≥20/μL with predominant lymphocytes, protein ≥2 g/L
    - ii) CT scan evidence of exudates, infarctions, hydrocephalus, and tuberculoma in various combinations
    - iii) Evidence of extra-CNS tuberculosis
    - iv) Response to antituberculosis therapy
  - C) Exclusion criteria: malaria, septic, fungal, and carcinomatous meningitides
- Definite tuberculous meningitis: A and C plus positive PCR for *M tuberculosis* or IgM ELISA , or AFB in CSF smear or culture
- Suggestive tuberculous meningitis: A, C, and three or more of B

Thwaites (2004)[10]

- Patients: HIV seropositive and negative, >14 years of age
- Case definition includes definite, probable, and possible tuberculous meningitis cases
- Definite tuberculous meningitis: clinical meningitis (nuchal rigidity and abnormal CSF parameters) and AFB in the CSF
- Probable tuberculous meningitis: clinical meningitis and one or more of the following:
  - i) Suspected active pulmonary tuberculosis on the basis of CXR
  - ii) AFB found in any sample other than from the CSF
  - iii) Clinical evidence of other extrapulmonary tuberculosis
- Possible tuberculous meningitis: clinical meningitis and four or more of the following:
  - i) History of tuberculousis
  - ii) Predominance of lymphocytes in the CSF
  - iii) Illness of more than 5 days in duration
  - iv) CSF to blood glucose ratio of less than 0·5
  - v) Altered consciousness
  - vi) Yellow CSF
  - vii) Focal neurological signs
- Patients were subsequently reclassified as having definite tuberculous meningitis if AFB were seen in or *M tuberculosis* was cultured from the CSF, and as not having tuberculous meningitis if another diagnosis was confirmed by microbiological or histopathological assessment

Adults and children

Nagesh Babu (2008)[21]

- Case definition includes definite and presumptive tuberculous meningitis cases
  - A) Clinical criteria: fever, headache, meningeal signs, and other clinical presentations of meningitis lasting for more than 2 weeks.
  - B) CSF criteria: typical features including pleocytosis (>20 cells/μL), lymphocytes >60%, protein >1 g/L, and CSF: blood glucose ratio of less than 0·6
  - C) Supportive criteria:
    - i) Isolation of *M tuberculosis* from body secretion other than CSF in smear or culture
    - ii) CXR findings of pulmonary tuberculosis (reticulonodular pattern in upper lobes with or without cavitary lesions)
    - iii) Hydrocephalous from brain CT scan
  - D) Negative bacterial and fungal cultures and negative India ink
- Definite tuberculous meningitis diagnostic criteria not stated
- Diagnosis of presumptive tuberculous meningitis requires A, B, one or more of C, and D to be fulfilled

Rafi (2007)[23]

- Patients: HIV seropositive and negative
- Case definition includes culture-confirmed and clinical tuberculous meningitis cases
- Diagnosis of clinical tuberculous meningitis requires A, B, and C:
  - A) Clinical findings: headache, fever, and vomiting for more than 3 weeks
  - B) CSF findings: pleocytosis and high protein concentration
  - C) Neuroimaging findings: the presence of a basal exudate with or without hydrocephalus

(Continues on next page)

having possible or probable tuberculous meningitis, depending on initial lumbar puncture or cerebral imaging findings. Results from subsequent tests will determine the patient's final diagnostic category—for example, a patient will move up to the definite tuberculous meningitis category if *M tuberculosis* is cultured from their CSF. Conversely, a patient will move down to the not tuberculous meningitis category if evidence for an alternative diagnosis is identified (panel 2).

Definite tuberculous meningitis

A diagnosis of definite tuberculous meningitis is made when AFB are seen, *M tuberculosis* is cultured, or *M tuberculosis* is detected by a reliable molecular method from the CSF in someone with symptoms or signs suggestive of the disease. AFB seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord are also diagnostic for tuberculous meningitis (mostly seen on autopsy). Identification

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(Continued from previous page)

3) Children only

van Well (2009)[1]

- Patients: HIV seropositive and negative
- Case definition includes definite and probable cases
- Definite tuberculous meningitis: *M tuberculosis* isolated from CSF
- Probable tuberculous meningitis: clinical signs of meningitis plus characteristic CSF findings (macroscopically clear, pleocytosis, raised protein, and reduced glucose), plus two or more of the following criteria:
  - i) Recent poor weight gain (crossing of percentiles on Road to Health card)
  - ii) Household contact with sputum smear-positive tuberculosis
  - iii) CT scan compatible with tuberculous meningitis
  - iv) CXR compatible with primary tuberculosis
  - v) Positive TST
  - vi) Other clinical specimens positive for AFB

Andronikou (2006)[20]

- Case definition includes definite and probable cases
  - A) Clinical criteria:
    - i) Household tuberculosis contact
    - ii) Positive Mantoux tests (>15 mm)
    - iii) Neurological features: depressed level of consciousness, focal neurological signs, raised intracranial pressure, seizures, or meningism
    - iv) Systemic upset (in combination with neurological findings): failure to thrive, fever, night sweats, proven pulmonary tuberculosis, proven abdominal tuberculosis, or evidence of extra-neurological involvement
    - v) Prolonged symptoms for more than 48h
  - B) CSF criteria:
    - i) Pleocytosis (>20 cells per µL) with lymphocyte predominance (>50%)
    - ii) Protein concentration greater than age-specific normal value; especially >1·0 g/L
    - iii) Chloride <120 mmol/L
    - iv) Glucose concentration less than 60% of concentration in blood
    - v) AFB on stains; negative India ink stains for cryptococcosis
    - vi) Culture positive for tuberculosis
- Definite tuberculous meningitis: culture-positive CSF for tuberculosis—ie, B(vi)
- Probable tuberculous meningitis: A (iii) and two other positives from A and three positives from B
- Patients are classified as not having tuberculous meningitis if criteria are not met or if an alternative diagnosis is made by culture or trial of therapy.

Saitoh (2005)[4]

- Diagnostic criteria for tuberculous meningitis:
- CSF pleocytosis (≥10 cells per µL) with one or more of the following:
  - i) Positive CSF culture for *M tuberculosis* complex organisms (*M tuberculosis* or *Mycobacterium bovis*)
  - ii) Positive *M tuberculosis* PCR in CSF
  - iii) Positive CSF smear for AFB
  - iv) Positive gastric aspirate *M tuberculosis* culture or AFB smear
  - v) Positive TST with clinical evidence of tuberculous meningitis including CT findings or close contact with individuals with known or suspected tuberculosis.

CSF=cerebrospinal fluid. AFB=acid fast bacilli. CXR=chest radiograph. TST=tuberculin skin test.

of *M tuberculosis* from CSF, either by detection of AFB or culture, is difficult, but the chances of positive diagnosis can be increased by doing more lumbar punctures. From an initial lumbar puncture sample, Kennedy and Fallon[28] recorded the sensitivity of microscopy to be 37% and the sensitivity of culture to be 52%. When up to four lumbar punctures were done, the sensitivity of microscopy increased to 87% and the sensitivity of culture increased to 83%. Increasing the volume of CSF obtained and meticulous microscopy (for at least 30 min) further increases the chance of positive diagnosis.[16] Although the benefits of fluorescence microscopy to detect AFB from sputum is well established,[29] data on its sensitivity for the detection of AFB from CSF are sparse.[30] Light-emitting diode fluorescence microscopy systems provide a simple and inexpensive alternative to costly mercury vapour bulbs,[29] but further studies are needed to assess its application in the diagnosis of tuberculous meningitis. The microscopic observation drug susceptibility assay is a liquid culture method that detects characteristic *M tuberculosis* morphology by use of an inverse light microscope.[31] This technique, which enables simultaneous detection of *M tuberculosis* and determination of drug susceptibility, might prove a rapid and sensitive diagnostic method for tuberculous meningitis.[32]

CSF molecular diagnostic methods, such as nucleic acid amplification tests[4,8,25–27] and *M tuberculosis* antibody detection assays,[8] have previously been included in diagnostic criteria for tuberculous meningitis. Because of their highly variable sensitivity and specificity, both antibody assays[33–35] and in-house nucleic acid amplification tests[18,23] are regarded as experimental. Commercial nucleic acid amplification tests generally show high specificities[36] and can therefore be used to establish a definitive diagnosis in someone with symptoms or signs suggestive of tuberculous meningitis. However, more data are urgently needed to establish the robustness of these tests in field conditions; the specificities of both culture and PCR methods might be compromised in areas endemic for tuberculosis because of an increased risk of sample cross-contamination.[37] The Xpert MTB/RIF assay (Cepheid, CA, USA), is one of several methods that uses real-time PCR to amplify and detect *M tuberculosis* and identify drug resistance. This is an easy, rapid method that was sensitive and highly specific in initial studies done in patients with pulmonary tuberculosis.[38] Studies are underway to validate these findings and to determine this assay's application in extrapulmonary tuberculosis.

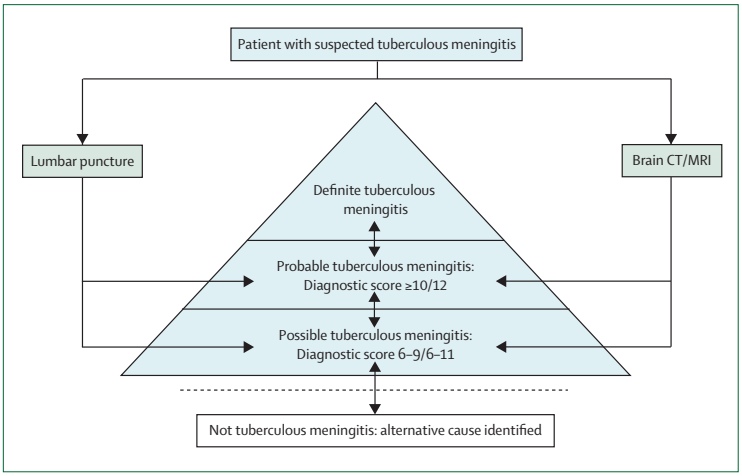

Figure 1: **Categories for patients with suspected tuberculous meningitis**
Patients suspected of tuberculous meningitis enter a study after lumbar puncture or brain imaging has been done in those meeting required criteria. Patients then move up or down the diagnostic pyramid as subsequent results become available and are classified into definite, probable, possible, or not tuberculous meningitis according to diagnostic criteria. Definite tuberculous meningitis: microbiological identification or evidence from commercial nucleic acid amplification tests of CNS *Mycobacterium tuberculosis* infection. Probable tuberculous meningitis: when imaging is available a diagnostic score of 12 or above is required, and when imaging is not available, a diagnostic score of 10 or above is required. Possible tuberculous meningitis: when imaging is available a diagnostic score of 6–11 is required, and when imaging is not available, a score of 6–9 is required.

In summary, a diagnosis of definite tuberculous meningitis should be made when one or more of the following criteria are met: AFB seen in the CSF, *M tuberculosis* cultured from CSF, or a CSF *M tuberculosis*-positive commercial nucleic acid amplification test from a patient who presents with symptoms or signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord together with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

Probable and possible tuberculous meningitis

We propose that the diagnosis of probable or possible tuberculous meningitis be determined by a diagnostic scoring system (table) that requires the presence of symptoms or signs indicative of meningitis plus additional clinical, CSF, or imaging criteria, with exclusion of the most likely alternative diagnoses. The number of points required for a diagnosis of probable tuberculous meningitis (≥12 when imaging is available or ≥10 when imaging is not available) and the points awarded in each category were decided by reviewing studies that quantified the diagnostic contribution of specific variables[5,19,25,39] and based on personal experience and consensus. The guiding principle was to encourage a uniform approach and to ensure diagnostic certainty of the highest possible standard, irrespective of the available resources. We have considered all available evidence on the differential diagnostic relevance of clinical variables to standardise definitions and establish uniformity that will allow direct comparison in future tuberculous meningitis studies. Thus, although the proposed case definition requires formal validation in both retrospective and prospective studies in various settings, its application should encourage identification in reports of the most important diagnostic variables and allow more accurate comparison of the results of different studies.

Clinical criteria

The clinical presentation of tuberculous meningitis is non-specific, especially in the early stages of disease. Adults and children typically present with fever, headache, irritability, neck stiffness, and vomiting.[1,5–7,11,39,40] These symptoms do not, however, distinguish tuberculosis from other forms of meningitis.[5,19,39,41] Systemic symptoms of *M tuberculosis* infection, such as lethargy, cough, weight loss (or poor weight gain in children), and night sweats might be suggestive of tuberculosis, but are also non-specific.[1,6,7,11] Unfortunately, many patients present with advanced disease and neurological signs, such as convulsions, an abnormal mental state, cranial nerve palsies, or limb paresis.[1,6,7,11,12] Although patients with HIV are more likely to present with disseminated tuberculosis than patients without HIV,[9,12] and might have clinical features suggestive of HIV/AIDS,[11,17] HIV infection does not seem to affect the neurological presentation of tuberculous meningitis.[9,11,12]

Four studies have attempted to define the clinical and laboratory features that distinguish tuberculous meningitis from bacterial[5,19,25,39] and other[19] causes of meningitis. The diagnostic value of headache to differentiate bacterial meningitis from tuberculous meningitis showed discrepant results.[5,39] In one adult study, cranial nerve palsies were more common in adult patients with tuberculous meningitis than in those with bacterial meningitis.[39] Optic atrophy, extra-pyramidal movements, and focal deficits were associated with tuberculous meningitis in one paediatric study,[19] but optic atrophy is a late occurrence and abnormal extra-pyramidal movements are rare. None of these findings have been validated in patients with HIV, and cryptococcal meningitis shares similar clinical features.[42,43]

The average duration between symptom onset and tuberculous meningitis presentation is 5–30 days[1,4–6,10,19] and does not differ between patients with and without HIV.[12,44] Symptom duration of more than 4 days,[25] 5 days,[5,39] or 6 days[19] has been used to discriminate between tuberculous meningitis and bacterial meningitis in HIV-seronegative adults and paediatric patients, but cannot differentiate tuberculous meningitis from cryptococcal meningitis, which also presents as subacute meningitis.[41,45]

A history of recent close contact with a person with infectious tuberculosis (within the past 12 months) is an important clue to the diagnosis of tuberculous meningitis in children, affecting more than 50%.[1,44,46,47] A positive tuberculin skin test also indicates probable *M tuberculosis* infection. However, the sensitivity of the tuberculin skin

test is variable; reported sensitivities range from 17% to 32% in mixed adult and paediatric populations[6,7] and 30% to 77% in children.[1,11,19,44,46] In infants, the sensitivity can be as high as 86%,[48] but specificity would be reduced in settings with universal BCG vaccination because of cross-reacting immune responses, which tend to wane with time. An HIV infection in patients with tuberculosis[49] or tuberculous meningitis[44,50] reduces the likelihood of positive tuberculin skin test results. Additionally, a positive tuberculin skin test does not differentiate *M tuberculosis* infection (which is a common event in tuberculosis endemic areas) from active disease.[51]

Peripheral blood interferon-gamma release assays are useful in the diagnosis of tuberculosis infection.[52] Generally, these tests have higher specificity than do tuberculin skin tests,[53] although sensitivities vary and interferon-gamma release assays do not make the crucial distinction between *M tuberculosis* infection and active disease. There is little data on the efficiency of the assays in severe disease such as tuberculous meningitis, and their role in the diagnosis of children or patients with HIV has not been established. In immunocompromised adults with extrapulmonary tuberculosis these assays have a sensitivity of 88% (95% CI 68–97%) and a specificity of 71% (51–86%),[54] and in adult patients with tuberculous meningitis they have a sensitivity of 69% (51–83%) and a specificity of 57% (42–70%).[55] However, further studies are needed to validate these findings. The rate of false negatives in patients with HIV with active tuberculosis increases as the CD4 cell count declines,[56] which is of particular importance in such patients with tuberculous meningitis, many of whom present with profound immunosuppression. A systematic review of commercially available antibody detection tests concluded that such tests have no role in the diagnosis of extrapulmonary tuberculosis.[57]

Response to tuberculosis treatment is generally included in classification systems[8,22,24] but is an unreliable diagnostic aid. In practice, many patients presenting with clinical features of meningitis receive antimicrobial treatment for both tuberculous meningitis and bacterial meningitis. Improvement of the patient's health after treatment will also not distinguish tuberculous meningitis from viral meningitis, which usually resolves spontaneously. Conversely, no response to treatment does not preclude the diagnosis of tuberculous meningitis; patients with multidrug-resistant tuberculous meningitis show little or no clinical improvement with standard tuberculosis treatment and their prognosis is poor.[58,59] Furthermore, at least 10% of patients with tuberculous meningitis have paradoxical deterioration after treatment initiation.[40,60] For these reasons, we have not included response to treatment in the consensus case definition.

In summary, all patients with features suggestive of meningitis (one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, or altered consciousness) and without a definite tuberculous meningitis diagnosis, should, according to our consensus case definition, be classified as probable, possible, or not tuberculous meningitis, depending on their total diagnostic score. Duration of neurological symptoms for more than 5 days is the most informative clinical criterion. A miliary picture on chest radiograph, the presence of lethargy, or other subtle signs in high-risk children (ie, children less than 3 years of age with documented *M tuberculosis* exposure) might also justify CSF examination or imaging studies. The score required for classification as probable tuberculous meningitis will vary according to the availability of cerebral imaging (≥12 when imaging is available or ≥10 when imaging is not available) to encourage a uniform approach and ensure diagnosis with the highest possible certainty, irrespective of the resources available.

***Panel 2:* Consensus tuberculous meningitis diagnosis**

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 fulfill criterion A or B:
  - A) Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; *Mycobacterium tuberculosis* cultured from the CSF; or a CSF positive commercial nucleic acid amplification test.
  - 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.

CSF=cerebrospinal fluid.

CSF criteria

Routine analysis of CSF in most patients with tuberculous meningitis shows clear appearance, pleocytosis (range=5–1000 cells per µL; median=50–450 cells per µL) with a lymphocyte predominance, a raised protein concentration (0·5–3 g/L), and a low glucose concentration (absolute value <2·2 mmol/L and a CSF to

	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 *Mycobacterium tuberculosis* cultured from another source—ie, 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)	

TST=tuberculin skin test. IGRA=interferon-gamma release assay. NAAT=nucleic acid amplification test. AFB=acid-fast bacilli. 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.

Table: **Diagnostic criteria for classification of definite, probable, possible, and not tuberculous meningitis**

plasma ratio <50% [median=~27%]).[1,5,6,13,17,19,61] Although lower CSF protein concentrations[13,14] and white cell counts[13,14,62] have been described in patients with HIV, most studies show similar findings compared to patients without HIV.[11,12,44,63–65] Atypical CSF findings have been described in both groups, including normal CSF glucose, protein, cell count, or a neutrophil predominance.[13,14,17,19,63–65] Rare cases of culture-proven tuberculous meningitis with no other CSF abnormalities have also been reported.[9] CSF findings that favour the diagnosis of tuberculosis over bacterial meningitis include clear appearance,[5] a white-cell count less than or equal to 900–1000/μL,[5,25,39] a neutrophil content less than 30–75%,[5,19,25,39] and a protein concentration greater than 1 g/L.[5] These studies did not, however, include patients with HIV and cryptococcal meningitis, many of whom have similar CSF findings.[45] Although CSF adenosine deaminase activity determination can be of benefit as a rule-in or rule-out test when values of less than 4 U/L and greater than 8 U/L are used, it cannot discriminate between tuberculous meningitis and bacterial meningitis.[66] False-positive results can also be found in patients infected with HIV who have other HIV-associated neurological diseases, such as cryptococcal meningitis, lymphomatous meningitis, and cytomegalovirus disease.[67]

The specificity of tuberculous meningitis diagnosis can be increased by molecular diagnostic tests. A systematic review and meta-analysis of commercial nucleic acid amplification tests for the diagnosis of tuberculous meningitis showed a combined average sensitivity of 56% and specificity of 98%.[36] Because of its high specificity, a positive commercial nucleic acid amplification test is regarded as a definitive test in patients with suspected tuberculous meningitis, and offers particular value in

patients who have previously received tuberculosis treatment.[68] Detection of *M tuberculosis* antigens[30,69,70] and antibodies[35,70] in CSF, and CSF interferon-gamma release assays[26,55,71] might be useful, but these results require verification in large studies from various settings.

In the consensus case definition, a maximum score of four points is assigned to CSF criteria that include: a clear CSF appearance; a leucocyte count of 10–500 cells per µL; a lymphocyte predominance (>50%); a protein concentration greater than 1 g/L; and a CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration of less than 2·2 mmol/L. A commercial *M tuberculosis* nucleic acid amplification test is regarded as a definitive diagnostic test for tuberculosis. At present, insufficient evidence exists for the inclusion of CSF antibody or interferon-gamma release assay tests.

Cerebral imaging criteria

The contribution of cerebral imaging to the diagnosis of tuberculous meningitis is well established, although its performance is not essential to establish a diagnosis of definite or probable disease. Abnormalities are most frequently detected in patients with severe disease.[72] On CT, hydrocephalus and basal meningeal enhancement are the most common radiological features of tuberculous meningitis. About 80% of children have hydrocephalus[3,72–74] and 75% basal meningeal enhancement;[1,3,75] whereas about 45% of adolescents and adults have hydrocephalus[7,8,72] and 8–34% basal meningeal enhancement.[7,8,72] Infarcts (8–44% of cases)[1,3,7,8,72–74] and tuberculoma (8–31%)[1,3,7,8,73,74] are also seen in many cases (figure 2). Andronikou[76] recorded the presence of a pre-contrast hyperdensity in the basal cisterns to be 100% specific of tuberculous meningitis in children, but this finding has not been validated in other studies. The combination of basal meningeal enhancement, infarcts, and hydrocephalus also had 100% specificity; basal meningeal enhancement was the most sensitive feature (89%). Kumar[74] reported similar results. MRI has higher sensitivity than CT for the detection of abnormalities such as meningeal enhancement, infarcts, and tuberculomas,[77,78] especially of lesions involving the brainstem. Thwaites[79] reported MRI findings in adult patients with tuberculous meningitis; 82% had basal meningeal enhancement and hydrocephalus was seen in 77% of cases. In the same study, tuberculoma developed in 74% of patients during treatment, most of which were asymptomatic. Children[11,44] and adults[14,27,63] with tuberculous meningitis and HIV show less hydrocephalus[11,14] and basal meningeal enhancement,[11,14] and a higher frequency of infarcts,[27] gyral enhancement,[44] and mass lesions compared with patients who do not have HIV.[63] Patients with HIV and tuberculous meningitis are also more likely to have cerebral atrophy,[11,14] which can be difficult to distinguish from communicating hydrocephalus. However, these findings are not consistent and some authors recorded no difference on imaging between the adults with and without HIV.[64] Alternative causes of meningitis, such as cryptococcal meningitis, cytomegalovirus encephalitis, toxoplasmosis, sarcoidosis, meningeal metastases, and lymphoma, which are more common in patients with HIV, can result in similar radiological findings.[61]

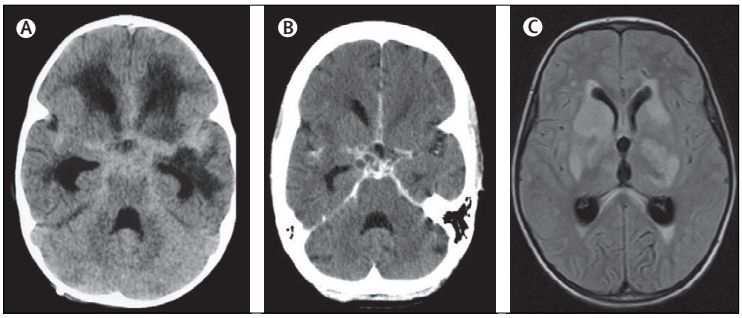

Figure 2: **Cerebral imaging abnormalities observed in tuberculous meningitis**
Non-contrast CT scan showing hyperdense material occupying the suprasellar and middle cerebral artery cisterns and hydrocephalus with periventricular lucency (A). Post-contrast CT scan showing basal meningeal enhancement and multiple right-sided suprasellar rim-enhancing lesions (B). MRI flair sequence showing bilateral basal ganglia infarcts (C). Adapted from Reference 76 with permisson from Springer.

The consensus case definition recommends that, when possible, CT or MRI brain scans should be done as part of the assessment for tuberculous meningitis. Recommended supportive diagnostic criteria include: hydrocephalus, basal meningeal enhancement, infarcts, tuberculoma, and pre-contrast basal hyperdensity (on CT imaging).

Evidence of tuberculosis elsewhere

The presence of tuberculosis outside the CNS increases the likelihood of tuberculous meningitis. Suggestive chest radiograph abnormalities are seen in 33–60%[1,6,7,27,65,72] of patients. A higher incidence of abnormal chest radiograph findings,[11] and extrapulmonary tuberculosis[12] have been identified in association with HIV infection. Additional imaging (ie, chest CT scan[80] and abdominal ultrasound[3,65]) can be useful to identify involvement of other organs.

Taking samples from sites of infection other than the CNS, such as lung, lymph node, liver, bone marrow, urine, ascitic fluid, and gastric fluid, further increases the chance of a positive diagnosis.[7,47] Gastric aspiration or induced sputum are particularly useful in children; in one study,[47] *M tuberculosis* was isolated in 68% of children with tuberculous meningitis. Positive *M tuberculosis* blood cultures seem very rare, even in immunocompromised patients with tuberculous meningitis.[59]

The consensus case definition states that every effort should be made to identify tuberculosis outside of the CNS. To do so, and when appropriate, clinicians should use: radiological features of tuberculosis on chest radiography, CT, MRI, or ultrasound; isolation of *M tuberculosis* from sputum, gastric aspirates, lymph nodes, ascitic fluid, urine, blood, bone marrow, or any other clinical specimen.

Exclusion of alternative diagnosis

The provision of an exhaustive list of possible differential diagnoses for tuberculous meningitis is beyond the scope of this article, but alternative conditions with which the disease is commonly confused, and which should be actively considered, are listed in the table. The diagnosis of severely immunocompromised individuals (eg, patients with HIV/AIDS), in whom more than one infection could be present, can be difficult. All patients should have a CSF Gram stain done (preferably with bacterial culture) to exclude pyogenic bacterial meningitis. In patients with HIV, India ink microscopy (preferably with cryptococcal antigen latex agglutination test or fungal culture) should also be done. Additional alternative diagnostic investigations that should be considered depending on age, immune status, and geographical region include neurosyphilis (venereal disease research laboratory or rapid plasma reagin assay with or without the fluorescent treponemal antibody absorption assay, micro-hemagglutination assay, or *Treponema pallidum* particle agglutination assay); viral meningoencephalitis (CSF viral PCR or culture); cerebral malaria (peripheral blood smears for malarial parasites); parasitic or eosinophilic meningitis (CSF serology for *Angiostrongylus cantonensis*, *Gnathostoma spinigerum*, toxocariasis, and cysticercosis); cerebral toxoplasmosis (cerebral imaging and positive serum serology for *Toxoplasma gondii* or histopathology); pyogenic bacterial brain abscess (cerebral imaging or histopathology); and malignancy (cytology or histopathology).

The consensus case definition recommends that an alternative diagnosis should be confirmed microbiologically (by stain, culture, or nucleic acid amplification tests when appropriate), serologically (eg, syphilis), or histopathologically (eg, lymphoma).

Conclusion

In view of the limitations of available diagnostic techniques and the urgent need for comparable tuberculous meningitis data from various settings, the consensus clinical case definition presented here, provides a robust and pragmatic case definition for use in future clinical research. Despite its limitations, we believe that ubiquitous application of this case definition, or at least meticulous reporting of the key variables listed, will improve methodological rigour, allow comparison of data, promote scientific communication, and encourage research collaboration. Strengthening the evidence base is essential if we are to improve the standard of care, and, ultimately, the clinical outcome for patients with tuberculous meningitis.

Search strategy and selection criteria

We searched PubMed for papers published from Jan 1, 1990, up to May 4, 2010. Search terms used included combinations of the following: "tuberculous meningitis", "*Mycobacterium tuberculosis*", "extra-pulmonary tuberculosis", "tuberculosis", 'central nervous system", "neurotuberculosis", "diagnosis", and "diagnostic criteria". We also searched the reference lists of articles retrieved. Relevant papers available in English were reviewed.

Contributors

All authors contributed equally to the preparation and writing of this Personal View.

Conflicts of interest

We declare that we have no conflicts of interest.

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Chapter 4

Chapter 4: Part 1

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Presentation and Outcome of Tuberculous Meningitis in a High HIV Prevalence Setting

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Abstract

Background: *Mycobacterium tuberculosis* is a common, devastating cause of meningitis in HIV-infected persons. Due to international rollout programs, access to antiretroviral therapy (ART) is increasing globally. Starting patients with HIV-associated tuberculous meningitis (TBM) on ART during tuberculosis (TB) treatment may increase survival in these patients. We undertook this study to describe causes of meningitis at a secondary-level hospital in a high HIV/TB co-infection setting and to determine predictors of mortality in patients with TBM.

Methods: A retrospective review of cerebrospinal fluid findings and clinical records over a six-month period (March 2009–August 2009). Definite, probable and possible TBM were diagnosed according to published case definitions.

Results: TBM was diagnosed in 120/211 patients (57%) with meningitis. In 106 HIV-infected patients with TBM, six-month all-cause mortality was lower in those who received antiretroviral therapy (ART) during TB treatment; hazard ratio = 0.30 (95% CI = 0.08–0.82). Factors associated with inpatient mortality in HIV-infected patients were 1) low $CD4^+$ count at presentation; adjusted odds ratio (AOR) = 1.4 (95% confidence interval [CI] = 1.03–1.96) per 50 cells/μL drop in $CD4^+$ count and, 2) higher British Medical Research Council TBM disease grade (2 or 3 versus 1); AOR = 4.8 (95% CI = 1.45–15.87).

Interpretation: Starting ART prior to or during TB treatment may be associated with lower mortality in patients with HIV-associated TBM. Advanced HIV and worse stage of TBM disease predict in-hospital mortality in patients presenting with TBM.

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Introduction

Meningitis causes significant mortality and morbidity in HIV-infected persons [1–4]. Tuberculous meningitis (TBM) accounts for a substantial proportion of cases, particularly in high tuberculosis (TB) prevalence areas [3]. Globally, access to antiretroviral therapy (ART) is rapidly increasing due to ART rollout programs [5]. Starting ART during TB treatment is associated with reduced mortality in HIV/TB co-infected patients [6,7]. However, few studies have reported the influence of ART on the outcome of patients with HIV-associated TBM [8–10]. In this study, we report the causes of meningitis at a secondary-level hospital in a high HIV/TB prevalence setting in the era of increasing availability of ART. We also describe the presentation and outcome of patients with TBM and investigate the predictors of mortality (including ART) in these patients.

Methods

Ethics statement

The ethics committee of the University of Cape Town (UCT) approved the study (REC REF 223/2010). As this was a retrospective folder review, and data were analysed anonymously outside of the clinical setting, the ethics committee of UCT waived the requirement for informed consent and informed consent was not obtained.

Setting and population

We conducted a retrospective study at GF Jooste Hospital, a 200-bed public sector referral hospital that serves adult patients from a community of approximately 1.3 million people. This predominantly low-income, high-density population is at the epicenter of the TB/HIV pandemic; in some parts of the referral

area the reported TB case notification rate exceeds 1500 cases per 100 000 people per year and the HIV seroprevalence at antenatal clinics reaches 30% [11]. All patients accessing public sector care with suspected meningitis are referred to GF Jooste Hospital for investigations, including a lumbar puncture (LP). Adult patients (≥18 years) who had a LP performed over a six-month period (1 March 2009–31 August 2009) were identified from laboratory logs and included in the study.

Procedure

As per standard protocol at the hospital laboratory [3], cerebrospinal fluid (CSF) samples underwent macroscopic examination, protein and glucose quantification, cell count, Gram stain, and bacterial and fungal culture. India ink staining and/or Crytococcus Latex Antigen Testing (CLAT) were also performed. If the clinical presentation or initial CSF findings were suggestive of TBM (as determined by the attending clinician), Ziehl-Neelsen (ZN) staining of sediment and/or *Mycobacterium tuberculosis* (*M. tuberculosis*) culture was performed. If acid-fast bacilli (AFB) were cultured from CSF, TB polymerase chain reaction (PCR) [Genotype MTBDRplus, Hain Lifesciences]) tests were performed to further identify mycobacteria species, and to determine first-line drug susceptibility (to rifampicin and isoniazid). In cases where rifampicin-resistant organisms were identified, additional drug susceptibility testing was performed by conventional methods. Syphilis serology (venereal disease research laboratory and/or *Treponema pallidum* hemagglutination assay), cytology and viral PCR examination were performed at attending clinician's discretion.

All CSF findings were reviewed. Microbiological diagnoses (i.e. where CSF analysis identified a specific etiological cause) were documented. Clinical records of patients with 'markedly abnormal' CSF who did not have a microbiological diagnosis were reviewed. In line with a previous study [3], CSF was considered to be 'markedly abnormal' when one or more of the following were present: 1) neutrophils >5 cells$\times 10^6$/L, 2) lymphocytes>20 cells$\times 10^6$/L, 3) protein >1 g/L, and 4) glucose <2.2 mmol/L. Patients who did not present with symptoms and/or signs of meningitis such as headache, photophobia, seizure, vomiting, altered mental state, neck stiffness or focal neurological deficit (e.g. patients with peripheral neuropathy) and those in whom an alternative diagnosis was made (e.g. subarachnoid hemorrhage), were excluded from the analysis. Data recorded for patients with TBM included medical and treatment history prior to admission, history of the presenting complaint (s), clinical examination, results of investigations, inpatient management and admission outcome. Additional information such as date of starting ART, was obtained from primary care clinic records. We used hospital medical notes, the National Health Laboratories Service database and the electronic hospital and primary care clinic (TB and ART) attendance registers to trace patients, in order to determine outcome (alive, dead or lost to follow-up) six months after LP was performed.

Patients received standardized TB treatment according to national treatment guidelines using Directly Observed Therapy Short-course (DOTS) either at the primary care TB clinic, or delivered to home by lay health care workers [12]. The duration of TB treatment (at least six to nine months) depended on the attending clinician's discretion. Patients with a new diagnosis of tuberculosis received isoniazid, rifampin, pyrazinamide, and ethambutol for two months (dosing schedules detailed in Table S1). This was followed by rifampicin and isoniazid for at least four months. The retreatment regimen included rifampicin, isoniazid, pyrazinamide, ethambutol and intramuscular streptomycin during the initial two months of treatment, followed by rifampicin, isoniazid, pyrazinamide and ethambutol for one month, followed by rifampicin, isoniazid and ethambutol for at least five months (dosing schedules detailed in Table S2). At the time of the study, national guidelines advised ART for all patients with a $CD4^+$ count of less than 200 cells/μL or World Health Organization (WHO) stage 4 disease [13]. First-line ART during this study was stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz was preferred for patients who were receiving rifampicin-based antituberculosis treatment.

Definitions

Definite TBM was diagnosed when 1) AFB were seen in CSF, 2) AFB or *M. tuberculosis* was cultured from CSF or 3) *M. tuberculosis* was detected by PCR from CSF. Probable and possible TBM were diagnosed according to modified published case definitions [10,14]. **Probable TBM** was diagnosed when: 1) a patient presented with clinical features of meningitis and 2) suggestive CSF findings of TBM (total white cell count >5 cells$\times 10^6$/L, protein >0.45 g/L and glucose <2.2 mmol/L), plus 3) one or more of the following i) chest radiograph findings consistent with pulmonary TB, ii) an extra-meningeal specimen positive for AFB, iii) other evidence of extra-meningeal TB (e.g. abdominal ultrasound features) or iv) brain computed tomography (CT) evidence of TBM including one or more of the following: basal meningeal enhancement, hydrocephalus or infarctions. **Possible TBM** was diagnosed when: 1) a patient presented with clinical features of meningitis and either 2) four or more of the following were present i) a history of TB ii) a predominance of CSF lymphocytes ($>50\%$), iii) illness duration of more than five days iv) CSF glucose <2.2 mmol/L, v) altered consciousness, vi) clear or yellow CSF with protein>1 g/L, vii) focal neurological signs, or 3) 'markedly abnormal' CSF (excluding isolated hypoglycemia) with evidence of TB elsewhere.

Patients were excluded from the probable and possible TBM groups if an alternative cause of meningitis was found, or if they improved with no treatment or alternative treatment in the absence of TB treatment. **Cryptococcal meningitis** (CM) was diagnosed when CSF India ink stain, CLAT or *Cryptococcus neoformans* culture was positive. **Bacterial meningitis** was diagnosed when: 1) bacteria were isolated from CSF or 2) a patient presented with clinical features of meningitis and i) a CSF polymorphonuclear cell predominance and showed a good response to antibacterial treatment in the absence of TB treatment, or ii) a CSF polymorphonuclear cell count >1000 cells$\times 10^6$/L, regardless of outcome. **Viral meningitis** was diagnosed when a patient presented with clinical meningitis and: 1) a virus was identified from CSF, or 2) a CSF lymphocytic predominance and had symptom resolution in the absence of antimicrobial treatment. **Loss to follow-up** was defined as being unable to trace a patient six months after LP, using the methods described above.

Statistical analysis

Univariate analysis was performed to 1) identify significant differences between patients who did, and did not die during hospitalization and at six months follow-up and 2) identify significant differences between patients with definite and those with probable/possible TBM. Continuous variables were compared using the Student t-test or Mann-Whitney *U* test, and categorical variables were compared by Fisher's exact test.

Variables associated with inpatient mortality ($p<0.2$) were evaluated using multivariate analysis. Stepwise logistic regression was used to identify variables predictive of inpatient mortality in all

TBM patients (regardless of HIV status) and subsequently, in HIV-infected patients only. A Cox proportional hazard model was used to assess the association of ART started before or during TB treatment with six-month mortality in HIV-infected patients who survived hospitalization. The validity of the model's assumptions was tested with Schoenfeld residuals.

A p-value$<$0.05 was considered statistically significant. Time to death was summarized by use of Kaplan-Meier estimates. The statistical analyses were performed with GraphPad Prism version 5 and STATA version 10.1 software.

Results

Causes of 'markedly abnormal' CSF

During the study period, 812 LPs were performed in 698 patients. CSF analysis was 'markedly abnormal' (n = 146), and/or identified a cause of meningitis (n = 107) in 253 patients. Figure 1 shows the reasons for exclusion (n = 42) and diagnoses in 211 patients who were diagnosed with meningitis. The most frequent microbiological diagnoses were CM, and TBM, which accounted for 45% (48/107), and 44% (47/107), of cases respectively. Fifty-seven percent (120/211) of patients with meningitis were diagnosed with definite (n = 47), probable (n = 35) or possible (n = 38) TBM.

Findings in patients with TBM

The demographic, clinical and investigative findings for patients with definite, probable and possible TBM are detailed in Tables 1 and 2. Eighty-eight percent of patients with TBM were HIV-infected with a median $CD4^+$ count of 79 cells/uL (interquartile range [IQR] = 39–137); 20 (19%) of these patients were receiving ART at the time of presentation. The majority of TBM cases (68%) presented with advanced TBM disease (British Medical Research Council [BMRC] disease grade 2 or 3) [15] 7 days (median, IQR = 3–15 days) after symptom onset. 26/115 (23%) of patients for whom this information was available were receiving TB treatment at time of presentation for a median duration of 106 days (IQR = 50–178). Disseminated TB was common; 87/114

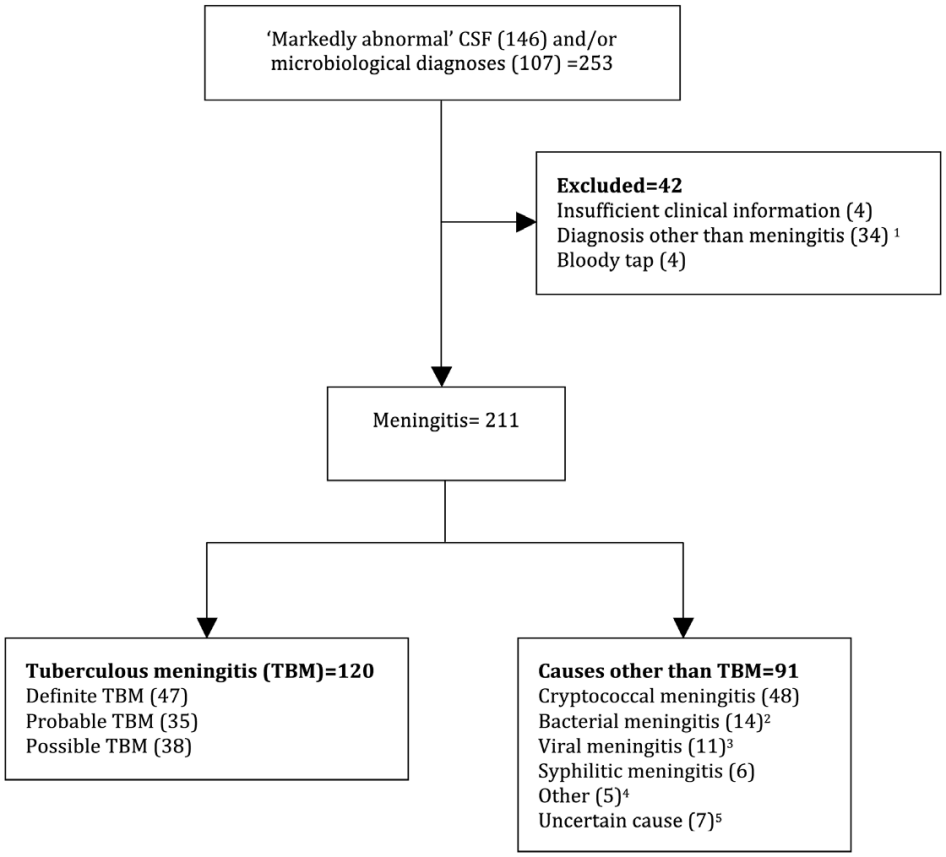

Figure 1. Flow diagram of differential diagnoses in patients with 'markedly abnormal' CSF and/or microbiological-confirmed meningitis. [1] Common alternative diagnoses include: hypoglycemia (n = 9), intracranial bleed (n = 7) and peripheral nerve disorders (n = 6). [2] Including 5 patients with CSF culture-confirmed bacterial meningitis. Organisms isolated include: *Streptococcus pneumonia* (n = 3), *beta-hemolytic Streptococcus* (n = 1), *Neisseria meningitides* (n = 1). [3] Including 1 patient with positive CSF polymerase chain reaction for both cytomegalovirus and herpes simplex-1 virus. [4] Other causes of meningitis include: Acute HIV infection (n = 1), *Toxoplasma gondii* meningoencephalitis (n = 1), disseminated Burkitt's lymphoma (n = 1), disseminated large B-cell lymphoma (n = 1), chronic resolving TBM immune reconstitution inflammatory syndrome (n = 1). [5] Including patients with the following differential diagnoses: 1) TBM with tuberculoma or toxoplasmosis (n = 1); 2) partially treated bacterial meningitis, viral meningitis or TBM (n = 3); and 3) viral meningitis or TBM (n = 3). CSF, cerebrospinal fluid.
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Table 1. Demographic and clinical characteristics of patients with definite, probable and possible tuberculous meningitis (TBM).

	Definite TBM (n = 47)		Probable TBM (n = 35)		Possible TBM (n = 38)	
Age, median (IQR)	35	(28–42)	36	(29–51)	38	(28–42)
Female, n/N (%)	22/47	(47)	16/35	(46)	22/38	(58)
HIV status, n/N (%)						
Infected	43/47	(91)	27/35	(77)	36/38	(95)
Uninfected	2/47	(4)	5/35	(14)	1/38	(3)
Unknown	2/47	(4)	3/35	(9)	1/38	(3)
$CD4^+$ cell count, median (IQR)[1]	63	(35–115)	79	(36–150)	109	(33–201)
On ART at presentation, n/N (%)[2]	9/41	(22)	6/27	(22)	5/35	(14)
Previous TB, n/N (%)	15/43	(35)	7/34	(21)	12/38	(32)
On TB treatment at time of LP, n/N (%)	9/43	(21)	8/34	(24)	9/38	(24)
Symptom onset to LP, median (IQR)	7	(4–15)	6	(3–21)	3	(2–11)
Neurological symptoms, n/N (%)						
Headache	26/42	(62)	18/34	(53)	17/38	(45)
Confusion[3]	23/42	(55)	21/34	(62)	17/38	(45)
Neck pain/stiffness[3]	12/42	(29)	8/34	(24)	5/38	(13)
Nausea/vomiting	15/42	(36)	11/34	(32)	12/38	(32)
Photophobia/blurred vision/diplopia	11/42	(26)	8/34	(24)	6/38	(16)
Seizures	7/42	(17)	2/34	(6)	6/38	(16)
Neurological signs, n/N (%)						
BMRC TBM Disease Grade[4]						
1	10/42	(24)	7/34	(21)	16/38	(42)
2	29/42	(69)	23/34	(68)	20/38	(53)
3	3/42	(7)	4/34	(12)	2/38	(5)
Confusion[5]	29/42	(69)	22/34	(65)	23/38	(61)
Neck stiffness[5]	31/42	(74)	23/34	(68)	19/38	(50)*
Focal neurological signs	9/42	(21)	17/34	(50)*	2/38	(5)**

IQR, interquartile range; n, number of patients; N, total number of patients for whom results were available; HIV, human immunodeficiency virus; ART, antiretroviral therapy; TB, tuberculosis; LP, lumbar puncture; D4T, stavudine 30 mg twice daily; 3TC, lamivudine 150 mg twice daily or 300 mg daily; EFV, efavirenz 600 mg nightly; AZT, zidovudine 300 mg twice daily; NEV, nevirapine 200 mg twice daily; ddI, didanosine 400 mg daily; LPV/rtv, lopinavir/ritonavir 800/200 mg twice daily.
*Significantly different ($p<0.05$) from patients with definite TBM;
**$p<0.01$.
[1]Only performed in HIV-infected patients.
[2]N includes HIV-infected patients only. Treatment regimens included: 1) D4T, 3TC, EFV (n = 11), 2) AZT, 3TC, NEV (n = 3), 3) D4T, 3TC, NEV (n = 3), 4) AZT, 3TC, EFV (n = 1), 5) AZT, 3TC, LPV/rtv (n = 1), 6) AZT, ddI, LPV/rtv (n = 1).
[3]Refers to symptoms reported by patient or family only.
[4]British Medical Research Council TBM disease grades: 1- Glasgow coma scale (GCS) 15 with no neurological deficit; 2- GCS 11–14 without neurological deficit, or GCS 15 with focal neurological deficit; 3- GCS≤10.[15]
[5]Refers to clinical findings on physical examination only.
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(76%) of patients presented with features of extra-meningeal TB. Chest radiograph abnormalities consistent with TB were observed in 74% (76/103) of patients. Abdominal ultrasound was performed in 27 patients, of which 25 (93%) showed features of TB. In addition, AFB were seen on microscopy, or *M. tuberculosis* was cultured, from one or more extra-meningeal specimens from 26 patients; specimens included sputum (n = 21), lymph node fine needle aspiration biopsy (n = 6), pleural fluid (n = 1), blood (n = 5) and urine (n = 3).

Atypical CSF findings in patients with definite TBM (n = 47) included a polymorphonuclear cell predominance (>50% of total leucocyte count) in six (13%), a glucose level of more than 2.2 mmol/L in 13 (28%), a protein concentration of less or equal to 0.45 g/L in three (6%) and a total leucocyte count of five or less cells$\times 10^6$/L in two (4%). No patient with definite TBM had completely normal CSF (both biochemistry and cell count). ZN staining was requested for CSF specimens from 88 patients, including 47 (100%) definite TBM, 24 (69%) probable TBM and 25 (66%) possible TBM cases. 76/88 of these specimens (86%) were insufficient for TB microscopy. Of the 12 TBM cases who had CSF direct smear examination performed, AFB were visualized in one. Cerebrospinal fluid *M. tuberculosis* culture was requested for 106 patients including 47 [100%] definite TBM, 31 [89%] probable TBM, and 28 [74%] possible TBM cases. Significantly less patients with probable and possible TBM had *M. tuberculosis* culture performed, compared to those with definite TBM, who by definition required a positive culture ($p = 0.02$, and $p = 0.0009$, respectively). Drug susceptibility testing for first-line TB drugs (rifampicin and isoniazid) was performed on 40/47 (85%) of isolates; 35 were susceptible to rifampicin and isoniazid, three were resistant to isoniazid, one was resistant to rifampicin, and one was resistant to both rifampicin and isoniazid (multidrug-

Table 2. Laboratory and radiological investigation findings of patients with definite, probable and possible tuberculous meningitis (TBM).

	Definite TBM (n = 47)		Probable TBM (n = 35)		Possible TBM (n = 38)	
Blood results, median (IQR)						
Hemoglobin (g/dL)	10.5	(9.1–13)	12	(10.7–13.2)*	10	(8–11.4)
White cell count (cells $\times 10^9$/L)	5.9	(4.3–8.5)	5.6	(4.5–7.7)	7.7	(5.4–10)
Sodium (mmol/L)	126	(123–130)	129	(127–133)**	130	(126–135)**
Cerebrospinal fluid results, median, (IQR)						
Protein (g/L)	2.6	(1.6–4.8)	2.4	(1.3–5.2)	1.2	(0.8–1.9)**
Glucose (mmol/L)	1.6	(0.9–2.4)	1.9	(1.3–2.8)	2.7	(2.1–3.2)**
Lymphocytes (cells $\times 10^6$/L)	77	(23–199)	59	(23–143)	12	(0–31)**
Polymorphonuclear cells (cells $\times 10^6$/L)	7	(0–39)	12	(0–12)	0	(0–3)**
Features of TB elsewhere, n/N (%)	35/42	(83)	21/34	(62)*	31/38	(82)
Chest radiograph abnormalities	34/38	(89)	18/27	(67)*	24/38	(63)
Abdominal ultrasound abnormalities[1]	6/6	(100)	11/11	(100)	8/10	(80)
Extra-meningeal AFB on microscopy/*M.tb* cultured	9/47	(19)	5/35	(14)	12/38	(32)
CT brain abnormalities,						
(excluding cerebral atrophy), n/N (%)[1]	11/16	(69)	21/23	(91)[2]	4/5	(80)[3]
Hydrocephalus	4/16	(25)	6/23	(26)	0/5	(0)
Meningeal enhancement	3/16	(19)	9/23	(39)	0/5	(0)
Infarct	5/16	(31)	12/23	(52)	1/5	(20)

IQR, interquartile range; n, number of patients; N, total number of patients for whom results were available; TB, tuberculosis; AFB, acid-fast bacilli; *M.tb*, *Mycobacterium tuberculosis*; CT, computed tomography.

*Significantly different ($p<0.05$) from patients with definite TBM,

**$p<0.01$.

[1]N includes total number of patients who underwent procedure.

[2]Significantly more patients with probable TBM had CT brain performed compared to patients with definite TBM, p = 0.007.

[3]Significantly less patients with possible TBM had CT brain performed compared to patients with definite TBM, p = 0.04.

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resistant [MDR] organisms). The latter two patients both died during hospitalization after starting regimen 2 TB treatment (prior to the availability of *M. tuberculosis* drug susceptibility testing results). In two additional patients MDR *M. tuberculosis* strains were cultured from extra-meningeal specimens prior to admission. One of these patients presented on a MDR TB drug regimen (i.e. ethambutol, pyrazinamide, ethionamide, ofloxacin and kanamycin) and was alive at 6-months follow-up. The *M. tuberculosis* drug resistance profile was unknown for the other patient at TBM presentation; the patient died during hospitalization after starting regimen 2 TB treatment.

Table 3 describes the management and outcome in patients with TBM. Five of 89 patients not receiving TB treatment at the time of presentation, failed to initiate TB treatment after LP; all five subsequently died. In one patient with definite TBM who did not commence TB treatment, the diagnosis was not considered initially as routine CSF investigations was mildly abnormal (only abnormality: lymphocyte count = 6×10^6/L) and there were no features of extra-pulmonary TB on chest radiograph. The remaining four patients (2-probable TBM, 2-possible TBM) died shortly after admission (within 24 hours), prior to TB drug initiation. The exact time of TB treatment initiation at presentation was known for 82/84 remaining patients: 11 patients started TB treatment 1–4 days prior to LP, but after symptom onset; 63 patients started TB treatment within 24 hours of LP; and 8 patients started TB treatment more than 24 hours after LP at a median time of three days (range, 2–8 days). Adjunctive corticosteroid treatment was started in 64/113 (57%) of patients during admission; significantly more patients with definite TBM received corticosteroids compared to those with possible TBM (71% versus 32%, p = 0.0007). The proportion of patients with probable TBM who received corticosteroids (70%) was similar to that of the definite TBM group. No patient received any other adjunctive therapy e.g. acetazolamide, or surgery. Overall inpatient mortality during hospitalization was 38% (45/120 patients), four days (median, IQR: 3–9 days) after LP (Figure 2). Among those discharged from hospital, 57% (31/54 patients) of HIV-infected patients (not on ART at time of presentation) initiated ART during six months of TB treatment. ART regimens for these patients are detailed in tables 1 and 3. At six-month follow-up, 48% of all TBM patients had died and 10% were lost to follow-up. Baseline characteristics did not differ significantly between patients who were retained in care and those who were lost to follow-up (data not shown). However, there was a trend to higher CD4+ counts in HIV-infected patients lost to follow-up compared to those who were not (median [IQR], 164 [71–250] cells/μL compared to 68 [35–144] cells/μL, p = 0.06).

Table 4 shows factors analyzed for association with inpatient mortality for all patients [n = 120] in univariate analysis. A higher BMRC TBM disease grade (2 or 3 versus 1: AOR [95% CI] = 3.0 [1.08–8.40], p = 0.04) remained predictive of mortality in multivariate analysis (logistic regression model p = 0.007, $R^2 = 0.12$). Table 5 shows factors analyzed for association with inpatient mortality for HIV-infected patients [n = 106] only. $CD4^+$ count (for every 50 cells/μL drop in $CD4^+$ count: AOR [95% confidence interval [CI]] = 1.4 [1.03–1.96], p = 0.03) and a higher BMRC TBM disease grade (2 or 3 versus 1: AOR [95% CI] = 4.8 [1.45–15.87], p = 0.01) remained predictive of mortality

Table 3. Management and outcome of patients with definite, probable and possible tuberculous meningitis (n = 120).

TB treatment		
On treatment at time of presentation, n/N (%)	26/115	(23)
Treatment started, n/N (%)[1]	84/89	(94)
Duration between symptom onset and starting treatment in days, median (IQR)	7	(3–13)
Corticosteroids started, n/N (%) **	64/113	(57)
ART		
Treatment started ≤6 months after starting TB treatment, n/N (%)[2]	31/54	(57)
Duration between diagnostic LP and starting ART in days, median (IQR)	42	(17–81)
Outcome[3]		
Inpatient mortality, n (%)	45	(38)
Duration from LP to death in days[4], median (IQR)	4	(3–9)
Six months, n (%)		
Alive	50	(42)
Dead	58	(48)
Lost to follow-up	12	(10)
Nine months, n (%)		
Alive	47	(39)
Dead	59	(49)
Lost to follow-up	14	(12)

TB, tuberculosis; n, number of patients; N, number of patients for whom results were available; IQR, interquartile range; LP, lumbar puncture; ART, antiretroviral therapy; TBM, tuberculous meningitis; D4T, stavudine 30 mg twice daily; 3TC, lamivudine 150 mg twice daily or 300 mg daily; EFV, efavirenz 600 mg nightly; AZT, zidovudine 300 mg twice daily; tenofovir 300 mg daily.

**Significantly more patients with definite TBM (71%) received corticosteroid treatment compared to patients with possible TBM (32%, $p<0.01$).

[1]N includes patients not on TB treatment at presentation.

[2]N includes HIV-infected patients not on ART at presentation who survived admission. Treatment regimens included: 1) D4T, 3TC, EFV (n = 14) 2) AZT, 3TC, EFV (n = 7) 3) 3TC, TDF, EFV (n = 3). Treatment regimes were not known for 7 patients.

[3]Outcomes reported for all patients (n = 120) with TBM.

[4]Only including patients who died during hospitalization.

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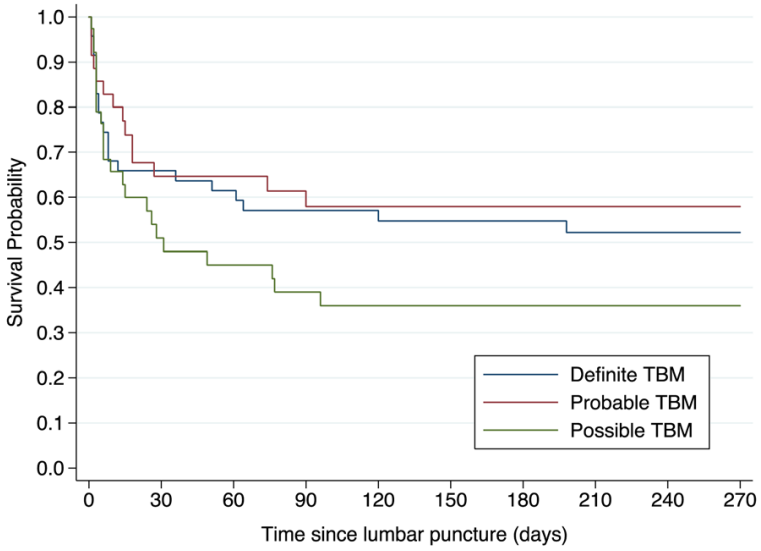

Figure 2. Kaplan-Meier survival curves of patients with definite, probable and possible tuberculous meningitis (TBM). Survival probability at 6-months was similar between patients with definite TBM and those with probable TBM (log-rank test p = 0.69), and possible TBM (log-rank test p = 0.15).

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Table 4. Univariate analysis of variables associated with inpatient mortality in all patients with definite, probable and possible tuberculous meningitis (n = 120).

	Died (n = 45)		Survived (n = 75)		P-value	OR[1]	(95% CI)
Age, median years (IQR) (N = 120)	37	(28–41)	35	(28–44)	0.97	-	-
Female, n (%) (N = 120)	24	(55)	36	(48)	0.71	1.2	(0.59–2.60)
History of previous TB, n (%) (N = 115)	13	(30)	21	(29)	1.00	1.1	(0.46–2.40)
On TB treatment at time of LP, n (%) (N = 115)	9	(21)	18	(25)	0.66	0.79	(0.32–1.97)
HIV-infected, N (%) (N = 114)	39	(98)	67	(91)	0.26	4.1	(0.48–34.38)
BMRC TBM disease grade 2 or 3, n (%)(N = 114)	36	(84)	45	(63)	0.03*	3.0	(1.16–7.63)
Definite TBM, n (%) (N = 120)	18	(40)	29	(39)	1.00	1.1	(0.50–2.25)
WCC, median cells $\times 10^9$/L (IQR)	6.2	(4.5–8.9)	6.0	(4.6–9)	0.83	-	-
Hemoglobin, median g/dL (IQR)	10.3	(8.8–12.3)	11	(9.6–12.8)	0.16	-	-
Serum sodium, median mmol/L (IQR)	127	(124–133)	129	(125–134)	0.48	-	-
CSF polymorphs, median cells $\times 10^6$ (IQR)	0	(0–14)	0	(0–14)	0.61	-	-
CSF lymphocytes, median cells $\times 10^6$ (IQR)	39	(8–144)	46	(16–125)	0.78	-	-
CSF protein, median g/L (IQR)	2.28	(1.51–4.87)	1.76	(1.05–3.08)	0.11	-	-
CSF glucose, median mmol/L (IQR)	1.8	(1–2.8)	2.2	(1.5–2.9)	0.19	-	-
Symptoms to TB treatment, median days (IQR)	7	(2–12)	6	(4–14)	0.37	-	-
Corticosteroids started, n (%)(N = 113)	21	(50)	43	(61)	0.33	0.7	(0.30–1.41)

n, number of patients; N, total number of patients for whom analysis was performed; IQR, interquartile range; TB, tuberculosis; LP, lumbar puncture; BMRC, British Medical Research Council; WCC, total blood white cell count ; CSF, cerebrospinal fluid.
p-value statistically significant (<0.05).
[1]Odds ratios (OR) and 95% confidence intervals (95%CI) reported for categorical variables.
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Table 5. Univariate analysis of variables associated with inpatient mortality in HIV-infected patients with definite, probable and possible tuberculous meningitis (n = 106).

	Died (n = 39)		Survived (n = 67)		P-value	OR[1]	(95% CI)
Age, median years (IQR) (N = 106)	37	(28–41)	34	(28–44)	0.88	-	-
Female, n (%) (N = 106)	19	(49)	38	(57)	0.54	0.7	(0.33–1.60)
History of previous TB, n (%) (N = 102)	13	(35)	19	(29)	0.66	1.3	(0.55–3.10)
On TB treatment at time of LP, n (%)(N = 101)	9	(24)	17	(27)	1.00	0.88	(0.35–2.26)
On ART at time of LP, n (% of HIV-infected) (N = 103)	8	(22)	12	(18)	0.80	1.2	(0.46–3.38)
CD4 at presentation, median cells/μL (IQR)	54	(23–143)	109	(40–170)	0.03*	-	-
BMRC TBM disease grade 2 or 3, n (%) (N = 100)	30	(81)	39	(62)	0.07	2.64	(1.002–6.94)
Definite TBM, n (%) (N = 106)	16	(41)	27	(40)	1.00	1.03	(0.46–2.30)
WCC, median cells $\times 10^9$/L (IQR)	6.2	(4.4–8.9)	5.6	(4.4–8.9)	0.60	-	-
Hemoglobin, median g/dL (IQR)	9.9	(8.5–11.5)	10.9	(9.4–12.6)	0.09	-	-
Serum sodium, median mmol/L (IQR)	127	(124–134)	129	(125–134)	0.96	-	-
CSF polymorphs, median cells $\times 10^6$ (IQR)	0	(0–16)	0	(0–12)	0.92	-	-
CSF lymphocytes, median cells $\times 10^6$ (IQR)	42	(7–135)	46	(10–130)	0.80	-	-
CSF protein, median g/L (IQR)	2.01	(1.26–3.00)	1.72	(1.04–3.09)	0.48	-	-
CSF glucose, median mmol/L (IQR)	1.93	(0.9–2.8)	2.2	(1.6–2.9)	0.22	-	-
Symptoms to TB treatment, median days (IQR)	7	(2–14)	6	(4–14)	0.40	-	-
Corticosteroids started, n (%)(N = 99)	19	(53)	36	(57)	0.68	0.84	(0.36–1.91)

n, number of patients; N, total number of patients for whom analysis was performed; IQR, interquartile range; TB, tuberculosis; LP, lumbar puncture; ART, antiretroviral therapy; BMRC, British Medical Research Council; CD4, $CD4^+$ cell count; WCC, total blood white cell count ; CSF, cerebrospinal fluid.
*p-value statistically significant (<0.05).
[1]Odds ratios (OR) and 95% confidence intervals (95%CI) reported for categorical variables.
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in multivariate analysis (logistic regression model p = 0.01, R^2 = 0.14).

Analysis of factors associated with six-month mortality is reported only for HIV-infected hospital survivors for whom outcome was known at six-months follow-up (n = 56, Table 6). Being either on ART at presentation, or having started ART during TB treatment, was negatively associated with six-month mortality (OR = 0.2, 95% CI = 0.05–0.81, p = 0.03). This association was confirmed in a Cox proportional hazard model (n = 66), which included 10 HIV-infected patients who were lost to follow-up after discharge (Figure 3, hazard ratio = 0.30, 95% CI = 0.08–0.82, p = 0.03). No additional factors were associated with six-months mortality when all patients (regardless of HIV-status) with a known outcome at six-month follow-up were included in analysis (data not shown).

Discussion

Several studies have reported CM as the most frequent cause of meningitis in HIV-infected patients [1–3,16,17]. In our study, TBM was the most common cause of meningitis (57%) when both microbiological-confirmed cases and cases diagnosed on clinical grounds were included. An earlier study conducted at our hospital (2006–2008) reported CM and TBM as the cause of microbiological-confirmed meningitis in 63%, and 28% of cases, respectively [3]. By comparison, we found TBM (44%) and CM (45%) to account for similar proportions of microbiological-confirmed cases. This change may reflect increasing ART access in the referral area (during 2009, 23 449 patients were commenced on ART in the Western Cape public health sector, compared to 19 527 patients during 2008 [*Catherine White, Western Cape ART Monitoring and Evaluation Programme- personal communication*]) and therefore fewer HIV-infected patients reaching the severity of immunosuppression associated with CM.

A higher BMRC TBM disease grade was predictive of death during hospitalization when all patients (regardless of HIV status) were included in the analysis. When the analysis was restricted to HIV-infected patients only, both a lower $CD4^+$ and a higher BMRC TBM disease grade were associated with death. The predictive value of worse TBM disease on the mortality of both HIV-infected [18,19] and uninfected [20,21] patients is well documented. Two previous studies also found an association between low $CD4^+$ count (less than 50 cells/μL [18] and less than 200 cells/μL [22]) and inpatient mortality in HIV-infected TBM patients. However, this finding has not always been reproduced [19,23]. Other factors previously reported associated with reduced hospital survival in HIV-associated TBM include disease duration of more than 14 days [22] and infection with MDR-TB strains [18]. In our study, prolonged symptom duration was not associated with inpatient mortality. Due to the low prevalence of MDR-TB (MDR-TB isolates identified in 3 patients; 1 from CSF and 2 from extra-meningeal specimens), its influence on mortality could not be assessed.

In this study, six-month mortality in HIV-infected TBM patients was significantly lower in patients who received ART during TB treatment. As most studies in HIV-infected TBM patients thus far were conducted in patients not receiving ART [24], few have assessed the influence of ART on outcome. Torok *et al.* [10] reported lack of ART prior to or during TB treatment to

Table 6. Univariate analysis of variables associated with six-month mortality in HIV infected patients with definite, probable and possible tuberculous meningitis (n = 56).[1]

	Died (n = 12)		Survived (n = 44)		P-value	OR[2]	(95% CI)
Age, median years (IQR) (N = 56)	36	(29–49)	34	(28–44)	0.58	-	-
Female, n (%) (N = 56)	3	(25)	19	(43)	0.33	0.4	(0.10–1.85)
History of previous TB, n (%) (N = 55)	5	(42)	13	(30)	0.50	1.6	(0.44–6.17)
On TB treatment at time of LP, n (%) (N = 54)	4	(33)	11	(26)	0.72	1.4	(0.36–5.62)
On ART at time of LP, n (% of HIV-infected) (N = 56)	1	(8)	10	(23)	0.42	0.3	(0.35–2.70)
CD4 at presentation, median cells/μL (IQR)	98	(18–160)	104	(46–159)	0.46	-	-
BMRC TBM disease grade 2 or 3, n (%)(N = 54)	6	(50)	26	(62)	0.52	0.6	(0.17–2.24)
Definite TBM, n (%) (N = 56)	3	(25)	20	(45)	0.32	0.4	(0.10–1.68)
WCC, median cells $\times 10^9$/L (IQR)	6.8	(4.8–9.4)	5.6	(4.1–9.4)	0.56	-	-
Hemoglobin, median g/dL (IQR)	9.7	(8.4–12.2)	10.6	(8.9–12.2)	0.60	-	-
Serum sodium, median mmol/L (IQR)	134	(126–135)	128	(123–131)	0.09	-	-
CSF polymorphs, median cells $\times 10^6$ (IQR)	0	(0–22)	2	(0–12)	0.42	-	-
CSF lymphocytes, median cells $\times 10^6$ (IQR)	17	(0–67)	79	(19–172)	0.06	-	-
CSF protein, median g/L (IQR)	1.28	(0.80–2.42)	1.91	(1.08–4.09)	0.12	-	-
CSF glucose, median mmol/L (IQR)	2.2	(1.5–2.9)	2.3	(1.7–3.2)	0.97	-	-
Symptoms to TB treatment, median days (IQR)	8	(4–48)	7	(4–17)	0.74	-	-
Corticosteroids started, n (%)(N = 54)	5	(42)	25	(60)	0.33	0.5	(0.13–1.79)
ART started prior to LP/during TB treatment, n (%) (N = 56)	5	(42)	34	(77)	0.03*	0.2	(0.05–0.81)

n, number of patients; N, total number of patients for whom analysis was performed; IQR, interquartile range; TB, tuberculosis; LP, lumbar puncture; ART, antiretroviral therapy; BMRC, British Medical Research Council; CD4, $CD4^+$ cell count; WCC, total blood white cell count ; CSF, cerebrospinal fluid.

*p-value statistically significant (<0.05).

[1]Analysis performed for HIV-infected patients who survived hospitalization for whom outcome was known at 6-month follow-up. One HIV-infected hospital survivor for whom ART treatment at TBM presentation was unknown excluded from analysis.

[2]Odds ratios (OR) and 95% confidence intervals (95%CI) reported for categorical variables.

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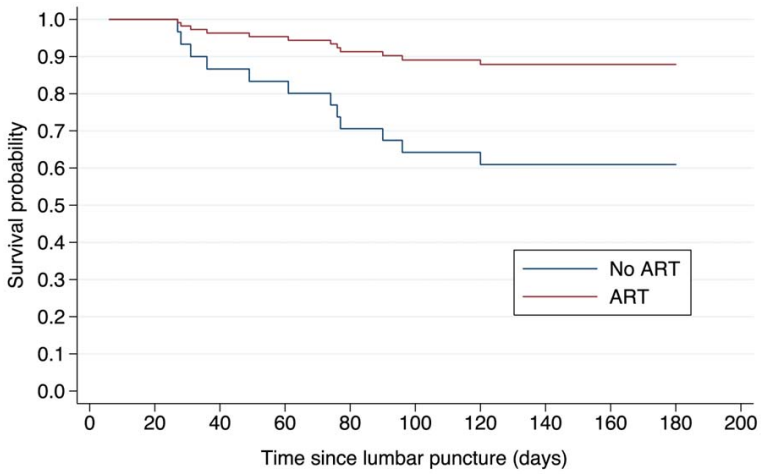

Figure 3. Cox proportional hazard model survival curves. ART: HIV-infected tuberculous meningitis (TBM) patients either on antiretroviral therapy (ART) at TBM presentation or started on ART during subsequent 6 months of antituberculosis (TB) treatment (n = 43). **No ART**: HIV-infected TBM patients not on ART at presentation nor started on ART during subsequent 6 months of TB treatment (n = 23). The model only included patients who survived hospitalization (n = 66). Hazard ratio for patients on ART = 0.30 (95% confidence interval 0.08–0.82, p-value = 0.03).
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be associated with earlier time to death by univariate, but not multivariate, analysis in adult TBM patients. However, a subsequent randomised trial conducted at the same site found no significant difference in nine-month mortality between HIV-infected TBM patients who started ART before or at two months of TB treatment (58% mortality), compared to the historical comparator group most of whom were not exposed to ART (67% mortality) [9,10]. Croda *et al.* [8] found a history of ART prior to TBM presentation to be predictive of death at nine-months follow-up. The authors postulate that this surprising finding most likely related to non-compliance to ART.

Contrary to inpatient mortality rates which are generally similar between HIV-infected and uninfected patients with TBM [22,23,25,26], six to nine-month outcome is substantially worse in HIV-infected patients [14,27–29]. HIV-related illnesses (other than TBM) probably account for a substantial proportion of deaths after hospital discharge in HIV-infected TBM patients, particularly those not on ART. As previous studies in HIV-associated TB have shown a clear survival benefit in patients receiving ART [6,7], it is intuitive to infer a causal relationship between ART and improved survival in our patient cohort. However, survival-bias might also have contributed to the association of ART and reduced mortality at six-month follow-up that we observed: those who survived were able to initiate ART.

Our study has several important limitations, which may have resulted in bias. Firstly, due to its retrospective nature, not all information was available in all cases. Specifically, a substantial proportion of chest radiographs (14%) were not available for review. This might have resulted in an underestimate of patients with probable TBM. The reasons for a substantial proportion of HIV-infected patients (43% of ART naïve patients who survived admission) failing to start ART after discharge could not be determined. Patients are not routinely followed-up at our facility after discharge from hospital; eligible patients are referred to their local ART clinics to start ART. Alternatively, patients who require prolonged admission may commence ART during admission to a step-down facility. Limited access to primary care clinic and step-down facility clinical records precluded the systematic collection of data regarding reasons for failing to start ART, drug toxicities and interactions, as well as co-morbidities after starting TB treatment and ART. Also, adherence to ART and the proportion of patients receiving directly observed TB treatment could not be assessed, and causes of death were not determined. Although similar to studies of TB patients previously conducted in our setting [30,31], the loss to follow-up rate (10%) was not insubstantial. No factors predictive of loss to follow-up could be confirmed by analysis of baseline characteristics of these patients compared to those retained in care. However, a trend to a lower $CD4^+$ count was observed in the latter group. Secondly, as study entry relied on CSF findings, patients with TBM who died prior to LP, and those who had a contraindication to LP based on brain CT, were not included in the analysis.

Thirdly, HIV itself often results in mild CSF abnormalities [32]. For this reason, we did not include patients with mildly abnormal CSF if a specific cause of meningitis was not found. However, it is well documented that a minority of patients with TBM may present with mildly abnormal, or completely normal CSF, especially in the context of HIV co-infection [8,22,23,33]. This group of patients would have been excluded from our study if CSF TB microscopy and culture were negative. Furthermore, the decision to perform CSF TB microscopy and culture was not uniform, being based on the attending clinician' s discretion. Significantly less patients with possible and probable TBM compared to definite TBM had CSF *M. tuberculosis* culture performed; this could have resulted in the misclassification of some patients with definite TBM who might have had *M. tuberculosis* cultured had culture been performed.

Fourthly, CSF findings and neurological signs, most notably focal neurological deficits (21% definite TBM versus 5% possible TBM [$p<0.01$]), differed significantly between patients with definite and possible TBM. This might reflect the inclusion of some patients with alternative diagnoses such as viral meningitis as possible TBM or, alternatively, be indicative of more severe disease in patients with definite TBM. Patients with possible TBM were also less likely, whilst those with probable TBM were more likely, to undergo brain CT compared to patients with definite

TBM ($p = 0.04$ and $p = 0.007$, respectively). This reflects the limited resources in our setting: brain imaging in the context of meningitis is usually prioritized to patients with severe disease or in whom an intracerebral space occupying lesion is suspected i.e. those with focal neurological deficits, severe depressed level of consciousness or seizures. Both the differences in CSF findings and the differences in the proportions of patients who underwent brain imaging between the definite and possible TBM groups could have resulted in bias, resulting in different corticosteroid prescription practices between the 2 groups (71% definite TBM compared to 32% possible TBM, [$p = 0.0007$]) and possibly differentially influencing outcomes. In our setting, corticosteroids are prescribed at the treating clinician's discretion.

Conclusions

In our setting where most patients with TBM are HIV co-infected, advanced HIV and worse stage of TBM disease are poor prognostic factors. Starting ART prior to or during TB treatment may be associated with lower mortality in TBM patients co-infected with HIV.

Supporting Information

Table S1 Treatment of new tuberculous meningitis cases (Regimen 1).[1]
(DOC)

Table S2 Treatment of re-treatment tuberculous meningitis cases (Regimen 2).[1]
(DOC)

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Author Contributions

Conceived and designed the experiments: SM DJP GM. Performed the experiments: SM DJP CS. Analyzed the data: SM DJP RJW GM. Contributed reagents/materials/analysis tools: SM DJP RJW GM. Wrote the paper: SM DJP CS RJW GM.

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Chapter 4: Part 2

CONCISE COMMUNICATION

Central nervous system disorders after starting antiretroviral therapy in South Africa

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Objective: To describe the spectrum of central nervous system (CNS) disease during the first year of antiretroviral therapy (ART) and to determine the contribution of neurological immune reconstitution inflammatory syndrome (IRIS).

Design: A prospective observational cohort study conducted over a 12-month period at a public sector referral hospital in South Africa.

Methods: HIV-seropositive patients who developed new or recurrent neurological or psychiatric symptom(s) or sign(s) within the first year of starting ART were enrolled. We used the number of patients starting ART in the referral area in the preceding year as the denominator to calculate the incidence of referral for neurological deterioration. Patients with delirium and peripheral neuropathy were excluded. Outcome at 6 months was recorded.

Results: Seventy-five patients were enrolled. The median nadir $CD4^+$ cell counts was 64 cells/μl. Fifty-nine percent of the patients were receiving antituberculosis treatment. The incidence of referral for CNS deterioration in the first year of ART was 23.3 cases [95% confidence interval (CI), 18.3–29.2] per 1000 patient-years at risk. CNS tuberculosis ($n=27$, 36%), cryptococcal meningitis ($n=18$, 24%), intracerebral space occupying lesions (other than tuberculoma) ($n=10$, 13%) and psychosis ($n=9$, 12%) were the most frequent diagnoses. Paradoxical neurological IRIS was diagnosed in 21 patients (28%), related to tuberculosis in 16 and cryptococcosis in five. At 6 months, 23% of the patients had died and 20% were lost to follow-up.

Conclusion: Opportunistic infections, notably tuberculosis and cryptococcosis, were the most frequent causes for neurological deterioration after starting ART. Neurological IRIS occurred in over a quarter of patients.

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Keywords: antiretroviral therapy, central nervous system diseases, HIV, immune reconstitution inflammatory syndrome, neurological disorders

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Introduction

Neurological disorders are important causes of morbidity and mortality in HIV-infected patients [1,2]. The use of combination antiretroviral therapy (ART) has significantly reduced the incidence and progression of HIV-associated dementia, as well as the incidence of opportunistic infections affecting the central nervous system (CNS) [3]. However, an early complication of ART is the immune reconstitution inflammatory syndrome (IRIS), which may affect the CNS [4,5]. IRIS occurs due to an exuberant inflammatory response directed toward opportunistic pathogens [6]. This results in the paradoxical worsening of a patient's condition despite adequate antimicrobial therapy (paradoxical IRIS) or the unmasking of an occult opportunistic infection with an unusually inflammatory presentation (unmasking IRIS) [7]. Neurological IRIS is described in several HIV-related CNS disorders such as tuberculosis (TB), cryptococcal meningitis, cytomegalovirus infection and progressive multifocal leukoencephalopathy [4,5].

Efavirenz (EFV) may cause CNS adverse events in more than 50% of the patients [8,9]. Furthermore, a Thai study reported an increased rate of primary CNS lymphoma and ischemic/hemorrhagic strokes in HIV-infected patients within the first 2 years of ART when compared with HIV-infected patients from the same setting in the pre-ART era [2].

In this study, we describe the spectrum of CNS disease during the first year of ART among patients presenting to a referral hospital in South Africa.

Participants and methods

A prospective observational study at GF Jooste Hospital, a secondary-level, public sector referral hospital in Cape Town, South Africa. GF Jooste Hospital serves adults from a population of approximately 1.3 million people who reside in high-density, low-income communities. The incidence of TB in these communities exceeds 1000 cases per 100 000 of the population per annum and the antenatal HIV seroprevalence can be 30% [10]. Eleven primary-level ART clinics are situated in the referral area. Most patients attending these clinics do not have health insurance. Thus, the public sector referral hospital serves most patients with significant clinical deterioration following ART initiation.

Between 1 November 2007 and 31 October 2008 (12 months), we enrolled adult (≥18 years of age) HIV-seropositive patients who were referred to our facility with new or recurrent neurological or psychiatric symptom(s) or sign(s) within the first year of starting ART. Patients with neurological deterioration due to peripheral neuropathy or 'delirium secondary to a general medical condition' were excluded. Doctors in the infectious diseases outpatient clinic and the admitting medical doctors were informed about the study and they screened all patients who presented to GF Jooste hospital with a medical illness. Patients who met the inclusion criteria were referred to a study physician. The study physicians also actively sort out referrals in the outpatient clinic and medical wards each day of the week (Monday–Friday). Data obtained by the study physician included demographic information, $CD4^+$ cell counts prior to ART initiation, ART regimen, previous/concurrent illnesses affecting the CNS, details of TB disease if present, medication use and details of neurological presentation. Thereafter, a neurological examination was performed by the study physician and investigations requested as determined by presentation. We subsequently used primary clinic and hospital medical notes, as well as the *National Health Laboratories Service* database to trace specimens, and the electronic hospital and primary clinic attendance registers to trace patients in order to determine the outcome 6 months after presentation. Data obtained prospectively (i.e. details regarding presentation and initial management), as well as retrospectively (i.e. outcome data), were recorded on a standardized data collection sheet. Data analysis was performed using Microsoft Excel. The incidence of referral for CNS deterioration was calculated with 95% Poisson confidence intervals (CIs) on the basis of the total number of patients initiating ART in the year preceding each study month at the 11 referring ART clinics, reduced by 10.9% to allow for losses to care. The data were obtained from monthly reports of total patients started on ART in each referring clinic and provincial cohort data on retention in care at 6 months on ART [11]. The Research Ethics Committee of the University of Cape Town approved this study.

Definitions

We defined paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) and cryptococcal meningitis immune reconstitution inflammatory syndrome (CM-IRIS) using consensus clinical case definitions [7,12,13]. The diagnosis of paradoxical TB-IRIS required diagnosis of active TB prior to ART initiation, response to antitubercular treatment and development of recurrent, new or worsening symptoms/signs of neurological TB within 3 months of starting ART. Paradoxical TB-IRIS was also diagnosed in patients who were diagnosed with TB after initiation of ART and subsequently developed a neurological paradoxical reaction after starting antitubercular therapy that did not have an alternative explanation. The diagnosis of paradoxical CM-IRIS required diagnosis of cryptococcal meningitis prior to ART, initial response to antifungal treatment with improvement of symptoms/signs and presentation with cryptococcal meningitis recurrence that was culture negative within 12 months of ART initiation. If there was a cryptococcal meningitis recurrence on ART and the cerebrospinal fluid (CSF)

cultured *Cryptococcus neoformans*, the onset of the event had to be within 3 months of initiation of antifungal therapy to be defined as CM-IRIS. The diagnosis of a culture-positive cryptococcal meningitis relapse on ART required re-presentation with CSF fungal culture-positive cryptococcal meningitis and occurrence more than 3 months after start of antifungal therapy.

The diagnosis of 'delirium secondary to a general medical condition' required acute confusion secondary to sepsis owing to a nonneurological infection or metabolic abnormality (e.g. hypoxia, hypoglycemia, renal or hepatic failure).

Results

Seventy-five patients presented with neurological deterioration within 1 year of starting ART. Between 1 November 2006 and 31 October 2007, the estimated person time at risk in the first year of ART was 3222 patient-years, accounting for losses to care. The incidence rate of referred patients with CNS deterioration in the first year on ART was, therefore, 23.3 cases (95% CI, 18.3–29.2) per 1000 patient-years at risk.

Table 1 summarizes the 75 patients' clinical and demographic characteristics. Forty-four patients (59%) were receiving antitubercular treatment at the time of neurological deterioration, 30 of whom had culture-confirmed disease. Drug susceptibility testing for rifampicin and isoniazid (INH) was performed on 18 isolates, which revealed drug susceptibility in 15 patients and multidrug-resistance (resistant to rifampicin and INH) in three patients. The four most frequent reasons for neurological deterioration were CNS TB, cryptococcal meningitis, intracerebral space occupying lesions (SOLs) and psychosis (Table 2).

Table 1. Characteristics and outcomes of 75 HIV-seropositive patients presenting with neurological deterioration within 1 year of initiating antiretroviral therapy.

Female, *n* (%)	40	(53)
Age in years, median (range)	33	(18–68)
Nadir $CD4^+$ cell counts (cells/μl), median (IQR)	64	(23–114)
WHO Staging, *n* (%)		
Stage II	2	(3)
Stage III	28	(37)
Stage IV	45	(60)
ART regimen, *n* (%)		
d4T/3TC/EFV	50	(67)
d4T/3TC/NVP	14	(19)
ZDV/3TC/EFV	6	(8)
ZDV/3TC/NVP	4	(5)
TDF/3TC/EFV	1	(1)
Current TB, *n* (%)[a]	44	(59)
Pulmonary/extrapulmonary disease[b], *n/n*	27/22	
Time from antitubercular treatment initiation to starting ART in days, median (IQR)[c]	56	(34–100)
Previous TB, *n* (%)	31	(41)
Previous CM, *n* (%)	11	(15)
Time from ART initiation to symptom onset in days, median (IQR)	23	(9–89)
Paradoxical TB-IRIS, $n=16$	16	(11–30)
Paradoxical CM-IRIS, $n=5$	58	(27–88)
Time from symptom onset to presentation in days, median (IQR)	4	(3–14)
Paradoxical TB-IRIS, n = 16	4	(3–16)
Paradoxical CM-IRIS, $n=5$	3	(2–4)
Outcome at 6 months, *n* (%)		
Alive	43	(57)
Dead[d]	17	(23)
Lost to follow-up[e]	15	(20)

3TC, lamivudine; ART, antiretroviral therapy; CM, cryptococcal meningitis; CM-IRIS, cryptococcal meningitis immune reconstitution inflammatory syndrome; d4T, stavudine; EFV, efavirenz; IQR, interquartile range; NVP, nevirapine; TB, tuberculosis; TB-IRIS, tuberculosis-associated immune reconstitution inflammatory syndrome; TDF, tenofovir; ZDV, zidovudine.

[a]Includes all patients on antitubercular treatment at the time of neurological deterioration [those who started prior to ART initiation ($n=40$) and those who started after ART initiation ($n=4$)].

[b]Extrapulmonary disease sites include nodal ($n=11$), central nervous system ($n=5$), abdominal ($n=4$), pleural ($n=4$) and pericardial ($n=2$). In five patients, both pulmonary and extrapulmonary disease were present. In three patients more than one extrapulmonary site of disease was present.

[c]For 40 patients who started antitubercular treatment prior to ART.

[d]Deaths occurred in the following patient categories: intracerebral space occupying lesion (other than tuberculoma) ($n=6$), new diagnosis of neurological TB ($n=2$), poor adherence to antitubercular treatment ($n=2$), multidrug-resistant TB ($n=1$), new diagnosis of CM ($n=3$), cytomegalovirus encephalitis ($n=1$) and HIV encephalopathy ($n=2$).

[e]Lost to follow-up occurred in the following patient categories: paradoxical TB-IRIS ($n=1$), multidrug-resistant TB ($n=2$), poor adherence to antitubercular treatment ($n=1$), paradoxical CM-IRIS ($n=1$), culture-positive CM relapse on ART ($n=1$), new diagnosis of CM ($n=1$), cerebral toxoplasmosis ($n=1$), intracerebral space occupying lesion of uncertain cause ($n=1$), EFV-induced psychosis ($n=2$), reactive psychosis ($n=1$), isolated abducens nerve palsy ($n=1$), varicella zoster virus radiculopathy ($n=1$) and bacterial meningitis ($n=1$).

Table 2. Causes of neurological deterioration within one year of antiretroviral therapy initiation in 75 HIV-seropositive patients.

	n	(%)
CNS TB	27	(36)
Paradoxical TB-IRIS[1]	16	(21)
TB meningitis ($n=8$)		
Tuberculoma ($n=6$)		
TB arachnoiditis ($n=1$)		
TB spinal epidural abscess ($n=1$)		
New diagnosis of neurological TB	4	(5)
TB meningitis ($n=3$)		
TB arachnoiditis ($n=1$)		
MDR-TB	2	(3)
Deterioration due to poor adherence	5	(7)
Cryptococcal meningitis (CSF culture positive[2])	18 [12]	(24)
Paradoxical CM-IRIS[3]	5 [1]	(7)
Culture-positive relapse on ART[4]	4 [4]	(5)
New diagnosis of CM	9 [7]	(12)
Intracerebral space occupying lesion[5]	10	(13)
Cerebral toxoplasmosis	1	(1)
Uncertain cause[6]	9	(12)
Psychosis	9	(12)
EFV-induced	5	(7)
HIV-induced	2	(3)
INH-induced	1	(1)
Reactive psychosis	1	(1)
Other	11	(15)
Seizure	3	(4)
CMV encephalitis	2	(3)
HIV encephalopathy	2	(3)
Isolated abducens nerve palsy	1	(1)
VZV radiculopathy	1	(1)
Bacterial meningitis	1	(1)
RIND	1	(1)

ART, antiretroviral therapy; CM, cryptococcal meningitis; CM-IRIS, cryptococcal meningitis immune reconstitution inflammatory syndrome; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; EFV, efavirenz; INH, isoniazid; MDR-TB, multidrug-resistant tuberculosis; RIND, reversible ischemic neurological deficit; TB, tuberculosis; TB-IRIS, tuberculosis-associated immune reconstitution inflammatory syndrome; VZV, varicella zoster virus.
[1,3,4]See definitions in the text.
[2]CSF culture positive for *Cryptococcus neoformans* at time of deterioration.
[5]Space occupying lesions other than tuberculoma.
[6]The most likely differential diagnoses included tuberculoma and toxoplasmosis.

Central nervous system tuberculosis

Twenty-seven patients (36%) presented with neurological deterioration related to TB. Paradoxical TB-IRIS was diagnosed in 16/75 patients (21%). Thirteen out of 16 of these patients received corticosteroids. At 6-months follow-up, 15/16 patients were alive and one was lost to follow-up. All patients diagnosed with tuberculoma either had a negative-serum immunoglobulin G (IgG) serological analysis for *toxoplasma* species or showed a good response to antitubercular treatment in the absence of treatment for toxoplasmosis.

Cryptococcal meningitis

Eighteen patients (24%) presented with deterioration related to cryptococcal meningitis. Five patients (7%) presented with paradoxical CM-IRIS, of whom one received corticosteroids. At 6-months follow-up, four of these five patients were alive and one was lost to follow-up.

Space occupying lesions (other than tuberculoma)

Ten patients (13%) presented with SOL due to toxoplasmosis ($n=1$) or of uncertain cause ($n=9$). The diagnosis of cerebral toxoplasmosis was on the basis of a response to antitoxoplasma treatment in the absence of antitubercular treatment. In the other nine patients, it could not be ascertained whether the SOL was related to TB or toxoplasmosis: four were already receiving antitubercular treatment and had antitoxoplasma treatment added, three were started on treatment for both TB and toxoplasmosis, one was treated only for toxoplasmosis, but died and one was only treated for TB, but died.

Psychosis

Nine patients (12%) presented with psychosis. An EFV-induced psychosis was the most likely cause in five. One patient was diagnosed with INH-induced psychosis and another was diagnosed with a reactive psychosis secondary to social stressors. A diagnosis of HIV-induced psychosis was presumed for two patients in whom no other cause could be identified.

Outcomes

At 6-months follow-up, 43 (57%) patients were alive, 17 (23%) were dead and 15 (20%) were lost to follow-up from the healthcare system (Table 1). The median interval from ART initiation, and presentation, to death was 67 days [interquartile range (IQR) 47–164 days] and 23 days (IQR 10–39 days), respectively.

Discussion

This is, to our knowledge, the first prospective study describing the spectrum of neurological disorders occurring within the first year of ART. TB and cryptococcal meningitis together accounted for at least 60% of cases. This is likely owing to the high incidence of TB in our setting, and profound immunosuppression at ART initiation; in 2007, 19% of adults starting ART in the Western Cape province had $CD4^+$ cell counts below 50 cells/μl, whereas the median $CD4^+$ cell counts of patients starting ART in two of the referring clinics were 131 cells/μl [14].

Paradoxical IRIS, which was associated with TB ($n=16$) and cryptococcal meningitis ($n=5$), accounted for 28% of cases. Paradoxical CM-IRIS may occur in up to 30% of patients following ART initiation [15–17]. We previously reported neurological TB-IRIS in 12% of patients who presented with TB-IRIS [12]. The median interval from starting ART to symptom onset in our patients with

TB-IRIS and CM-IRIS was 16 and 58 days, respectively. These findings are similar to previous reports [12,15]. The management of neurological IRIS is problematic; no diagnostic test exists and treatment strategies are based on anecdotal case reports [18]. Eighty-one percent of patients with TB-IRIS and 20% of patients with CM-IRIS in our cohort received corticosteroid therapy, which may be of benefit in patients with neurological TB-IRIS [12,18]. High mortality rates have been associated with both CM-IRIS (up to 66%) [17] and neurological TB-IRIS (at least 13%, at 6-months follow-up) [12]. No patients in our cohort who presented with IRIS died during 6-months follow-up and only one patient with TB-IRIS and one with CM-IRIS were lost to follow-up at 6 months.

New or expanding SOL developed in 21% of patients ($n = 16$). Confirming the cause of SOL is difficult in our setting in which access to stereotactic brain biopsy is limited. Similar to previous studies [12], we relied on available evidence to make the diagnosis. The major differential diagnoses of SOL are cerebral toxoplasmosis (diagnosed in one patient) and tuberculoma (diagnosed in six patients) [19]. In more that half of our patients (9/16), the diagnosis was uncertain. The lack of definitive diagnosis in most patients, and the associated high mortality in these patients (6/9), emphasize the challenge of managing patients with SOL.

Neurological deterioration is an important cause of clinical deterioration and death after starting ART. The referral rate of 23.3 cases per 1000 patient-years at risk is most likely an underestimate of the true incidence of neurological deterioration. Although significant neurological presentation related to a CNS cause results in referral to our facility, patients with mild symptoms or signs are not always referred; patients who are too confused or otherwise unwell to seek medical help may die at home; and patients may attend other hospitals or move out of the referral area. Furthermore, new or worsening peripheral neuropathy is a common cause for neurological deterioration after starting ART [20]. However, as most peripheral neuropathies are managed at primary care level, we did not include these patients in our study. In this study, the challenges posed by the management of patients with neurological deterioration are reflected in poor outcome (23% died), and high rate of loss to follow-up (20%), at 6 months. In comparison, among all patients starting ART in our setting, mortality and loss to follow-up is considerably lower. The cumulative mortality rate during the first year of ART (from 2004 to 2007 in the largest ART clinics in our referral area) was 8%, and 3–5% of patients were lost to follow-up during the first year of ART [21]. In a busy ART program, clinic attendance and adherence to ART require patient mobility, insight and motivation. Neurological deterioration makes this difficult, especially if there is inadequate treatment support from family or friends. Patients with psychosis or confusion may not have the insight to seek medical help and may default ART and other medical therapies. Furthermore, patients with weakness or other neurological impairment may be physically incapable of seeking medical care independently. This may contribute to the high loss to follow-up rate we observed.

Conclusion

In our setting, opportunistic infections, notably TB and cryptococcosis, were the most important causes for neurological deterioration during the first year of ART. Over a quarter of patients were related to paradoxical IRIS. Our study has particular relevance to ART programs in high TB prevalence regions. We highlight the challenges associated with the management of these in patients in resource-constrained settings.

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Chapter 5

Frequency, Severity, and Prediction of Tuberculous Meningitis Immune Reconstitution Inflammatory Syndrome

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Background. Tuberculosis immune reconstitution inflammatory syndrome (IRIS) is a common cause of deterioration in human immunodeficiency virus (HIV)–infected patients receiving tuberculosis treatment after starting antiretroviral therapy (ART). Potentially life-threatening neurological involvement occurs frequently and has been suggested as a reason to defer ART.

Methods. We conducted a prospective study of HIV-infected, ART-naive patients with tuberculous meningitis (TBM). At presentation, patients started tuberculosis treatment and prednisone; ART was initiated 2 weeks later. Clinical and laboratory findings were compared between patients who developed TBM-IRIS (TBM-IRIS patients) and those who did not (non-TBM-IRIS patients). A logistic regression model was developed to predict TBM-IRIS.

Results. Forty-seven percent (16/34) of TBM patients developed TBM-IRIS, which manifested with severe features of inflammation. At TBM diagnosis, TBM-IRIS patients had higher cerebrospinal fluid (CSF) neutrophil counts compared with non-TBM-IRIS patients (median, 50 vs 3 cells $\times 10^6$/L, $P = .02$). *Mycobacterium tuberculosis* was cultured from CSF of 15 TBM-IRIS patients (94%) compared with 6 non-TBM-IRIS patients (33%) at time of TBM diagnosis; relative risk of developing TBM-IRIS if CSF was *Mycobacterium tuberculosis* culture positive = 9.3 (95% confidence interval [CI], 1.4–62.2). The combination of high CSF tumor necrosis factor (TNF)-α and low interferon (IFN)-γ at TBM diagnosis predicted TBM-IRIS (area under the curve = 0.91 [95% CI, .53–.99]).

Conclusions. TBM-IRIS is a frequent, severe complication of ART in HIV-associated TBM and is characterized by high CSF neutrophil counts and *Mycobacterium tuberculosis* culture positivity at TBM presentation. The combination of CSF IFN-γ and TNF-α concentrations may predict TBM-IRIS and thereby be a means to individualize patients to early or deferred ART.

Keywords. meningitis; tuberculosis; neutrophils; pathogenesis.

Paradoxical tuberculosis immune reconstitution inflammatory syndrome (IRIS) occurs in 8%–43% of human immunodeficiency virus (HIV)–infected patients receiving tuberculosis treatment after starting antiretroviral therapy (ART) [1–4]. Tuberculosis IRIS results from rapid restoration of *Mycobacterium tuberculosis*–specific immune responses, but its pathogenesis remains poorly understood [1, 5, 6].

Neurological tuberculosis IRIS occurs in a substantial proportion (12%) of tuberculosis IRIS cases and is the commonest cause of central nervous system (CNS) deterioration during the first year of ART in settings of high tuberculosis/HIV prevalence [7, 8]. Mortality is high (up to 30%) in those affected [8]. Manifestations of neurological tuberculosis IRIS include meningitis

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[7–11], intracranial tuberculomata [7, 8, 12–14], brain abscesses [12, 15], radiculomyelitis [7, 8, 11], and spinal epidural abscesses [7]. There are no prospective studies describing tuberculous meningitis (TBM) IRIS; only isolated cases [9–15] and 1 case series of neurological tuberculosis IRIS [8, 16] have been published. Although consensus now exists that ART should be started early (around 2 weeks) in HIV/tuberculosis-coinfected patients with severe immunosuppression, a potential exception is TBM because of the perceived risk of TBM-IRIS [17, 18]. However, the frequency and severity of this complication are not well documented and no means exist to predict the syndrome.

We therefore investigated clinical and laboratory findings in ART-naive HIV-infected patients who presented with TBM. We undertook serial cerebrospinal fluid (CSF) sampling in patients who did and did not develop TBM-IRIS.

MATERIALS AND METHODS

Setting

This prospective, observational study was performed at GF Jooste Hospital, a public sector referral hospital in Cape Town. The hospital serves a low-income, high-density population in which the tuberculosis notification rate exceeds 1.5% per year with 70% of tuberculosis cases coinfected with HIV [19].

Participants

ART-naive HIV-infected patients aged ≥18 years presenting with meningitis from March 2009 through October 2010 were screened for study inclusion. HIV infection was diagnosed using 2 rapid HIV antibody tests and confirmed by HIV load. Definite TBM was diagnosed when acid-fast bacilli were seen, or when *M. tuberculosis* was cultured from CSF. Probable TBM was diagnosed when a patient showed clinical, laboratory, and radiological features of TBM in the absence of other infective causes for presentation [20]. Paradoxical TBM-IRIS was diagnosed according to a published definition for tuberculosis IRIS modified for meningitis [7, 8]. The definition had 3 components: (1) TBM diagnosis before starting ART and improvement on tuberculosis treatment prior to ART initiation; (2) onset of TBM-IRIS manifestations (ie, new, recurrent, or worsening clinical features of TBM) within 3 months of ART initiation; and (3) exclusion of alternative causes for clinical deterioration.

Patients were ineligible if they had a contraindication to lumbar puncture, including unequal pressures between individual brain compartments on brain imaging, or severe TBM (ie, modified British Medical Research Council [BMRC] grade III disease severity) [21]. The University of Cape Town Research Ethics Committee approved the study and written informed consent was obtained from all patients or their relatives.

Procedures

Demographic data and history of tuberculosis disease, HIV infection, and other systemic illnesses and medications were recorded. Patients underwent general physical and neurological examination. Chest radiography, phlebotomy, and lumbar puncture were performed. In patients with suspected raised intracranial pressure or focal neurological deficits, brain computed tomography scanning was performed prior to lumbar puncture. CSF analysis included biochemistry, cytology, microbiology (including microscopy and culture for fungi and pyogenic bacteria), syphilis serology, HIV load, and *Cryptococcus* latex agglutination titer. Ziehl-Neelsen staining of sediment and *M. tuberculosis* culture was performed. If mycobacteria were cultured from CSF, tuberculosis polymerase chain reaction (PCR; Genotype MTBDRplus, Hain Lifesciences) was performed to determine susceptibility to rifampicin and isoniazid. CSF varicella zoster virus PCR was performed if the etiology was suspected. CSF was also stored at −80°C and analyzed for a range of inflammatory markers on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA) using customized Milliplex kits (Millipore, St Charles, MO) according to the manufacturer's instructions.

At TBM diagnosis, patients started tuberculosis treatment according to national guidelines [22] and prednisone (1.5 mg/kg/day). After 2 weeks of treatment and prior to initiation of ART, patients were assessed for improvement on tuberculosis treatment. The initial ART regimen was stavudine, lamivudine, and efavirenz. Later during the study, tenofovir replaced stavudine according to revised national guidelines. CSF investigations performed at TBM diagnosis were repeated at the time of ART initiation, 2 weeks later, and at time of TBM-IRIS presentation and 2 weeks thereafter. Prednisone was reduced to 0.75 mg/kg/day 4 weeks after starting ART and discontinued 2 weeks thereafter, unless the patient developed TBM-IRIS. At TBM-IRIS presentation, investigations were performed to exclude alternative causes of deterioration. Prednisone was either recommenced or the dose increased. Patients were followed for the duration of tuberculosis treatment (9 months); routine visits were at 2 weeks, 4 weeks, 6 weeks, 12 weeks, 6 months, and 9 months after TBM diagnosis. Patients were seen more frequently during deterioration.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism version 5, R version 2.14.1, and StatXact version 9 software packages. Categorical variables were compared using χ^2 or Fisher exact test. Continuous variables were compared between groups and time points within groups, using the Wilcoxon rank sum and Wilcoxon matched pairs tests, respectively. Adjusted relative risks (RRs) were evaluated using Cochran-Mantel-Haenszel tests and tests of homogeneity when

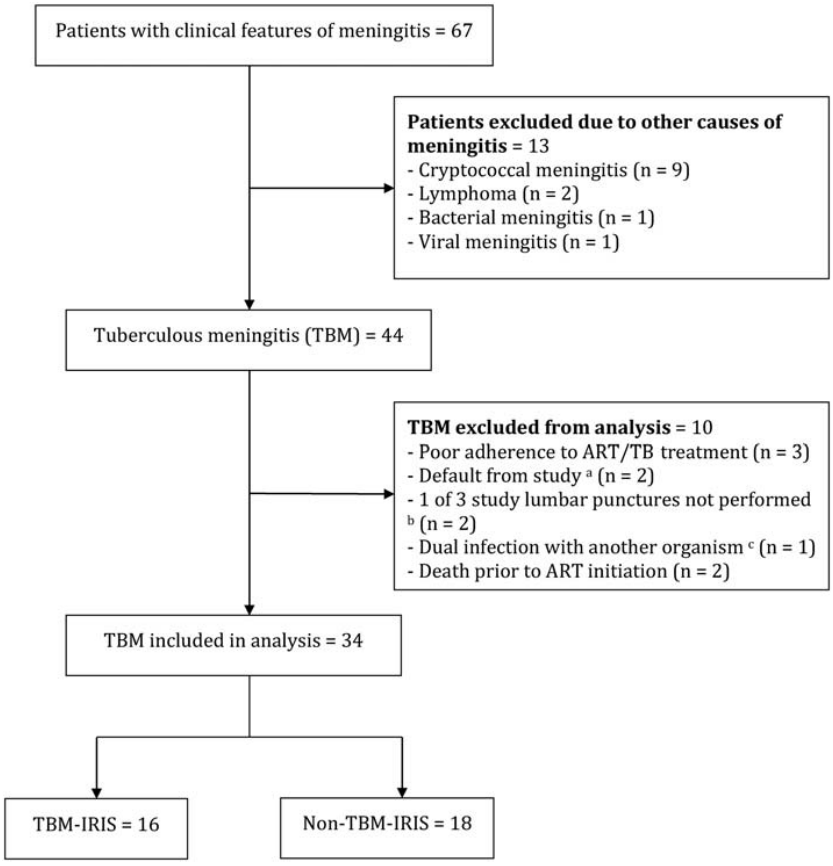

Figure 1. Flow diagram of patients with features of meningitis (eg, headache, confusion, vomiting, and/or neck stiffness) screened for study inclusion. [a]Patients defaulted within 3 months of starting antiretroviral therapy (ART). [b]Time points of lumbar punctures include tuberculous meningitis (TBM) diagnosis, ART initiation, and 2 weeks after starting ART. [c]Cerebrospinal fluid varicella-zoster virus polymerase chain reaction was performed in 1 patient with who had shingles at time of TBM presentation, which was positive. Abbreviations: ART; antiretroviral therapy, IRIS; immune reconstitution inflammatory syndrome; TB; tuberculosis; TBM, tuberculous meningitis.

considering categorical risk factors. Log-binomial models were fitted to continuous risk factors. Significance testing was done using 2-sided P values with $P < .05$ taken as significant.

The predictive accuracy of CSF neutrophil counts at TBM diagnosis for TBM-IRIS was assessed using nonparametric area under the receiver operating characteristic curve (AUC). Additionally, a model to predict TBM-IRIS risk was developed from 5 prespecified cytokines measured in CSF at time of TBM diagnosis. Interleukin 6 (IL-6), interleukin 10, interleukin 12p40, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) were selected as candidate markers of TBM-IRIS based on previous studies [23, 24]. We prespecified the analysis for evaluating the multivariate cytokine model as follows. Significant cytokines comparing TBM-IRIS and non-TBM-IRIS using Wilcoxon rank sum tests were selected for a logistic regression model. Nonsignificant cytokines were dropped, resulting in a final model. The entire model building process was evaluated using leave-one-out cross-validation, the bootstrap method [25], and the permutation test to provide a cross-validated (nonparametric) estimate of the AUC, P values, and 95% confidence intervals (CIs). As a secondary analysis, we examined whether the addition of CSF neutrophils and/or lymphocyte counts would improve the model's predictive ability.

RESULTS

TBM Presentation

The final diagnoses and reasons for exclusion of patients with meningitis are shown in Figure 1. Thirty-four patients were included in the final analysis; 15 (44%) were female and the median age was 33 years (interquartile range [IQR], 29–44). Sixteen patients (47%) developed TBM-IRIS (TBM-IRIS

Table 1. Demographic and Baseline Characteristics of Patients Who Developed Tuberculous Meningitis Immune Reconstitution Inflammatory Syndrome and Those Who Did Not

	TBM-IRIS	Non-TBM-IRIS
	No. (%)	No. (%)
Female	9 (56)	6 (33)
Age, y, median (IQR)	33 (30–46)	31 (25–41)
Duration between tuberculosis treatment and ART, d, median (IQR)[a]	15 (14–16)	15 (14–20)
Previous tuberculosis	5 (31)	6 (33)
Neurological symptoms		
Duration of neurological symptoms, d, median (IQR)	19 (6–31)	9 (6–21)
Nausea/vomiting	13 (81)	8 (44)
Headache	16 (100)	14 (78)
Visual disturbances	6 (38)	5 (28)
Confusion	7 (44)	6 (33)
Neck pain/stiffness	13 (81)	14 (78)
Systemic symptoms		
Chest symptoms	13 (81)	8 (44)
Night sweats	12 (75)	8 (44)
Abdominal symptoms	9 (56)	5 (28)
Weight loss	14 (88)	12 (67)
Clinical findings		
Body mass index, median (IQR)	18.4 (17.2–23.3)	20.7 (19.0–23.3)
BMRC disease grade 1[b]	7 (44)	11 (61)
Focal neurological signs[c]	4 (25)	3 (17)
Blood investigations		
Sodium, mmol/L, median (IQR)	123 (121–129)	130 (128–134)
Hemoglobin, g/dL, median (IQR)	10.2 (8.5–11.7)	12.4 (9.3–13.4)
Other investigations		
CXR abnormalities	13 (81)	9 (50)
Features of extra CNS tuberculosis[d]	14 (88)	12 (67)
Abdominal ultrasound, abnormal/number performed	5/5 (100)	6/8 (75)
Brain CT abnormal/number performed[e]	4/5 (80)	5/9 (56)

Data are presented as No. (%) unless otherwise specified. Definitions for TBM-IRIS and non-TBM-IRIS are taken from [16] and [18], respectively.

Abbreviations: ART, antiretroviral therapy; BMRC, British Medical Research Council; CNS, central nervous system; CT, computed tomography; CXR, chest radiograph; IQR, interquartile range; TBM-IRIS, tuberculous meningitis immune reconstitution inflammatory syndrome.

[a] ART regimens included stavudine (D4T), lamivudine (3TC), and efavirenz (EFV; n = 19); tenofovir, 3TC, and EFV (n = 9); zidovudine, 3TC, and EFV (n = 5); and D4T, 3TC, and lopinavir/ritonavir (n = 1).

[b] BMRC grade I: Glasgow Coma Scale (GCS) score of 15 with no focal neurologic signs; grade II: GCS score of 11–14 or GCS of 15 with focal neurologic signs; grade III: GCS score of ≤10 [21].

[c] Including cranial nerve palsies (n = 4), hemiparesis (n = 1), cerebellar signs (n = 2).

[d] Including number of patients with 1 or more of the following: chest radiograph or abdominal ultrasound features of tuberculosis and acid-fast bacilli seen or *M. tuberculosis* cultured from specimen other than cerebrospinal fluid.

[e] Including features compatible with TBM: hydrocephalus, meningeal enhancement, tuberculoma, and infarct.

patients) whereas 18 did not (non-TBM-IRIS patients). Tables 1 and 2 show the demographic and baseline characteristics of these 2 groups. Baseline characteristics were similar between groups, although patients who developed TBM-IRIS had a longer duration of symptoms (median, 19 vs 9 days) and more frequent features of extrameningeal tuberculosis, such as chest symptoms (81% vs 44%) and chest radiographic abnormalities (81% vs 50%). Five TBM-IRIS patients (31%) and 3 non-TBM-IRIS patients (17%) developed features of extrameningeal tuberculosis IRIS.

TBM-IRIS Presentation

Features of TBM-IRIS developed a median of 14 days (IQR, 4–20) after starting ART. Symptoms and signs included new or worsening headache (n = 12), confusion (n = 6), neck pain/stiffness (n = 11), generalized tonic-clonic seizures (n = 4),

Table 2. Serial Blood and Cerebrospinal Fluid Findings in Patients Who Developed Tuberculous Meningitis Immune Reconstitution Inflammatory Syndrome (TBM-IRIS) and Those Who Did Not (non-TBM-IRIS)

	TBM-IRIS	Non-TBM-IRIS	
	Median (IQR)	Median (IQR)	*P* Value[c]
Blood			
C-reactive protein, mg/L			
TBM diagnosis	45 (13–98)	25 (1–71)	.22
ART start	6 (4–15)	8 (1–20)	.64
2 wk after ART start	56 (20–105)	14 (6–63)	.07
CD4 count, cells/μL			
TBM diagnosis	131 (52–169)	102 (69–278)	.79
ART start	93 (65–158)	145 (64–231)	.40
2 wk after ART start	158 (139–193)	178 (103–261)	.68
HIV load, $\log_{10}$			
TBM diagnosis	5.39 (4.75–6.16)	5.60 (4.76–5.72)	.83
ART start	5.61 (5.26–6.26)	5.30 (4.90–6.04)	.13
2 wk after ART start	3.15 (2.50–3.36)	2.71 (2.43–2.98)	.13
Cerebrospinal fluid			
Protein, g/L			
TBM diagnosis	2.70 (1.80–5.11)	1.88 (1.28–3.31)	.24
ART start	1.63 (1.22–2.67)	0.94 (0.82–1.63)	.03
2 wk after ART start/IRIS presentation[a]	3.11 (1.99–22.83)	0.61 (0.37–1.76)	<.001
2 wk after IRIS presentation[b]	2.62 (2.24–12.74)	…	…
Glucose (CSF blood ratio)			
TBM diagnosis	0.24 (0.15–0.31)	0.47 (0.28–0.57)	.01
ART start	0.51 (0.43–0.55)	0.48 (0.38–0.57)	.63
2 wk after ART start/IRIS presentation[a]	0.39 (0.33–0.43)	0.53 (0.37–0.59)	.05
2 wk after IRIS presentation[b]	0.38 (0.34–0.48)	…	…
Neutrophil counts, $\times 10^6$/L			
TBM diagnosis	50 (15–86)	3 (0–44)	.02
ART start	1 (0–4)	0 (0–5)	.32
2 wk after ART start/IRIS presentation[a]	42 (17–244)	0 (0–3)	<.001
2 wk after IRIS presentation[b]	3 (0–12)	…	…
Neutrophil proportion, % of cells per sample			
TBM diagnosis	36 (6–53)	0 (0–12)	.009
ART start	1 (1–11)	0 (0–18)	.61
2 wk after ART start/IRIS presentation[a]	9 (4–22)	1 (0–33)	.35
Lymphocyte counts, $\times 10^6$/L			
TBM diagnosis	218 (63–366)	93 (24–274)	.36
ART start	110 (44–225)	41 (18–79)	.02
2 wk after ART start/IRIS presentation[a]	177 (69–363)	25 (6–52)	<.001
2 wk after IRIS presentation[b]	147 (98–405)	…	…
HIV load, $\log_{10}$			
TBM diagnosis	6.35 (5.80–6.73)	5.60 (4.81–6.54)	.05
ART start	5.56 (5.16–5.95)	5.40 (4.93–5.82)	.27
2 wk after ART start	3.00 (2.24–3.67)	2.55 (2.42–2.80)	.21

Definitions for TBM-IRIS and non-TBM-IRIS are taken from [16] and [18], respectively.

Abbreviations: ART, antiretroviral therapy; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; TBM, tuberculous meningitis.

[a] For TBM-IRIS patients, results are reported for lumbar puncture performed at TBM-IRIS presentation, which occurred a median of 14 days after ART initiation.

[b] In 5 TBM-IRIS patients, lumbar puncture was not performed 2 weeks after TBM-IRIS because of death (n = 1), contraindication to lumbar puncture (n = 3), and patient admitted to alternative facility (n = 1).

[c] $P < .05$ was considered statistically significant.

vomiting (n = 5), paraparesis (n = 3), myoclonic jerks (n = 1), dysconjugate eye movements (n = 1), and aphasia (n = 1). At time of TBM-IRIS presentation, 15 patients underwent brain imaging, including computed tomography (n = 14) or magnetic resonance imaging (n = 1). Imaging showed features of TBM in 14 of these patients. Magnetic resonance imaging of the spine was performed in 2 patients with paraparesis; both had features of radiculomyelitis (Supplementary Figure 1).

Serial Blood and CSF Findings

Baseline blood investigations were similar between TBM-IRIS and non-TBM-IRIS patients with the exception of serum sodium concentrations, which were lower in TBM-IRIS patients (median, 123 vs 130 mmol/L, $P = .01$, Table 1). Table 2 and Figure 2 show serial blood and CSF findings. There was a significant rise in CD4 counts between starting ART and 2 weeks later in both TBM-IRIS (median, 93–158 cells/μL, $P = .009$) and non-TBM-IRIS (median, 145–178 cells/μL, $P = .04$) patients. Between these time points, blood and CSF HIV loads decreased significantly ($P < .001$) in both groups.

At TBM diagnosis, TBM-IRIS patients had higher CSF cell counts, in particular neutrophils (median, 50 vs 3 cells $\times 10^6$/L, $P = .02$, Table 2). Similarly, neutrophil percentages from individual samples were higher in TBM-IRIS patients compared with non-TBM-IRIS patients (median, 36% vs 0%, $P = .009$). CSF to blood glucose ratios were lower in TBM-IRIS patients (median, 0.24 vs 0.47, $P = .005$). In both groups, CSF parameters initially improved on tuberculosis treatment (Table 2 and Figure 2). However, at TBM-IRIS presentation, TBM-IRIS patients showed findings of recurrent inflammation. In this group, lymphocyte and neutrophil counts at TBM-IRIS presentation were similar, and protein concentrations higher, compared with the same parameters at TBM diagnosis (median protein, 3.11 g/L at TBM-IRIS vs 2.70 g/L at TBM diagnosis, $P = .007$).

Mycobacterium tuberculosis was cultured from the CSF of 15 TBM-IRIS patients (94%) and 6 non-TBM-IRIS patients (33%) at TBM diagnosis; the risk of developing TBM-IRIS if CSF *M. tuberculosis* culture was positive at this time point was 71.4% (15/21), compared with a risk of 7.7% (1/13), corresponding to an RR of 9.3 (95% CI, 1.4–62.2, $P = .004$). Additional analyses considered the RRs of culture positivity adjusting for the following known or potential risk factors for tuberculosis IRIS: baseline viral load (median, ≥330 000 copies/mL, equivalent to $\log_{10} = 5.52$, vs <330 000), CD4 count (median, ≤137 cells/μL vs >137), evidence of disseminated disease in the form of an abnormal chest radiograph suggesting miliary disease, and duration of illness (median duration, ≥2 weeks vs <2 weeks). *Mycobacterium tuberculosis* culture positivity remained a significant risk factor after adjusting for these factors (Supplementary Table 1). There was no evidence of differences in the RRs according to these factors after considering culture status. Some TBM-IRIS patients remained culture positive after 2 weeks (n = 7) and 4 weeks (n = 2) of tuberculosis treatment. No non-TBM-IRIS patients were culture positive after starting tuberculosis treatment. All cultures were fully drug sensitive with the exception of one, which was monoresistant to isoniazid.

Analysis of Baseline CSF Neutrophil Count and Cytokine Concentrations to Predict TBM-IRIS

Concentrations of the prespecified cytokines from which the model to predict TBM-IRIS was developed are shown in Supplementary Table 2 and Supplementary Figure 2. The final multivariate logistic regression model included IFN-γ and TNF-α and produced a cross-validated AUC of 0.91 (95% CI, .53–.99, $P = .02$), indicating high diagnostic accuracy when jointly considering these 2 cytokines to differentiate TBM-IRIS from non-TBM-IRIS at time of TBM diagnosis. The odds ratio (OR) for TNF-α was 1.85 (per 10 pg/mL, $P = .006$), indicating an 85% increase in the odds of IRIS for every 10 pg/mL increase in TNF-α (after adjusting for IFN-γ). The OR for IFN-γ was 0.64 (per 100 pg/mL, $P = .01$), indicating decreased odds of IRIS with increasing IFN-γ (after adjusting for TNF-α). Figure 3 provides a heatmap representation of the predicted probabilities of the resulting model with the observed values overlaid. Neutrophil counts produced an AUC of 0.72 (95% CI, .54–.90, $P = .03$), indicating modest discriminatory accuracy for TBM-IRIS. However, including CSF neutrophil and lymphocyte counts in the model did not improve its ability to predict TBM-IRIS.

Management and Outcome

In 13 patients prescribed prednisone (0.75–1.5 mg/kg/day), the dose was increased at TBM-IRIS diagnosis. Prednisone was restarted at a dose of 1.5 mg/kg/day in the other 3 TBM-IRIS patients. The median total duration of corticosteroid treatment in TBM-IRIS patients was 109 days (IQR, 69–141) compared with 35 days (IQR, 20–43) in non-TBM-IRIS patients. ART was interrupted during TBM-IRIS in 1 patient because of brainstem involvement. This patient made a full recovery and had no recurrent symptoms after recommencement of ART under prednisone cover. At 9 months' follow-up, all non-TBM-IRIS patients were alive (including 1 who had defaulted study follow-up but continued tuberculosis treatment from a primary care tuberculosis clinic), 2 had marked cognitive impairment (international HIV dementia scale <10) [26], and 1 patient had marked cognitive impairment with residual hemiparesis. Twelve IRIS patients (75%) were alive at 9 months' follow-up, 2 patients showed marked cognitive impairment, 1 patient defaulted study follow-up but was alive, and 1 had marked cognitive impairment, residual

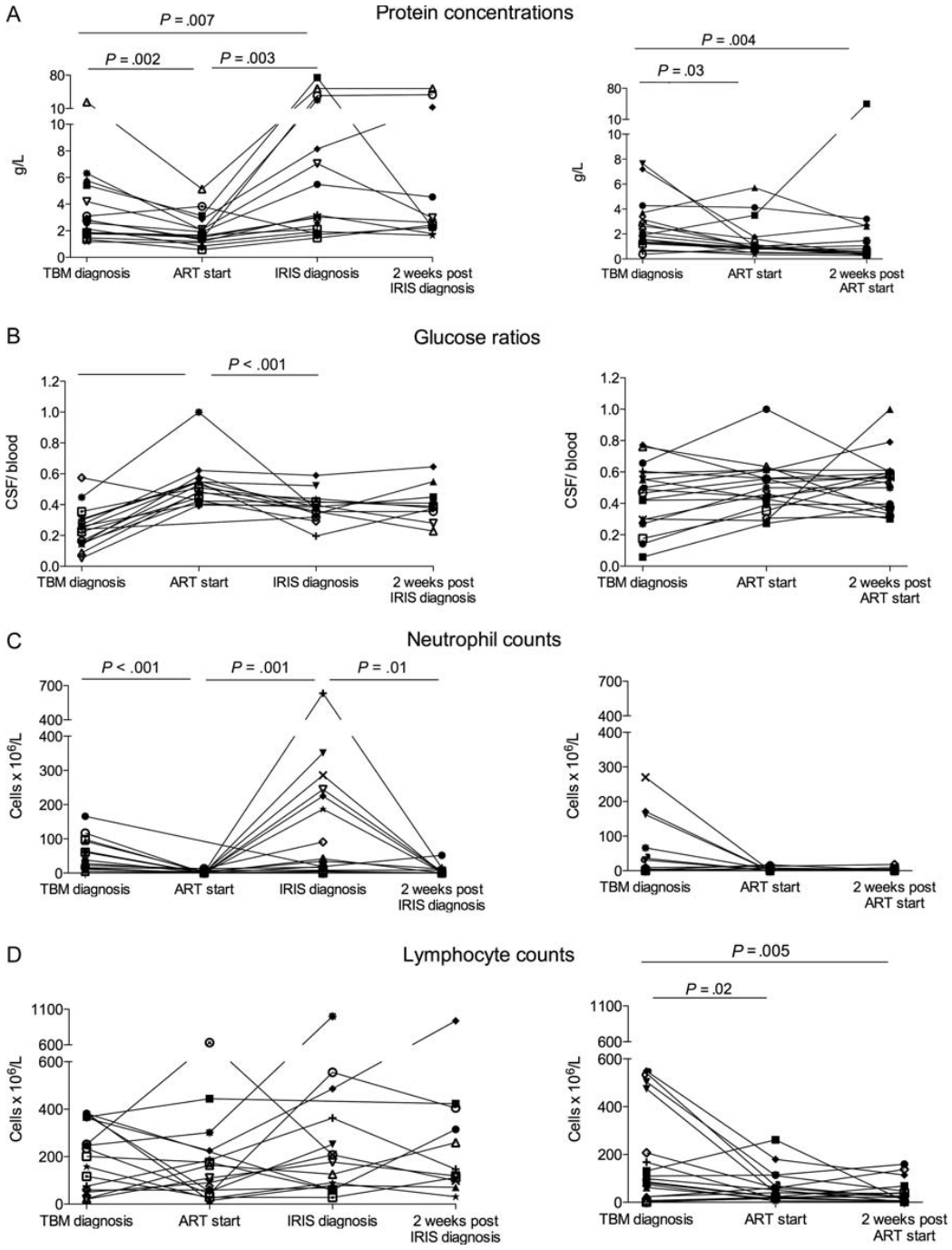

Figure 2. Serial cerebrospinal fluid (CSF) findings in patients who developed tuberculous meningitis immune reconstitution inflammatory syndrome (left), and those who did not (right), including protein concentrations (*A*), CSF to blood glucose ratios (*B*), neutrophil counts (*C*), and lymphocyte counts (*D*). Significant differences ($P < .05$) between time points within groups are indicated. Abbreviations: ART; antiretroviral therapy, CSF cerebrospinal fluid; IRIS; immune reconstitution inflammatory syndrome; TBM, tuberculous meningitis.

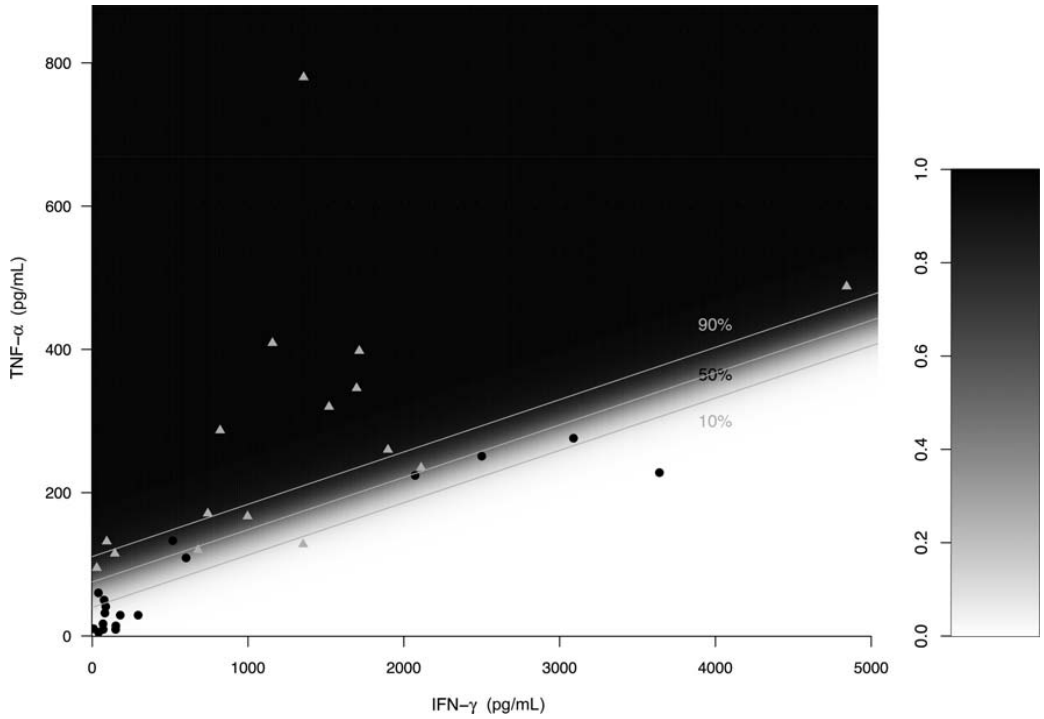

Figure 3. Predictive model for tuberculous meningitis immune reconstitution inflammatory syndrome (TBM-IRIS; patients depicted by gray triangles) and non-TBM-IRIS (patients depicted by black circles). Tumor necrosis factor–α and interferon-γ concentrations (pg/mL) are reported. Darker gray indicates greater probability of TBM-IRIS, while lighter gray indicates greater probability of not developing TBM-IRIS. Probabilities associated with shading are indicated by the legend. The middle line indicates 50% chance of TBM-IRIS, while the upper and lower gray lines indicate probabilities of 90% and 10%, respectively. Observe that when using the median line to classify patients as TBM-IRIS or non-TBM-IRIS, all but 2 TBM-IRIS and 1 non-TBM-IRIS patients are correctly classified. Several points in the lower left were moved marginally to the right so that all subjects are clearly identifiable. Abbreviations: IFN, interferon; TNF, tumor necrosis factor.

hemiparesis, and hearing impairment. Death occurred in 4 (25%) TBM-IRIS patients at 33, 53, 60, and 118 days after TBM diagnosis and was related to TBM-IRIS in 2 patients. The 2 other deaths were due to a road traffic accident (n = 1) and unknown cause (n = 1). Kaplan-Meier survival analysis by TBM-IRIS vs non-IRIS (with a log-rank hypothesis test of the difference in survival between these 2) was nonsignificant.

DISCUSSION

This is the first prospective study of TBM-IRIS. In our cohort, tuberculosis IRIS presenting as TBM-IRIS (47%), as well as tuberculosis IRIS involving any organ system (56%), was more frequent than in previous studies [2–4, 27]. Extrapulmonary tuberculosis is a risk factor for tuberculosis IRIS [14, 28], and our cohort included only patients with TBM. A shorter interval between starting tuberculosis treatment and ART (which was 2 weeks in our study) similarly increases the risk of tuberculosis IRIS [2–4]. The high TBM-IRIS incidence we observed is striking considering that all TBM-IRIS patients were taking prednisone (1.5 mg/kg/day) at time of ART initiation and 13 of these patients (81%) were taking prednisone (0.75–1.5 mg/kg/day) at the time of developing TBM-IRIS. Adjunctive corticosteroids have been shown to reduce mortality in TBM and tuberculosis pericarditis, presumably by reducing pathological host immune responses [21, 29]. In paradoxical tuberculosis IRIS, the symptomatic benefit of corticosteroids was demonstrated in a randomized trial in which prednisone was compared to placebo [30]. For these reasons, we anticipated that prednisone would decrease the risk of tuberculosis IRIS.

TBM-IRIS was associated with a poor outcome; 2 patients (13%) died as a result of TBM-IRIS, all-cause mortality at 9 months was 25%, and 3 of 11 (27%) survivors examined at 9 months' follow-up were severely disabled, compared with no deaths and 3 of 17 (18%) patients with severe morbidity in the non-TBM-IRIS group. Our findings are similar to a previous study performed in neurological tuberculosis IRIS [8]. The poor outcome in at least 44% of TBM-IRIS cases emphasizes

the need to predict and prevent, and improve the treatment of, TBM-IRIS.

Low serum sodium concentration is associated with death in HIV-associated TBM [31]. In our study, serum sodium concentration was lower in TBM-IRIS patients compared with non-TBM-IRIS patients at the time of TBM diagnosis. This may reflect the higher degree of tuberculosis dissemination observed in TBM-IRIS patients, which could have contributed to their risk of developing TBM-IRIS.

Our finding of an association between higher CSF neutrophils at TBM presentation and subsequent development of TBM-IRIS provides important and novel insight into the pathogenesis of tuberculosis IRIS. Not only were neutrophil counts higher in TBM-IRIS patients compared with non-TBM-IRIS patients, but neutrophil percentages for individual patients were similarly raised in TBM-IRIS. The neutrophil counts showed dynamic fluctuations over time in TBM-IRIS patients with a marked decrease on tuberculosis treatment, and a striking increase at TBM-IRIS onset. Similar changes in lymphocyte counts were not observed. Studies of tuberculosis IRIS have hitherto focused on the contribution of helper T-cell type 1 lymphocyte responses [32, 33]. However, a role for myeloid cells in tuberculosis IRIS is suggested by a case report of a patient who died from unmasking pulmonary tuberculosis IRIS; postmortem histological examination of diseased lung showed a marked macrophage infiltrate [34]. We have found cytokines of predominantly myeloid origin (IL-6 and TNF-α) to be consistently elevated in patients with tuberculosis IRIS, compared with those who did not develop IRIS [23]. Oliver et al [6] also reported an association between plasma cytokines (interleukin 18) and chemokines (CXCL-10) of the innate immune system and tuberculosis IRIS. In an animal model, immune reconstitution following transfer of mycobacteria-specific CD4 T cells to T-cell–deficient mice infected with *Mycobacterium avium* was associated with marked increases of both blood and lung CD11b cells (likely representing inflammatory monocytes and neutrophils) [35]. Our results suggest that neutrophils contribute to tuberculosis IRIS pathogenesis. The combination of high CSF TNF-α and low IFN-γ concentrations at the time of TBM diagnosis predicted TBM-IRIS in this cohort. Simmons et al [36] reported a negative correlation between CSF IFN-γ and mortality in HIV-infected patients with TBM. Conversely, a positive correlation was found between CSF IFN-γ and TNF-α concentrations and TBM disease severity by others [37].

Several studies have shown an association between disseminated and extrapulmonary tuberculosis and subsequent tuberculosis IRIS [14, 28, 38, 39]. At TBM diagnosis, CSF *M. tuberculosis* culture positivity, which reflects mycobacterial antigen load, was a major risk factor for developing TBM-IRIS (RR = 9.3). Furthermore, 7 TBM-IRIS patients (44%) were persistently CSF *M. tuberculosis* culture positive after 2 weeks of tuberculosis treatment and 2 patients (13%) remained culture positive after 4 weeks of tuberculosis treatment. This strongly supports the inference that a high *M. tuberculosis* bacillary load at time of starting ART is a risk factor for tuberculosis IRIS [40]. The findings suggest it important to optimize tuberculosis treatment prior to starting ART in patients at high risk of developing TBM-IRIS.

We acknowledge several limitations. Because of the relatively small sample size, the study may not have been powered to detect further differences between IRIS and non-TBM-IRIS patients. Only patients with less severe disease (BMRC TBM grade 1 and 2) and those without contraindications to lumbar puncture were enrolled, resulting in the exclusion of a significant proportion of TBM patients presenting in our setting [41]; our results may therefore not be generalizable to ART-naive patients presenting with severe HIV-associated TBM. The model to predict TBM-IRIS needs further validation and exploration with independent data.

In conclusion TBM-IRIS complicated the course of treatment of HIV-associated TBM in nearly half our patients, despite the use of adjunctive corticosteroid therapy. The manifestations were severe, fatal in 2 cases. The occurrence of TBM-IRIS associated with CSF *M. tuberculosis* culture positivity and a high neutrophil count at both baseline and at the time of TBM-IRIS. The baseline relationship between CSF TNF-α and IFN-γ predicted TBM-IRIS. These observations provide novel insight into the pathogenesis of this condition and provide rationale to individualize ART beyond 2 weeks in this devastating, partly iatrogenic, condition.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Chapter 6

Neutrophil-associated central nervous system inflammation in tuberculous meningitis immune reconstitution inflammatory syndrome: A prospective observational study

Introduction

As a consequence of rapid scale-up of antiretroviral therapy (ART) programs, tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is a significant contributor to the health-care burden in high TB/HIV co-infection settings.[1-3] Paradoxical TB-IRIS presents as clinical deterioration after starting ART in TB patients who have improved or stabilized on TB treatment prior to ART initiation.[4] This deterioration occurs in the context of a rapid restoration of *Mycobacterium tuberculosis*-specific immune responses. The central nervous system (CNS) is commonly affected by TB-IRIS with 12-31% of TB-IRIS cases experiencing neurological deterioration.[5-7] Neurological TB-IRIS is the most severe form of TB-IRIS, with an associated mortality of 13-75%,[3, 5-7] as opposed to the 3.2% estimate for all forms of paradoxical TB-IRIS combined.[8]

Although recent studies have advanced our understanding of TB-IRIS through the identification of several cellular, immunological and genetic factors that associate with the syndrome, the immunopathogenesis remains incompletely defined and no diagnostic test exists.[9] Thus far explorations of the immune response in TB-IRIS pathogenesis have been of those measured in the blood compartment, which is likely an incomplete representation of the immune response in affected tissues, e.g. lungs or CNS. The objective of this study was to better understand the pathogenesis of tuberculous meningitis (TBM)-IRIS. We hypothesised that cerebrospinal

fluid (CSF) inflammatory mediator concentrations will be increased in TBM patients who develop TBM-IRIS, compared to patients who do not develop TBM-IRIS.

Methods

Study design, setting and participants

We conducted a prospective, observational study at a public sector referral hospital in Cape Town, South Africa. ART-naïve HIV-infected adults who presented with TBM were recruited over a 20-month period. A detailed description of the clinical methods has previously been published.[3] Tuberculous meningitis was diagnosed according to a published case definition.[10] ART-naïve HIV-infected adults without meningitis who presented with symptoms and/or signs necessitating a lumbar puncture (LP) were enrolled as control participants. The University of Cape Town Human Research Ethics Committee approved the study (232/2008). Additional details on participants are available in the appendix.

Procedure

We investigated serial inflammatory mediators in CSF (40 mediators) and blood (33 mediators) from HIV-associated TBM patients who started ART while on TB treatment. Paired samples were collected from patients who presented with TBM at three to five timepoints shown in Figure 1. Samples were collected at one timepoint in control participants. Samples were stored at -80°C and analyzed in batches as detailed below.

The primary outcome measure was CSF mediator concentrations in TBM patients who did and did not develop TBM-IRIS at time of TBM-IRIS (TBM-

IRIS patients) and two weeks after ART initiation (TBM-non-IRIS patients). The two weeks after ART initiation timepoint in TBM-non-IRIS patients was informed by the median time (14 days after ART initiation) reported for TB-IRIS development.[4] Secondary analyses of CSF and blood results were as follows: i) Baseline results were compared between TBM patients and control patients; ii) Within the TBM group, results were compared between TBM-IRIS and TBM-non-IRIS patients at TBM diagnosis and 2 weeks later (i.e. day of starting ART); Within the TBM-IRIS and TBM-non-IRIS groups, results were compared longitudinally between all timepoints; Within all groups (i.e. TBM, control, TBM-IRIS and TBM-non-IRIS) blood results were compared to CSF results at all timepoints.

Mediators analysed in CSF and serum by Luminex multiplex included: tumor necrosis factor (TNF), interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-10, IL-13, IL-1β, IL-6, IL-12p40, IL-17, IFN-α2, C-C chemokine 2 ligand (CCL2), CCL3, CCL4, C-X-C chemokine ligand 1-3 (CXCL1-3), CXCL8, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage (GM)-CSF. Matrix metalloproteinases (MMP)-1, -2, -3, -7, -9, -10, -12, and -13, and tissue inhibitors of matrix metalloproteinases (TIMP)-1 and -2 were measured in CSF and plasma by Luminex multiplex. Luminex assays were performed in 96-well filter plates on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA) using customized Milliplex™ kits (Millipore, St Charles, MO, USA). Mediators measured by ELISA in CSF and serum included: CXCL10, IL-22 and IL-23 (R & D Systems, Minneapolis, USA); IL-21 (BioLegend, San Diego, USA); and IL-12p70 and IL-17A (high sensitivity assay) (eBioscience, Inc., San Diego, USA). CSF was also analysed by ELISA for IL-18 (Medical and Biological Laboratories Co, Naka-ku Nagoya, Japan) and neutrophil-associated mediators: cathepsin G (DRG

Diagnostics, Marburg, Germany); lipocalin-2 (RayBiotech inc. Norcross, USA); and LL-37, human neutrophil peptides (HNP) 1-3, complement (C) 5a, and S100A8/A9 (Hycult Biotech inc., Uden, The Netherlands). See appendix for supplementary methods.

Statistical analysis

As no TBM-IRIS pilot data were available for statistical powering at study inception, we based our power calculations on previous TBM studies that determined CSF cytokine concentrations at TBM diagnosis[11] and fold reductions in CSF cytokine concentrations on TB treatment.[12] We estimated that for the primary analysis, a sample size of 15 patients in each group (TBM-IRIS and TBM-non-IRIS) was needed to detect a 2·1 fold difference in mediator concentrations, with β=0·8 and α=0·05. Statistical analysis was performed using GraphPad Prism version 5 (Graphpad software, inc.) and R version 3·0.[13] Variables were compared between groups using Wilcoxon rank-sum tests. Variables between timepoints, and between blood and CSF compartments, within groups were compared using Wilcoxon matched-pairs tests. Correlations were estimated by Kendall's tau (τ). Unsupervised agglomerative hierarchical clustering using complete linkage and Euclidean distance measure on all included mediators in CSF and individuals was performed. Clusterwise stability was assessed by two resampling schemes (bootstrap and subsetting; 100 resampling runs) and the Jaccard similarities computed.[14, 15] Throughout, an unadjusted p-value of less than 0·05 was used as a nominal threshold for statistical significance, except for the correlation analysis which reports false discovery rate-adjusted (Benjamini and Hochberg) p-values.[16] Due to the large number of statistical tests the p-value should be used for guidance in interpretation rather than finality. See appendix for details on multivariate analysis.

Role of the funding source

The sponsors had no role in study design; data collection, analysis and interpretation; writing of the manuscript or the decision to submit for publication and accept no liability whatsoever in this regard. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

Results

Details of recruitment, clinical characteristics and follow-up were previously reported for the TBM group.[3] Eighteen patients with no meningitis were enrolled as controls. These patients were seen once (at presentation) only. Due to limited space on mediator analysis plates, 4/18 control patients were excluded from the analysis. To match the CD4 counts of TBM patients, the 14 controls with the lowest CD4 counts were selected for analysis. The demographic characteristics and baseline blood results of TBM (n=34) and control (n=14) patients are presented in Table 1. Sixteen TBM patients developed TBM-IRIS, a median of 14 days (IQR, 4-20) after starting ART, whilst 18 did not.[3] Of note, 15/16 TBM-IRIS and 6/18 TBM-non-IRIS patients cultured *M. tuberculosis* from CSF at TBM diagnosis.

IL-2, IL-4, IL-12p70, IL-13, IL-21, IL-23, MMP-12, and MMP-13 were excluded from all analyses due to minimal or no detection in CSF and blood at all timepoints (see appendix for further details). When comparing TBM patients (i.e. TBM-IRIS and TBM-non-IRIS combined) to patients with no meningitis at presentation, TBM patients had significantly higher ($p<0{\cdot}05$) CSF concentrations of 28/32 mediators; only CCL2 and IL-17 were similar, and IL-18 and C5a had medians equal to zero in both groups (appendix

Table S2). Conversely, only 6/25 mediators in blood were different between groups; IFN-γ, IL-6, CXCL8, CXCL10, and MMP-3 concentrations were higher in TBM patients ($p<0{\cdot}05$), whilst CCL2 was higher in controls ($p=0{\cdot}045$).

Within the TBM group at the time of TBM diagnosis, cytokine and chemokine concentrations were significantly higher in CSF than in blood with the exception of IL-12p40, which showed comparable levels in blood and CSF, and CXCL1-3, which showed a higher trend ($p=0{\cdot}056$) in blood (appendix Table S2). Conversely, of the MMP, MMP-1, -2 -3, -7 and -10 levels were significantly higher in blood compared to CSF, and only MMP-9 as well as its inhibitor TIMP-1, were increased in CSF relative to blood ($p<0{\cdot}001$). Within the control group, blood cytokine, chemokine, MMP, and TIMP concentrations were either similar to, or increased compared to CSF concentrations, with the exceptions of G-CSF, IFN-α2, CCL2, CCL3, and CXCL8 that were higher in CSF. Consistent with the combined TBM group, a highly compartmentalized inflammatory response was seen in both subgroups when dividing the TBM group into those who did and did not subsequently develop TBM-IRIS (Figure 2 and appendix Tables S3, S4 and S5).

At the time of TBM diagnosis the group who subsequently developed TBM-IRIS showed significantly increased CSF Th1 (IFN-γ, IL-18) and other pro-inflammatory cytokines (TNF, IL-6, IL-1β, G-CSF and GM-CSF), IFN-α2, chemokines (CCL2, -3, -4 and CXCL1-3, -8, -10), neutrophil-associated mediators (HNP1-3, lipocalin-2, C5a and S100A8/A9), MMP-1, -7 and -10, and TIMP-1 and -2, compared to TBM patients who did not develop TBM-IRIS (Figure 2 and appendix Table S3). Figure 3 and appendix Table

S6 show the changes in CSF mediator concentrations over time for TBM-IRIS and TBM-non-IRIS patients. In both groups there was a significant decrease of the majority of mediators tested in CSF during TB treatment prior to starting ART. However, IL-12p40 and IL-17A showed a significant increase from baseline in TBM-IRIS, but not in TBM-non-IRIS patients, prior to starting ART.

At TBM-IRIS presentation (compared to two weeks after ART initiation in TBM-non-IRIS patients), TBM-IRIS patients showed significantly higher CSF concentrations for all mediators (31/32) except IFN-α2 (Figure 2 and appendix Table S5). ART was associated with marked rises in CSF inflammatory mediator concentrations in TBM-IRIS patients, despite adjunctive corticosteroid therapy (13/16 patients were still receiving prednisone), such that concentrations reached similar or higher levels at TBM-IRIS presentation, approximately two weeks after ART initiation, compared to those at TBM presentation (appendix Table S6A). Conversely, in TBM-non-IRIS there were no significant CSF changes observed between starting ART and two weeks thereafter (appendix Table S6B). Moreover, when comparing findings between samples taken two weeks after starting ART to those from TBM diagnosis in TBM-non-IRIS, a significant decrease in CSF concentrations of G-CSF, GM-CSF, CCL3, CCL4, TNF, IFN-γ, IL-6, CXCL8, CXCL10, lipocalin-2, S100A8/A9, MMP-10, and TIMP-1 was noted. This suggests that the rise in CSF mediators observed in TBM-IRIS two weeks following ART reflected IRIS development, not simply ART prescription.

Samples taken two weeks after TBM-IRIS presentation were available for analysis in 10/16 TBM-IRIS patients as death (n=1) and contra-indication

to LP (n=5) precluded CSF sampling in the others. Compared to mediator concentrations at TBM-IRIS presentation (when prednisone was restarted or the dose increased), the only significant changes in CSF mediators were decreased concentrations of G-CSF (p=0·039) and LL-37 (p=0·016), two weeks post TBM-IRIS.

At TBM diagnosis, blood concentrations were similar between TBM-IRIS and TBM-non-IRIS with the exception of IFN-α2 (p=0·030), CXCL8 (p=0·0049), and MMP-1 (p=0·037), which were higher in TBM-IRIS patients (Figure 2 and appendix Table S3). CXCL8 remained elevated in TBM-IRIS at time of starting ART (p=0·017; Figure 2 and appendix Table S4). Two weeks after starting ART, CCL4, TNF, IFN-γ, MMP-7 (p-value for all<0·05, >0·01), and CXCL8 and CXCL10 (p-value for both <0·01, >0·001) were elevated in blood of TBM-IRIS compared to TBM-non-IRIS patients, whilst TIMP-2 was higher in TBM-non-IRIS (p=0·046, Figure 2 and appendix Table S5).

Unsupervised hierarchical clustering of TBM patients by CSF mediators showed that TBM-non-IRIS patients who were culture positive for *M. tuberculosis* from CSF at TBM diagnosis tended to cluster with TBM-IRIS patients at all timepoints (Figure 4 and Jaccard similarity indexes are shown in appendix Table S7). TBM-non-IRIS patients who were culture-positive showed mediator profiles more similar to TBM-IRIS patients than to culture-negative TBM-non-IRIS patients, at TBM diagnosis (appendix Table S8), and further had increasing concentrations of a number of mediators after starting ART (Figure 3). Appendix Table S9 shows the similarities between CSF findings in CSF culture-positive TBM-non-IRIS patients and TBM-IRIS patients, and the differences between culture-positive and culture-negative TBM-non-IRIS patients, two weeks after starting ART. Of note,

neutrophil counts and S100A8/A9 distinguished culture-positive TBM-non-IRIS patients from TBM-IRIS patients: neutrophils differentiated them at baseline (median [IQR], 3 [0-14] vs 38 [11-117] cells x 10^6/L, p=0·020) and at two weeks after ART initiation (2 [0-11] vs 52 [17-244] cells x 10^6/L, p=0·0027), while S100A8/A9 differentiated them two weeks after ART (15346 [0-19152] vs 33500 [27000-48000] pg/ml, p=0·001).

Correlation analyses showed no biologically significant correlations between neutrophil and lymphocyte counts and mediator concentrations in CSF of TBM-IRIS and TBM-non-IRIS patients over time (appendix Figures S1-3 and Table S10).

Discussion

This is the only comprehensive analysis to date of serial CSF and blood immune mediators in TBM-IRIS. Neutrophils, lymphocytes and total protein concentrations,[3] as well as 31/32 analysed mediators (all except IFN-α2), were elevated in TBM-IRIS compared to TBM-non-IRIS patients two weeks after starting ART. This widespread upregulation of diverse mediators of diverse cellular functions suggests that both innate and adaptive immune responses are involved in TBM-IRIS pathogenesis.

Adjunctive corticosteroid treatment is associated with reduced short-term mortality in HIV-uninfected patients with TBM,[17] and symptomatic improvement in TB-IRIS.[1] In our study, an increased inflammatory response was observed after ART initiation in TBM-IRIS patients despite adjunctive corticosteroid therapy. Furthermore, a decrease of only two mediators was observed two weeks after corticosteroids were increased or restarted at TBM-IRIS presentation. Previous studies in TBM patients also found little

effect of corticosteroids on CSF cytokine or chemokine concentrations.[12] Cerebrospinal fluid concentrations of MMP-9 were shown to decrease in patients on dexamethasone compared to controls early during the treatment of TBM and it was postulated that this may represent a mechanism by which corticosteroids improve outcome in these patients.[18] However, in our study MMP-9 did not change after TB treatment (plus corticosteroid) initiation in either TBM-IRIS or TBM-non-IRIS patients, and increased significantly after ART initiation in the TBM-IRIS group. These findings suggest that immunomodulatory treatment options more potent and specific than corticosteroids need to be derived for the prevention and/or management of TBM-IRIS.

As previously reported for extra-pulmonary TB, including TBM,[11] pleural[19] and pericardial TB,[20] a highly compartmentalized inflammatory response in CSF was seen in patients with TBM. Relatively fewer differences between TBM-IRIS and TBM-non-IRIS groups were observed for mediators in blood compared to the differences observed in CSF. Corticosteroids do modulate blood inflammatory responses in TB/HIV co-infected persons[21, 22] and likely further attenuated differences between these groups subsequent to TBM presentation once patients were started on TB treatment and corticosteroids.

High baseline CSF mycobacterial load (reflected by *M. tuberculosis* culture positivity) is a risk factor for subsequent TBM-IRIS in TBM patients.[3] This is similar to cryptococcal meningitis (CM)-IRIS where high CSF fungal loads (reflected by quantitative culture) at time of CM diagnosis also predict subsequent IRIS.[23] However, unlike the highly inflammatory baseline presentation being predictive of TBM-IRIS, paradoxical CM-IRIS

is predicted by a paucity of inflammation at CM diagnosis as evidenced by a lack of CSF leukocytes and/or normal protein level [23,24] and lower serum levels of pro-inflammatory cytokines and chemokines (IL-6, CXCL8, TNF, and IFN-γ).[24] These differing findings in TBM and CM suggest more than one pathogenic mechanism underlies neurological forms of IRIS.

In this study TBM-non-IRIS patients who were culture-positive for *M. tuberculosis* from CSF at TBM diagnosis tended to have higher baseline mediator concentrations than culture-negative TBM-non-IRIS patients and showed a recurrent inflammatory response after ART initiation, reaching levels of magnitude comparable to those in TBM-IRIS two weeks after ART initiation. These increases in mediator concentrations were not seen in culture-negative TBM-non-IRIS patients. Drug choice, doses and the route of administration in TBM are based on the same principles as pulmonary TB, rather than being informed by randomized controlled trials in TBM.[25] Our data supports increased research into antimicrobial regimens containing higher-than-normal dose rifampicin as well as a fluoroquinolone.[26, 27] Given that mycobacterial load drives inflammation on ART it is plausible that improving early mycobacterial clearance from the CNS in HIV-associated TBM patients through a more potent TBM drug regimen may decrease the risk of TBM-IRIS.

Although initial studies explored the role of lymphocytes in TB-IRIS pathogenesis, increasing evidence implicates the innate immune system as an important contributor,[9] and it is proposed that neutrophils mediate TB pathology.[28] Based on these results and the association we found between CSF neutrophils and TBM-IRIS,[3] we investigated neutrophil-associated mediators in the CSF of our cohort. S100A8/A9, HNP 1-3,

lipocalin-2 and C5a were increased at TBM diagnosis, and all measured neutrophil-associated mediators were increased two weeks after starting ART, in TBM-IRIS compared to TBM-non-IRIS patients. Unlike the other neutrophil-associated mediators but similar to neutrophils, S100A8/A9 was also increased in TBM-IRIS compared to TBM-non-IRIS patients with baseline CSF culture-positivity two weeks after ART initiation, when TBM-IRIS usually presents. Given these findings, we hypothesise that S100A8/A9, through its interaction with neutrophils, contributes to the recurrent CSF inflammatory response that manifests as clinical deterioration in patients with TBM-IRIS. S100A8/A9 is a protein complex that appears to have prominent immune regulatory properties, such as neutrophil chemoattraction and stimulation.[29] Several studies have shown that serum S100A8/A9 is increased in HIV-uninfected pulmonary TB patients compared to controls, and that concentrations correlated with radiographic severity.[30, 31] In a murine model of TB, IL-17-induced S100A8/A9 was a key factor in neutrophil accumulation and exacerbated lung inflammation through inducing pro-inflammatory cytokines.[31] Given that IL-17 may therefore be important in TBM-IRIS pathogenesis, but considering the low concentrations previously found during *in vivo* studies in blood[20, 32] as well as site-of-disease samples (i.e. pericardial and pleural fluid)[20] in TB patients, we measured IL-17A with a high sensitivity assay. Cerebrospinal fluid IL-17A concentrations increased significantly over time in the TBM-IRIS group, reaching concentrations more than eight-fold that of TBM diagnosis at TBM-IRIS presentation; while an opposite trend was seen in TBM-non-IRIS patients. These findings support previous suggestions that IL-17 may be important in TB-IRIS pathogenesis.[32]

The role of MMP as key mediators of TB pathology has attracted interest

recently,[33] and their association with TB-IRIS has been reported.[34] MMP degrade extracellular matrix components,[35] and their activity is regulated by synchronously expressed TIMP.[36] In our study, MMP-1 and -9 and TIMP-1 and -2 were persistently elevated in the CSF of TBM-IRIS compared to TBM-non-IRIS patients at all timepoints. Furthermore, all MMP as well as the MMP-9:TIMP-1 ratio showed a significant increase after starting ART in CSF of TBM-IRIS patients. In a previous study of TB-IRIS patients, MMP-7 was elevated in serum from TB-IRIS patients *in vivo*.[34] We hypothesize that MMP contribute to the excessive CSF inflammation observed in TBM-IRIS patients; evidence suggests that MMP not only facilitate blood-brain-barrier disruption, which results in leucocyte influx,[37,38] but also upregulate microglial secretion of cytokines.[39] Furthermore, elevated CSF MMP-9[40-42] and MMP-2[40] concentrations and increased MMP-9:TIMP-1 ratios[41] have been associated with poorer outcome, including death[42] and neurological complications, in TBM patients.[40, 41]

While this is the first comprehensive analysis hitherto of disease site coupled with blood immune mediators in TB-IRIS, we acknowledge certain limitations. We did not include HIV-uninfected patients with TBM as controls and we excluded patients with neurological deterioration prior to starting ART. We were therefore unable to compare the inflammatory response in TBM-IRIS to that of the "paradoxical reaction" characterized by neurological deterioration and which can occur in both HIV-infected and -uninfected patients with TBM after starting TB treatment.[43] This is a descriptive study of mediators in TBM patients over time; cellular contributions to mediator production in CSF and blood compartment were not determined by functional experiments and will be the focus of future studies.

Our findings have several important implications. Firstly, adjunctive oral corticosteroid therapy during TBM treatment is insufficient to prevent, or to rapidly reduce, the marked inflammatory response that characterizes TBM-IRIS; in addition to investigations of different corticosteroid doses and routes of administration in the management of severe TB-IRIS cases by randomized trials, alternative immunomodulatory therapies should be explored for the prevention and treatment of the disease. Secondly, although the adaptive immune system appears to be activated in TBM-IRIS, the sequence of immunological events in the CNS suggests a major contribution of the innate response in this condition. CSF neutrophils, which may be driven through a IL-17 and S100A8/A9-dependent pathway, associate with the most severe CNS inflammation manifesting as TBM-IRIS; these findings are likely to direct future research into TB-IRIS immunopathogenesis and management strategies.

Contributors

G.M., R.J.W., S.M. and D.J.P. designed the clinical study, S.M., G.M., Z.I., C.S., and D.J.P. assessed participants and collected specimens; R.J.W., S.M., and K.A.W. designed the laboratory experiments; S.M. and K.A.W. performed the laboratory experiments; S.M., K.A.W., G.M., A.K.C., M.L., A.D., and R.J.W. analysed and interpreted the data; M.L. and S.M. generated the figures; S.M. wrote the first draft; and K.A.W., G.M., Z.I., C.S., D.J.P., A.K.C., M.L., A.D., and R.J.W. critically revised the manuscript and approved the final version.

Conflicts of interest

We have no conflicts of interest to declare.

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Panel: Research in context

Systematic review

We searched Pubmed up to Dec 2013, with no date limits set, with the terms " tuberculosis and immune reconstitution inflammatory syndrome", "tuberculosis and immune restoration disease", tuberculosis-associated immune reconstitution syndrome", "tuberculous meningitis", and "neurological tuberculosis". We also searched reference lists of identified papers. We included studies published in English that assessed tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) disease and those that assessed immunopathogenic mechanisms of TB-IRIS and tuberculous meningitis (TBM). We only included studies for which we had access to the full text version. A systematic review of the immunopathogenesis of TB-IRIS performed by our group was

recently published.[9] Systematic review of the literature further revealed that, although several studies have investigated blood immune markers in TB-IRIS, only one study of site-of-disease findings in TB-IRIS exists.[3] This study, performed on the same cohort of TBM patients presented in this manuscript, suggested an important role for neutrophils and baseline cerebrospinal fluid mycobacterial load in TBM-IRIS pathogenesis.

Interpretation

Aside from our previous publication,[3] we know of no other study to investigate inflammatory markers from the site-of-disease in patients with TB-IRIS. Findings from this study contribute significantly to knowledge of the immunopathogenic process that occurs in the central nervous system of patients with TBM-IRIS; our results extend data from our previous study by providing additional evidence for a prominent role of the innate immune system in TB-IRIS pathogenesis. These results are likely to guide future immunopathogenic, and ultimately therapeutic studies in TB-IRIS, regardless of the disease site.

Figure legends and footnotes

Figure 1. Flow diagram of timepoints when lumbar puncture and phlebotomy were performed in tuberculous meningitis (TBM) patients.
Drug interventions are indicated at each timepoint (grey boxes)
Procedures were performed at a minimum of three timepoints: 1) TBM diagnosis; 2) antiretroviral treatment (ART) initiation; and 3) two weeks after ART initiation or TBM-IRIS presentation, which ever occurred first. Patients who developed TBM-IRIS after the "two weeks post ART initiation" timepoint underwent repeat procedures at TBM-IRIS presentation. Unless a lumbar puncture was contra-indicated, procedures were repeated in TBM-IRIS patients two weeks after TBM-IRIS presentation.

Figure 2. Boxplots of mediator concentrations over time in A) cerebrospinal fluid and B) blood [†] **of patients who developed TBM-IRIS (red)* and those who did not (blue).**
Definitions of abbreviations: TBM, tuberculous meningitis; ART, antiretroviral therapy; wks; weeks
The assays' limits of detection have been substituted for zero values. Note the left y-axis is a log 10 scale. The right y-axis indicates timepoints of sample collection. Within graphs, boxes with horizontal lines represent interquartile ranges and medians. Raw data points are overlaid. See Tables E3, E4 and E5 for p-values of analyses between groups.
[†] Blood mediators with medians equal to zero at all timepoints in both groups are not shown.
* For patients who developed TBM-IRIS, the "2 weeks post ART" timepoint indicates findings at time of TBM-IRIS that developed at a median of 14 days (interquartile range, 4-20) after starting ART.

Concentrations of all mediators were measured as picograms per milliliter with the exception of Cathepsin G, which was measured in units per milliliter.

Figure 3. Representative examples of changes over time of cerebrospinal fluid (CSF) mediators in patients who developed TBM-IRIS (n=16, red) and those who did not (n=18, blue).

Definitions of abbreviations: TBM, tuberculous meningitis; ART, antiretroviral therapy

For patients who developed TBM-IRIS, the "2 weeks post ART" timepoint indicates findings at time of TBM-IRIS that developed at a median of 14 days (interquartile range, 4-20) after starting ART.

Note that some TBM-non-IRIS patients showed an increase in mediator concentration and MMP-9:TIMP-1 ratio after starting ART; these patients were those who were culture positive for *M. tuberculosis* from CSF at time of TBM diagnosis.

Figure 4. Unsupervised hierarchical clustering of tuberculous meningitis (TBM) at A) TBM diagnosis, B) ART start and C) TBM-IRIS presentation or 2 weeks after ART (non-TBM-IRIS patients).

IRIS status: Indicates patients who developed TBM-IRIS in red and those who did not in blue.

TB Culture status: Black and green boxes indicate cerebrospinal fluid *M. tuberculosis* culture positivity and negativity, respectively at time of TBM diagnosis.

Results from cerebrospinal fluid analyses are reported. Values plotted are natural log plus one. Corresponding concentrations in picograms per milliliter are indicated on the color key. Concentrations of all mediators were measured as picograms per milliliter with the exception of Cathepsin

G, which was measured in units per milliliter. Neutrophils and lymphocytes were measured as cells x 10^6/L.

Table 1. Baseline characteristics of patients presenting with tuberculous meningitis and controls without meningitis

	Tuberculous meningitis (n=34)		**No meningitis** * (n=14)		p-value
Age, years	33	(28-44)	34	(27-38)	0.95
Female, n (%)	15	(44)	9	(64)	0.34
Body mass index	20.0	(18.3-22.7)	20.5	(19.9-27.3)	0.17
Blood investigations					
Sodium (mmol/L)	129	(123-131)	135	(133-137)	<0.0001
Hemoglobin (g/dl)	11.4	(8.8-13.1)	12.4	(10.8-13.4)	0.10
C-reactive protein (mg/L)	40	(6-78)	5	(1-8)	0.0052
CD4 count (cells/μL)	113	(69-199)	129	(75-180)	0.91
HIV viral load (log_{10})	5.46	(4.82-5.89)	4.87	(4.44-5.44)	0.061
Cerebrospinal fluid (CSF) investigations					
Lymphocyte count (x 10^6/L)	177	(87-339)	6	(2-13)	<0.0001
Neutrophil count (x 10^6/L)	20	(2-42)	0	(0-0)	<0.0001
Protein (g/L)	1.94	(1.29-3.06)	0.51	(0.38-0.87)	<0.0001
Glucose (CSF:blood ratio)	0.30	(0.17-0.5)	0.53	(0.5-0.74)	0.0002

* Diagnoses include: HIV-associated psychosis (n=4), tension headache (n=4), generalized tonic-clonic seizures (n=2), meningioma (n=1), stroke (n=1), depression (n=1) and HIV-associated neurocognitive disorder (n=1). Median and interquartile range is presented unless otherwise specified. P-values were calculated for comparisons between groups using Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. A p-value <0.05 was considered statistically significant.

Figure 1

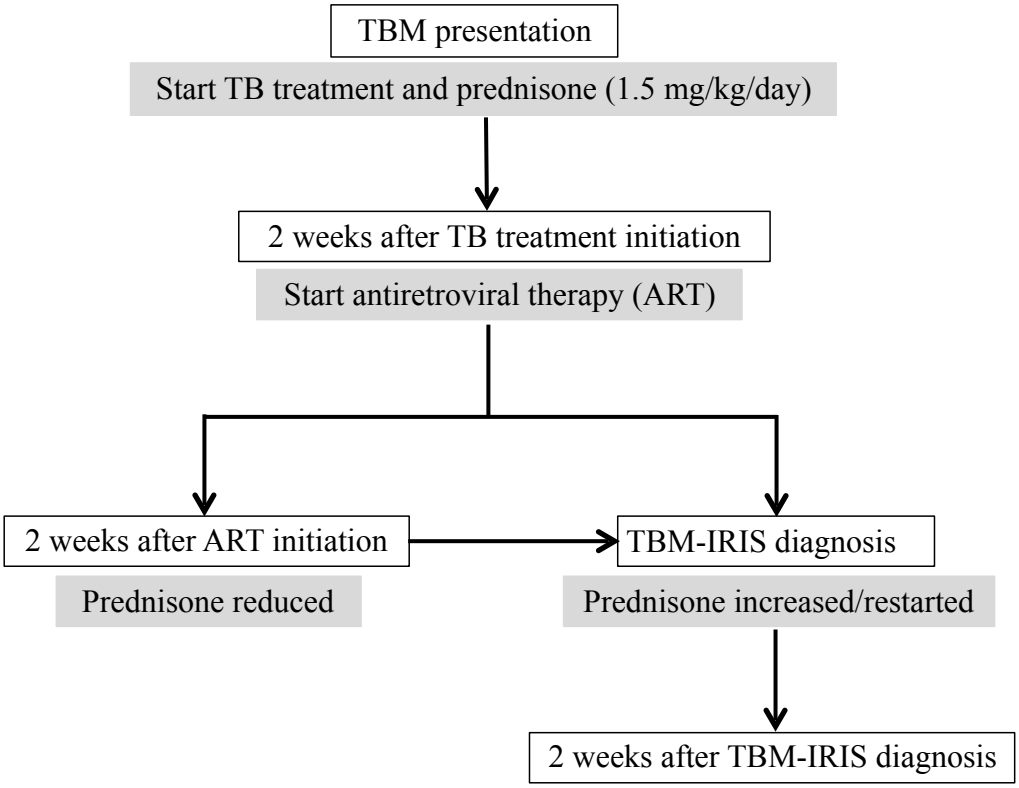
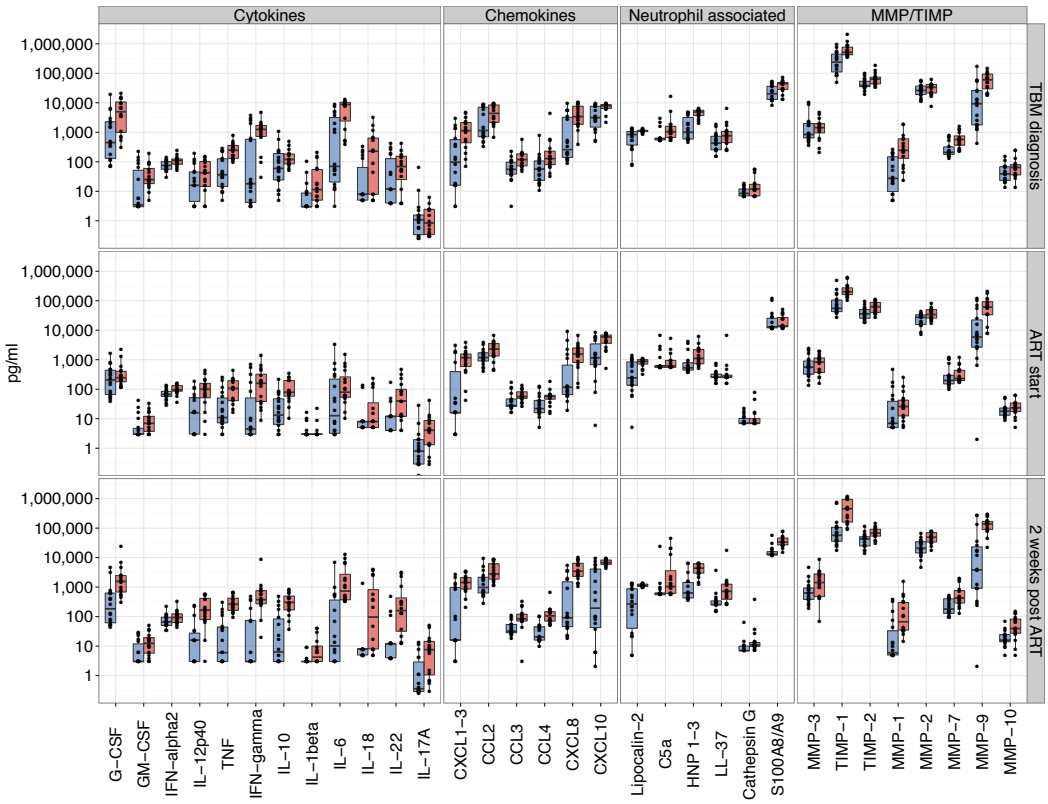

TBM presentation
Start TB treatment and prednisone (1.5 mg/kg/day)
2 weeks after TB treatment initiation
Start antiretroviral therapy (ART)
2 weeks after ART initiation
Prednisone reduced
TBM-IRIS diagnosis
Prednisone increased/restarted
2 weeks after TBM-IRIS diagnosis

Figure 2

A


Cytokines
Chemokines
Neutrophil associated
MMP/TIMP
TBM diagnosis
ART start
2 weeks post ART
pg/ml
1,000,000
100,000
10,000
1,000
100
10
1
G−CSF
GM−CSF
IFN−alpha2
IL−12p40
TNF
IFN−gamma
IL−10
IL−1beta
IL−6
IL−18
IL−22
IL−17A
CXCL1−3
CCL2
CCL3
CCL4
CXCL8
CXCL10
Lipocalin−2
C5a
HNP 1−3
LL−37
Cathepsin G
S100A8/A9
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TIMP−1
TIMP−2
MMP−1
MMP−2
MMP−7
MMP−9
MMP−10

B

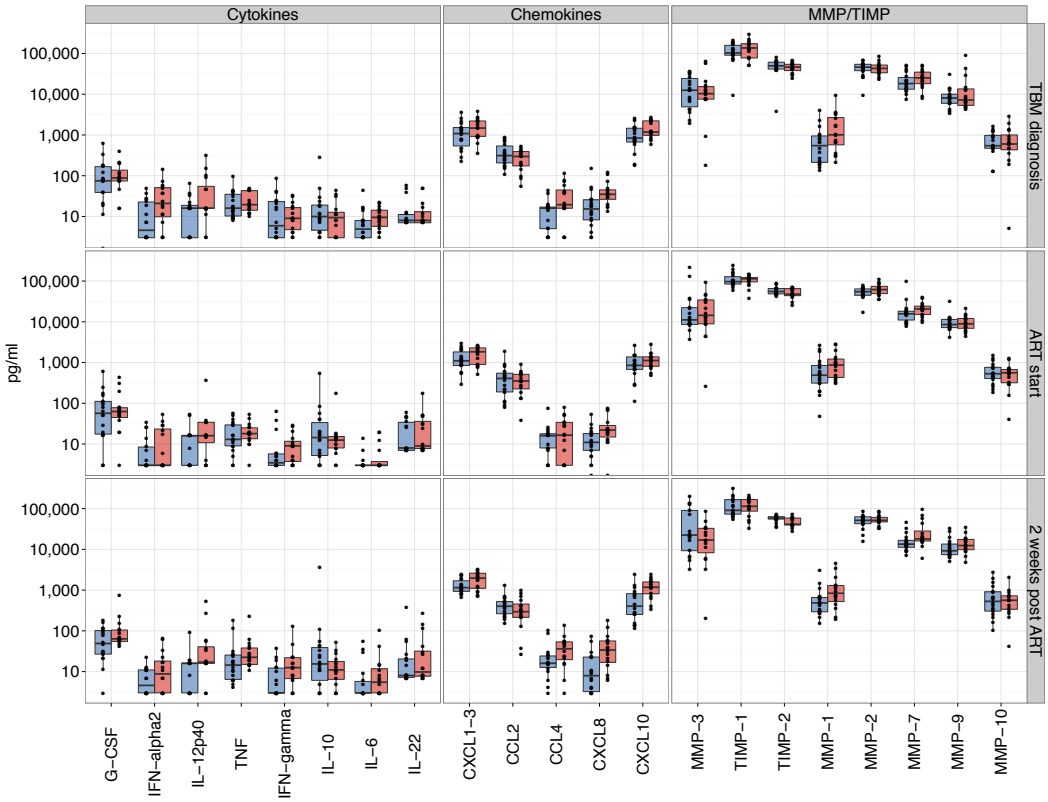

Cytokines
Chemokines
MMP/TIMP
TBM diagnosis
ART start
2 weeks post ART
pg/ml
100,000
10,000
1,000
100
10
G−CSF
IFN−alpha2
IL−12p40
TNF
IFN−gamma
IL−10
IL−6
IL−22
CXCL1−3
CCL2
CCL4
CXCL8
CXCL10
MMP−3
TIMP−1
TIMP−2
MMP−1
MMP−2
MMP−7
MMP−9
MMP−10

Figure 3

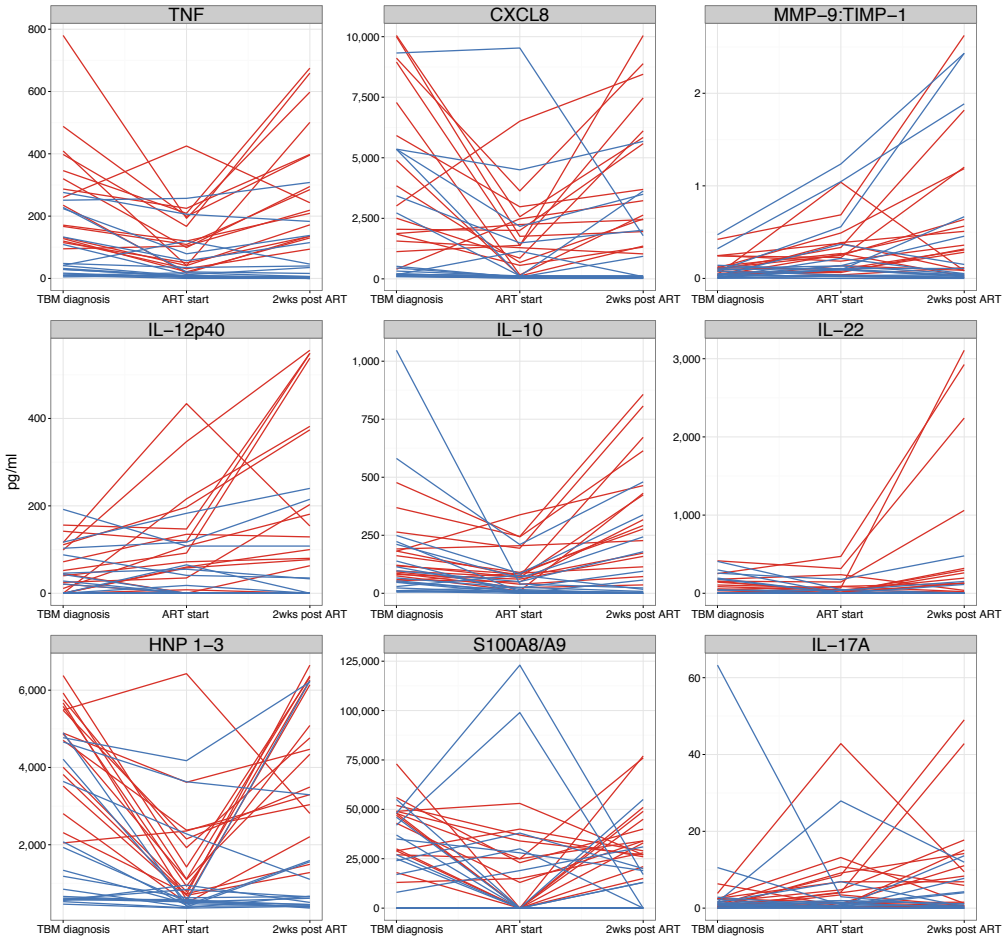
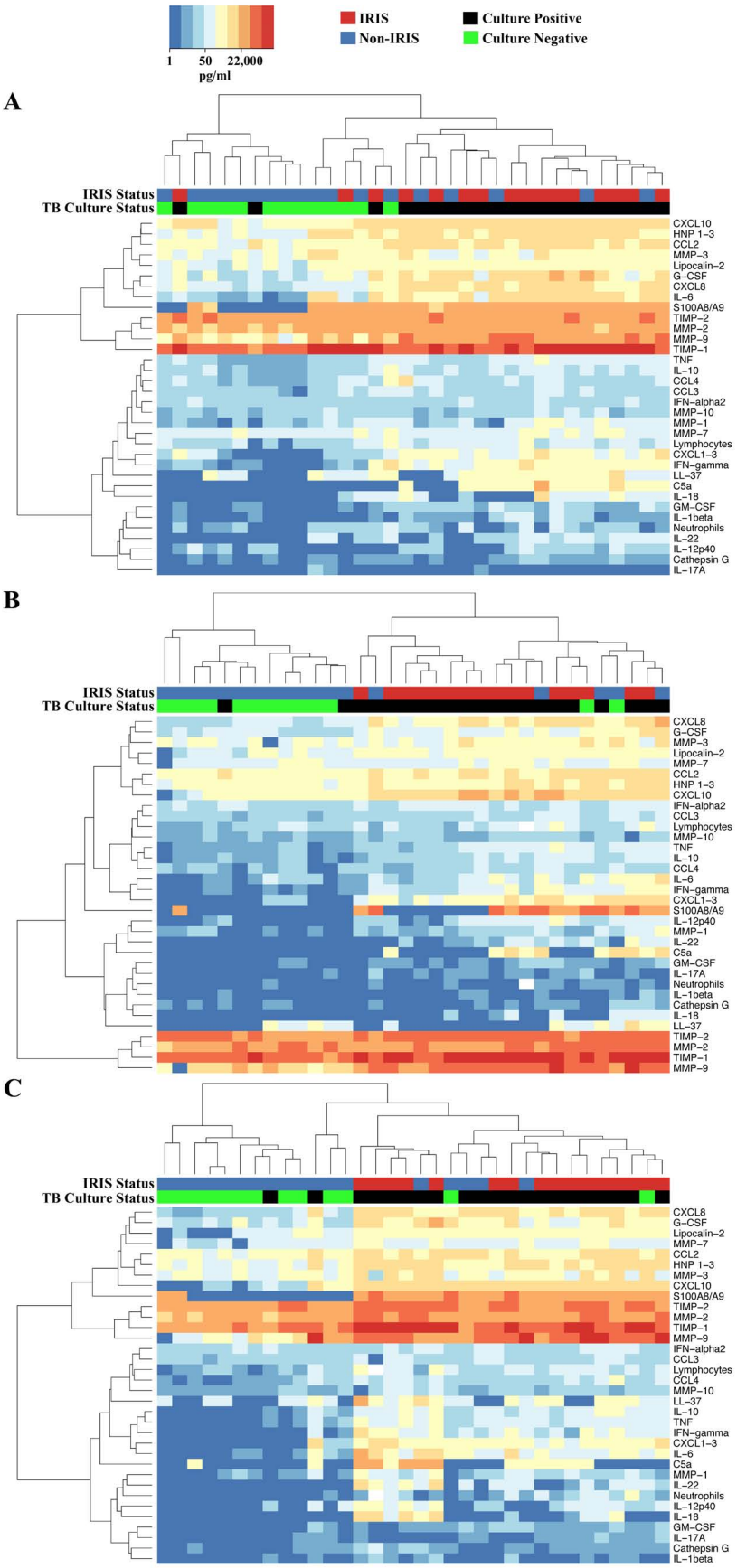

TNF
CXCL8
MMP−9:TIMP−1
IL−12p40
IL−10
IL−22
HNP 1−3
S100A8/A9
IL−17A
pg/ml
TBM diagnosis
ART start
2wks post ART

Figure 4


IRIS
Non-IRIS
Culture Positive
Culture Negative
1
50
22,000
pg/ml
A
IRIS Status
TB Culture Status
CXCL10
HNP 1–3
CCL2
MMP–3
Lipocalin–2
G–CSF
CXCL8
IL–6
S100A8/A9
TIMP–2
MMP–2
MMP–9
TIMP–1
TNF
IL–10
CCL4
CCL3
IFN–alpha2
MMP–10
MMP–1
MMP–7
Lymphocytes
CXCL1–3
IFN–gamma
LL–37
C5a
IL–18
GM–CSF
IL–1beta
Neutrophils
IL–22
IL–12p40
Cathepsin G
IL–17A
B
IRIS Status
TB Culture Status
CXCL8
G–CSF
MMP–3
Lipocalin–2
MMP–7
CCL2
HNP 1–3
CXCL10
IFN–alpha2
CCL3
Lymphocytes
MMP–10
TNF
IL–10
CCL4
IL–6
IFN–gamma
CXCL1–3
S100A8/A9
IL–12p40
MMP–1
IL–22
C5a
GM–CSF
IL–17A
Neutrophils
IL–1beta
Cathepsin G
IL–18
LL–37
TIMP–2
MMP–2
TIMP–1
MMP–9
C
IRIS Status
TB Culture Status
CXCL8
G–CSF
Lipocalin–2
MMP–7
CCL2
HNP 1–3
MMP–3
CXCL10
S100A8/A9
TIMP–2
MMP–2
TIMP–1
MMP–9
IFN–alpha2
CCL3
Lymphocytes
CCL4
MMP–10
LL–37
IL–10
TNF
IFN–gamma
CXCL1–3
IL–6
C5a
MMP–1
IL–22
Neutrophils
IL–12p40
IL–18
GM–CSF
IL–17A
Cathepsin G
IL–1beta

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Supplementary Data for Chapter 6

Supplement-Methods

Participants

ART-naïve HIV-infected adults (≥ 18 years) who presented with tuberculous meningitis (TBM) were recruited from March 2009 to October 2010. Lumbar puncture (LP) was repeated within 48 hours of TBM presentation in patients who had already a diagnostic LP performed. Definite TBM was retrospectively diagnosed when Mycobacterium tuberculosis was cultured from cerebrospinal fluid (CSF). Probable TBM was diagnosed when a patient showed clinical, laboratory or radiological features of TBM in the absence of other infective causes for presentation including bacteria, fungi, syphilis and if suspected, viruses.[1] Paradoxical TBM-immune reconstitution inflammatory syndrome (IRIS) was diagnosed according to a published definition for TB-IRIS modified for meningitis.[2,3] The definition had 3 components: (1) TBM diagnosis before starting ART and improvement on TB treatment prior to ART initiation; (2) onset of TBM-IRIS manifestations (ie, new, recurrent, or worsening clinical features of TBM) within 3 months of ART initiation; and (3) exclusion of alternative causes for clinical deterioration. As control participants, we enrolled HIV-infected ART-naïve adults (≥ 18 years) without a meningitis diagnosis who presented with symptoms and/or signs necessitating a lumbar puncture such as headache, cognitive decline or seizures. HIV infection itself is often associated with a mild to moderate CSF lymphocytic pleocytosis;[4] we therefore did not exclude patients with a mild lymphocytosis (up to 25 cells x 10^6/L) as controls. Written informed consent was obtained from all patients or their relatives, if a patient was unable to provide informed consent.

Luminex multiplex and ELISA assays

All assays were performed according to manufacturer's instructions. For Luminex multiplex experiments, all CSF and blood samples from an individual patient were assayed on the same plate. Mediator concentrations were calculated with reference to a standard curve for each mediator derived from a range of concentrations of mediator standards assayed in the same manner as CSF and blood samples. Manufacturer supplied internal controls were used to validate standard curves. For ELISA experiments CSF and blood samples were assayed on separate plates. The sensitivities of the assays are presented in Table

S1.

Supplement-Results

Mediators not detected

The median concentrations of IL-4, IL-12p70, IL-13, IL-21, IL-23 and MMP-12 were lower than the assay limit of detection in both blood and CSF in all subgroups (i.e. TBM-IRIS, TBM-non-IRIS and no meningitis patients) at all timepoints, whilst IL-2 (median <7pg/ml in TBM-IRIS patients at TBM diagnosis and starting ART) and MMP-13 (median=12pg/ml in TBM-IRIS patients at TBM diagnosis) were minimally detected only in CSF of TBM-IRIS patients at the indicated timepoints. These mediators were therefore excluded from further analysis.

Statistical analysis

A logistic multivariate model was developed (not shown) to predict TBM-IRIS from analytes measured in CSF at time of TBM diagnosis. The resulting model was extremely unstable due to a high degree of collinearity (as measured by variance inflation factor) and a small number of very high leverage individuals whereby removal resulted in instability in coefficient estimates. The variable selection process was flawed as standard methods (e.g. starting with those variables significant after a univariate test) would rest on a univariate test with low power (due to large proportion of values below the limit of detection). We therefore chose not to report the multivariate model as a supplement to univariate analyses.

Correlation between CSF mediators and white blood cell counts

Figures S1, S2 and S3 show correlation between neutrophil and lymphocyte counts and mediator concentrations measured in CSF in TBM-IRIS and TBM-non-IRIS patients over time. Benjamini and Hochberg adjusted p-values are provided for correlation between cell types and mediators in Table S10.[5] Notably, no significant correlation was observed between either cell type and any mediator in TBM-IRIS patients at any timepoint. A lack of significant correlation between mediators and cells was also observed in TBM-non-IRIS patients at TBM diagnosis. Although a statistically significant correlation between a minority of mediators and cell counts was observed at further timepoints in TBM-

non-IRIS patients, these correlations were unlikely to be of biological significance, rather reflective of multiple zero values in comparator data.

Supplement-Tables and Figures

Supplementary Table 1. Sensitivities of Luminex multiplex and ELISA assays performed on mediators determined in cerebrospinal fluid (CSF) and blood

	CSF	Blood
Performed by LUMINEX multiplex †		
G-CSF	3·2-16	3·2-16
GM-CSF	3·2	3·2
IFN-α2	3·2	3·2
IL-12p40	3·2-16	3·2-16
TNF	3·2	3·2
IFN-γ	3·2	3·2
IL-10	3·2	3·2
IL-1β	3·2	3·2
IL-6	3·2	3·2
IL-17	3·2	3·2
IL-2	3·2	3·2
IL-4	3·2	3·2
IL-13	3·2	3·2
CCL2	3·2	3·2
CCL3	3·2-16	3·2-16
CCL4	3·2-16	3·2-16
CXCL8	3·2	3·2
CXCL1-3	3·2-16	3·2-16
MMP-1	4·9	4·9
MMP-2	12-3125	12-3125
MMP-3	49-195	49-195
MMP-7	98	98
MMP-9	2·4	2·4
MMP-10	4·9	4·9
MMP-12	391-1563	391-1563
MMP-13	9·8	9·8
TIMP-1	19·5	19·5
TIMP-2	48·8	48·8
Performed by ELISA *		
IL-12p70	2·5-6·5	5·1-6·5
IL-17A	0·26-0·29	0·28-0·35
IL-21	22·6-33·9	20·9-26·4
IL-22	3·8-12·3	6·8-7·5
IL-23	32-43·7	5·5-46·7
CXCL10	2·1-6·1	8·3-11·8
IL-18	4·9-7·7	-
C5a	549-617	-
Lipocalin-2	4·9-20·6	-
Cathepsin G	6·9-7·2	-
HNP 1-3	247-360	-
LL-37	156-255	-
S100A8/A9	1150-1300	-

Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter
† For Luminex multiplex experiments, all CSF and blood samples from an individual patient were assayed on the same plate
* For ELISA experiments CSF and blood samples were assayed on separate plates

Supplementary Table 2. Baseline cerebrospinal fluid (CSF) and blood (BI) mediator concentrations in patients with tuberculous meningitis (TBM) and controls with no meningitis

	TBM (n=34)					No meningitis (n=14)						
Mediator	**Blood**		**CSF**		**BI vs CSF**	**Blood**		**CSF**		**BI vs CSF**	**BI vs BI**	**CSF vs CSF**
pg/ml †	median	(IQR)	median	(IQR)	p-value	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	80	(45 -169)	1748	(362-6536)	<0·0001	42	(21-95)	140	(62-231)	0·025	0·084	**< 0·0001**
GM-CSF	0	(0-5)	21	(0-69)	<0·0001	0	(0-1)	0	(0-0)	-	-	**< 0·0001**
IFN-α2	11	(0-37)	97	(62-114)	<0·0001	4	(0-11)	58	(47-70)	0·001	0·15	**0·0013**
IL-12p40	0	(0-34)	23	(0-91)	0·18	0	(0-0)	0	(0-13)	-	-	**0·015**
TNF	18	(12-42)	130	(31-264)	<0·0001	16	(9-32)	6	(3-13)	0·052	0·38	**< 0·0001**
IFN-γ	8	(0-21)	640	(16-1701)	<0·0001	0	(0-0)	0	(0-0)	-	**0·0001**	**< 0·0001**
IL-10	10	(4-19)	96	(51-196)	<0·0001	6	(0-16)	0	(0-18)	1	0·29	**< 0·0001**
IL-1β	0	(0-1)	6	(0-18)	0·0005	0	(0-0)	0	(0-0)	-	-	**0·0011**
IL-6	7	(4-14)	2504	(58-8883)	<0·0001	0	(0-4)	0	(0-6)	-	**0·001**	**< 0·0001**
IL-22	0	(0-12)	42	(0-160)	0·0011	0	(0-34)	0	(0-0)	-	-	**0·0007**
IL-18	-	-	0	(0-250)	-	-	-	0	(0-0)	-	-	-
IL-17A	0	(0-0)	1	(0-25-2·35)	< 0·0001	0	(0-0·10)	0·23	(0-1·58)	0·29	-	0·13
CXCL1-3	1246	(772-2150)	442	(64-1558)	0·056	1156	(551-1882)	0	(0-48)	0·0001	0·55	**< 0·0001**
CCL2	302	(180-412)	2935	(870-7936)	<0·0001	402	(308-612)	1345	(909-3156)	0·0001	**0·045**	0·18
CCL3	0	(0-0)	82	(49-132)	<0·0001	0	(0-0)	24	(22-36)	0·0011	-	**< 0·0001**
CCL4	16	(0-36)	90	(38-151)	<0·0001	18	(0-34)	13	(5-26)	0·78	0·84	**< 0·0001**
CXCL8	24	(12-44)	1959	(204-5353)	<0·0001	9	(5-18)	61	(39-163)	0·0001	**0·0033**	**< 0·0001**
CXCL10	1113	(739-1620)	6844	(2731-8499)	<0·0001	730	(553-1034)	347	(68-1380)	0·30	**0·039**	**< 0·0001**
HNP 1-3	-	-	3578	(803-4892)	-	-	-	526	(462-615)	-	-	**< 0·0001**
LL-37	-	-	568	(0-949)	-	-	-	0	(0-52)	-	-	**0·0003**

Cathepsin G	-	-	11	(0-15)	-	-	-	0	(0-8)	-	-	**0·0078**
Lipocalin-2	-	-	1066	(748-1169)	-	-	-	63	(7-124)	-	-	**< 0·0001**
C5a	-	-	0	(0-1320)	-	-	-	0	(0-0)	-	-	-
S100A8/A9	-	-	29000	(6000-48000)	-	-	-	0	(0-3250)	-	-	**0·0002**
MMP-1	710	(287-1681)	142	(23-269)	< 0·0001	436	(277-626)	2	(0-8)	0·0002	0·14	**< 0·0001**
MMP-2	45490	(33600-53920)	28790	(19410-40760)	0·0006	50140	(46750-55670)	13900	(11990-17540)	0·0002	0·080	**0·0001**
MMP-3	11290	(7258-20430)	1280	(632-2040)	< 0·0001	5807	(2482-10460)	403	(279-660)	0·0002	**0·023**	**< 0·0001**
MMP-7	21260	(14040-34860)	314	(205-642)	< 0·0001	12660	(9526-24930)	150	(74-278)	0·0002	0·058	**0·0017**
MMP-9	7914	(5822-12550)	25390	(7110-69610)	0·0004	10380	(9065-13050)	2821	(585-6352)	0·0034	0·055	**0·0001**
MMP-10	584	(461-1064)	46	(27-75)	< 0·0001	892	(522-1351)	16	(9-23)	0·0002	0·18	**< 0·0001**
TIMP-1	123600	(77620-170900)	420100	(188500-589900)	< 0·0001	109500	(80670-123200)	40150	(22170-79780)	0·0081	0·17	**< 0·0001**
TIMP-2	49800	(40300-58040)	48260	(36330-68640)	0·25	59820	(43900-61980)	33370	(22780-39830)	0·0046	0·085	**0·0012**

Definitions of abbreviations: IQR, interquartile range
P-values for analysis between blood and CSF:
Concentrations significantly ($p<0·05$) increased in CSF compared to blood
Concentrations significantly ($p<0·05$) decreased in CSF compared to blood
P-values for analysis between groups:
Significant ($p<0·05$) differences between groups are indicated in bold
† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter
Results shown are mediator concentrations assayed with Luminex multiplex or ELISA
Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary Table 3. Mediator concentrations in cerebrospinal fluid (CSF) and blood (BI) of TBM-IRIS and TBM-non-IRIS patients at TBM diagnosis

	CSF					Blood					TBM-IRIS	TBM-non-IRIS
Mediator	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**CSF vs CSF**	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**BI vs BI**	**BI vs CSF**	**BI vs CSF**
pg/ml †	median	(IQR)	median	(IQR)	p-value	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	5102	(808-12860)	451	(118-2528)	**0·0035**	88	(73-180)	75	(34-169)	0·43	0·0005	0·0011
GM-CSF	25	(17-74)	2	(0-70)	**0·032**	0	(0-8)	0	(0-4)	-	0·0021	0·0373
IFN-α2	112	(81-127)	76	(53-104)	**0·016**	21	(8-54)	4	(0-24)	**0·03**	0·0005	0·0002
IL-12p40	42	(3-108)	0	(0-56)	0·14	6	(0-81)	0	(0-20)	0·20	0·53	-
TNF	248	(129-385)	36	(13-156)	**0·0002**	20	(13-43)	16	(10-38)	0·27	0·0005	0·025
IFN-γ	1256	(695-1710)	18	(4-970)	**0·0067**	9	(4-18)	6	(0-24)	0·68	0·0005	0·047
IL-10	120	(82-189)	60	(20-214)	0·11	10	(0-14)	10	(4-20)	0·43	0·0005	0·0074
IL-1β	12	(5-57)	0	(0-9)	**0·015**	0	(0-9)	0	(0-0)	-	0·0097	-
IL-6	8816	(1668-10550)	71	(16-3939)	**0·0003**	10	(5-15)	5	(0-10)	0·059	0·0005	0·0005
IL-22	71	(8-154)	0	(0-184)	0·16	0	(0-16)	0	(0-12)	-	0·0032	-
IL-18	228	(0-647)	0	(0-137)	**0·0089**	-	-	-	-	-	-	-
IL17A	0·86	(0·34-2·50)	1·08	(0-1·74)	0·92	0	(0-0)	0	(0-0)	-	0·0002	0·0007
CXCL1-3	1140	(354-2284)	99	(0-806)	**0·0035**	1478	(889-2274)	1086	(479-1631)	0·18	0·70	0·024
CCL2	4524	(2031-8556)	1160	(699-7598)	**0·042**	296	(156-394)	312	(190-614)	0·28	0·0005	0·0004
CCL3	118	(72-190)	56	(38-102)	**0·0052**	0	(0-0)	0	(0-0)	-	0·0021	0·0003
CCL4	128	(73-264)	56	(22-114)	**0·010**	20	(0-54)	6	(0-18)	0·12	0·0005	0·0004
CXCL8	3428	(1854-8536)	264	(132-3910)	**0·0044**	35	(22-47)	16	(8-27)	**0·0049**	0·0005	0·0002
CXCL10	7901	(6311-8996)	3152	(1273-7806)	**0·0089**	1180	(914-2280)	842	(642-1476)	0·095	0·0005	0·0008
HNP 1-3	4879	(3593-5648)	1018	(585-3782)	**< 0·0001**	-	-	-	-	-	-	-
LL-37	762	(442-1117)	428	(0-778)	0·064	-	-	-	-	-	-	-

Cathepsin G	12	(2-18)	8	(0-12)	0·10	-	-	-	-	-	-	-
Lipocalin-2	1114	(1046-1192)	845	(314-1135)	**0·017**	-	-	-	-	-	-	-
C5a	1044	(172-1752)	0	(0-0)	**0·0053**	-	-	-	-	-	-	-
S100A8/A9	45000	(27500-49000)	20500	(0-38250)	**0·0096**	-	-	-	-	0·49	-	-
MMP-1	246	(97-634)	28	(8-167)	**0·0004**	1010	(439-3075)	556	(204-1004)	**0·037**	0·0007	0·0004
MMP-2	33550	(20570-41630)	26310	(18670-38790)	0·60	43130	(33530-52250)	46170	(35340-54770)	0·77	0·016	0·01
MMP-3	1442	(744-2006)	844	(613-2111)	0·60	10340	(7515-16100)	12530	(4328-26850)	0·82	0·0006	0·0003
MMP-7	570	(309-914)	209	(172-400·5)	**0·0012**	25160	(17120-35270)	18260	(12900-29180)	0·21	0·0005	0·0002
MMP-9	60480	(24540-95750)	9368	(1681-33420)	**0·0006**	7258	(5126-14450)	8066	(5974-10640)	0·82	0·0015	0·49
MMP-10	64	(36-85)	39	(23-69)	0·10	600	(381-1194)	544	(465-1064)	0·97	0·0006	0·0002
TIMP-1	514000	(415900-835900)	246100	(99360-484900)	**0·0009**	136900	(75910-181600)	103200	(86550-170800)	0·40	0·0005	0·010
TIMP-2	63620	(42400-78370)	38280	(34320-57320)	**0·012**	46560	(34590-55280)	49800	(41090-61620)	0·29	0·019	0·41
MMP-9/TIMP-1	0·10	(0·04-0·22)	0·04	(0·01-0·1)	0·51	-	-	-	-	-	-	-

Definitions of abbreviations: IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased in CSF compared to blood

Concentrations significantly ($p<0·05$) decreased in CSF compared to blood

P-values for analysis between groups:

Significant ($p<0·05$) differences between groups are indicated in bold

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary table 4. Mediator concentrations in cerebrospinal fluid (CSF) and blood (BI) of TBM-IRIS and TBM-non-IRIS patients at time of starting ART

	CSF					Blood					TBM-IRIS	TBM-non-IRIS
Mediator	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**CSF vs CSF**	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**BI vs BI**	**BI vs CSF**	**BI vs CSF**
pg/ml †	median	(IQR)	median	(IQR)	p-value	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	248	(156-423)	208	(62-469)	0·37	63	(41-78)	57	(13-125)	0·73	0·0077	0·019
GM-CSF	7	(1-15)	0	(0-6)	0·051	0	(0-3)	0	(0-0)	-	0·012	-
IFN-α2	96	(81-132)	66	(54-89)	**0·0092**	3	(0-26)	2	(0-10)	0·38	0·0005	0·0002
IL-12p40	100	(41-184)	0	(0-58)	**0·0023**	0	(0-35)	0	(0-0)	0·12	0·012	-
TNF	106	(41-198)	11	(7-62)	**0·0023**	18	(12-25)	13	(8-31)	0·33	0·0015	0·71
IFN-γ	159	(36-403)	4	(0-109)	**0·0029**	9	(1-13)	2	(0-6)	0·13	0·0005	0·064
IL-10	79	(52-202)	14	(6-54)	**0·0009**	12	(8-16)	14	(4-40)	0·60	0·0035	0·76
IL-1β	0	(0-0)	0	(0-1)	-	0	(0-0)	0	(0-0)	-	-	-
IL-6	82	(51-386)	14	(2-282)	**0·043**	0	(0-6)	0	(0-0)	-	0·0005	0·0026
IL-22	40	(2-130)	0	(0-0)	**0·0013**	5	(0-44)	0	(0-39)	0·56	0·10	-
IL-18	0	(0-95)	0	(0-0)	-	-	-	-	-	-	-	-
IL-17A	4·06	(1·24-9·00)	0·67	(0-1·94)	**0·0089**	0	(0-0)	0	(0-0)	0·19	< 0·0001	0·0005
CXCL1-3	1177	(487-1636)	0	(0-1048)	**0·0039**	1835	(874-2338)	1101	(838-2004)	0·24	0·24	0·013
CCL2	2298	(1307-3827)	1191	(836-1784)	**0·042**	353	(197-531)	408	(165-572)	0·92	0·0005	0·0005
CCL3	56	(46-93)	28	(26-53)	**0·016**	0	(0-0)	0	(0-0)	-	0·0041	0·0002
CCL4	57	(40-65)	22	(10-46)	**0·016**	14	(0-34)	8	(0-19)	0·31	0·0009	0·02
CXCL8	1571	(722-2545)	116	(64-1225)	**0·0028**	20	(12-32)	10	(5-19)	**0·017**	0·0005	0·0002
CXCL10	5902	(3005-7282)	1163	(640-4666)	**0·0024**	1122	(808-1418)	854	(644-1463)	0·25	0·0006	0·11
HNP 1-3	1106	(716-2305)	531	(427-870)	**0·005**	-	-	-	-	-	-	-
LL-37	0	(0-311)	103	(0-342)	0·57	-	-	-	-	-	-	-

Cathepsin G	0	(0-14)	4	(0-11)	0·85	-	-	-	-	-	-	-
Lipocalin-2	877	(679-1013)	242	(125-928)	**0·02**	-	-	-	-	-	-	-
C5a	0	(0-952)	0	(0-290)	-	-	-	-	-	-	-	-
S100A8/A9	14000	(0-31750)	0	(0-28500)	0·39	-	-	-	-	0·17	-	-
MMP-1	26	(12-46)	7	(0-51)	**0·046**	871	(419-1322)	488	(282-923)	0·12	0·0005	0·0004
MMP-2	34500	(24550-51040)	27320	(15190-33620)	**0·03**	61850	(46660-79090)	54870	(44320-65790)	0·19	0·0029	0·0003
MMP-3	829	(365-1219)	558	(323-964)	0·37	14530	(8630-34650)	11160	(8472-25700)	0·70	0·0005	0·0002
MMP-7	230	(194-463)	199	(151-413)	0·21	20690	(14750-25310)	15730	(9946-18610)	0·059	0·0005	0·0002
MMP-9	60810	(33280-104300)	5943	(2606-32570)	**0·0009**	8906	(6400-12810)	8574	(7190-11780)	0·96	0·0012	0·79
MMP-10	24	(16-34)	18	(13-23)	0·15	560	(305-691)	530	(372-801)	0·73	0·0005	0·0002
TIMP-1	202200	(152400-298900)	54890	(42140-120800)	**< 0·0001**	114100	(87390-122400)	96620	(83560-139900)	0·65	0·0010	0·24
TIMP-2	62180	(39500-94550)	35820	(24340-52650)	**0·0039**	47610	(43690-65850)	55550	(47620-66060)	0·21	0·066	0·0069
MMP-9/TIMP-1	0·26	(0·11-0·38)	0·10	(0·03-0·25)	0·056	-	-	-	-	-	-	-

Definitions of abbreviations: ART, antiretroviral therapy; IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased in CSF compared to blood

Concentrations significantly ($p<0·05$) decreased in CSF compared to blood

P-values for analysis between groups:

Significant ($p<0·05$) differences between groups are indicated in bold

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary Table 5. Mediator concentrations in cerebrospinal fluid (CSF) and blood (BI) of TBM-IRIS and TBM-non-IRIS patients at TBM-IRIS or 2 weeks after ART initiation (TBM-non-IRIS)

	CSF					Blood					TBM-IRIS	TBM-non-IRIS
Mediator	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**CSF vs CSF**	**TBM-IRIS (n=16)**		**TBM-non-IRIS (n=18)**		**BI vs BI**	**BI vs CSF**	**BI vs CSF**
pg/ml †	median	(IQR)	median	(IQR)	p-value	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	1572	(622-2554)	196	(57-640)	**0·0008**	64	(55-108)	49	(25-106)	0·15	0·0005	0·002
GM-CSF	12	(5-22)	0	(0-16)	**0·019**	0	(0-7)	0	(0-0)	-	0·020	-
IFN-α2	94	(63-128)	66	(50-100)	0·062	9	(0-21)	4	(0-11)	0·18	0·0005	0·0002
IL-12p40	168	(78-499)	0	(0-34)	**0·0003**	9	(0-50)	0	(0-2)	0·066	0·0041	-
TNF	264	(145-475)	6	(0-63)	**< 0·0001**	22	(15-40)	14	(6-26)	**0·045**	0·0005	0·85
IFN-γ	376	(268-788)	0	(0-105)	**< 0·0001**	12	(6-22)	0	(0-13)	**0·030**	0·0005	-
IL-10	304	(162-576)	6	(0-116)	**0·0002**	11	(6-19)	16	(4-42)	0·33	0·0005	0·93
IL-1β	3	(0-13)	0	(0-0)	**0·029**	0	(0-3)	0	(0-0)	-	0·032	-
IL-6	772	(366-5152)	10	(0-500)	**0·0006**	6	(0-13)	0	(0-7)	0·23	0·0005	0·034
IL-22	162	(25-875)	0	(0-0)	**0·0001**	6	(0-39)	0	(0-22)	0·52	0·0001	-
IL-18	123	(0-2030)	0	(0-0)	**0·0008**	-	-	-	-	-	-	-
IL-17A	7·6	(0·81-14·9)	0·36	(0-4·07)	**0·0017**	0	(0-0)	0	(0-0)	0·54	0·0005	0·0068
CXCL1-3	1435	(850-2242)	0	(0-1079)	**0·0011**	1966	(987-2605)	1150	(897-1852)	0·095	0·39	0·012
CCL2	2792	(1798-7032)	1005	(606-2274)	**0·0039**	296	(211-526)	402	(253-552)	0·32	0·0005	0·0006
CCL3	84	(68-124)	32	(28-56)	**0·0008**	0	(0-0)	0	(0-0)	-	0·0009	0·0002
CCL4	108	(67-155)	20	(14-46)	**< 0·0001**	36	(16-55)	15	(3-25)	**0·047**	0·0005	0·099
CXCL8	3461	(2075-7132)	91	(43-1839)	**0·0001**	34	(15-58)	8	(2-23)	**0·0041**	0·0005	0·0002
CXCL10	6786	(5734-8379)	233	(28-4605)	**0·0001**	1175	(744-1694)	406	(227-859)	**0·0014**	0·0005	0·26
HNP 1-3	4406	(2867-6228)	633	(417-1568)	**< 0·0001**	-	-	-	-	-	-	-
LL-37	719	(314-1323)	252	(0-353)	**0·0071**	-	-	-	-	-	-	-

Cathepsin G	11	(9-14)	0	(0-10)	**0·0068**	-	-	-	-	-	-	-
Lipocalin-2	1150	(1050-1280)	274	(21-943)	**0·0012**	-	-	-	-	-	-	-
C5a	1050	(0-10350)	0	(0-204)	**0·015**	-	-	-	-	-	-	-
S100A8/A9	33500	(27000-48000)	0	(0-17500)	**< 0·0001**	-	-	-	-	0·46	-	-
MMP-1	73	(34-316)	6	(0-52)	**0·0013**	844	(466-1318)	486	(286-665)	0·060	0·0012	0·0004
MMP-2	49010	(32970-70180)	20800	(13480-35820)	**0·0004**	51490	(46720-64140)	51440	(42300-64190)	0·70	0·55	0·0041
MMP-3	1453	(461-2846)	632	(371-1006)	**0·036**	16970	(6158-33490)	22420	(8006-110400)	0·31	< 0·0001	0·0003
MMP-7	426	(282-816)	183	(120-414)	**0·0058**	18020	(16020-39510)	13460	(10560-17510)	**0·022**	0·0005	0·0002
MMP-9	139000	(84410-180400)	3828	(772-28150)	**0·0001**	12400	(9281-18330)	9232	(7250-14260)	0·18	0·0006	0·90
MMP-10	40	(26-93)	16	(14-25)	**0·0039**	563	(312-751)	533	(284-992)	0·89	0·0005	0·0002
TIMP-1	453100	(149700-952100)	57380	(33390-110200)	**< 0·0001**	114200	(72400-170700)	90760	(71450-171800)	0·75	0·0021	0·15
TIMP-2	68380	(52210-91680)	42990	(24410-52720)	**0·0024**	41280	(38990-59190)	58130	(54690-63580)	**0·046**	0·0035	0·041
MMP-9/TIMP-1	0·34	(0·09-1·05)	0·04	(0·01-0·51)	**0·029**	-	-	-	-	-	-	-

Definitions of abbreviations: ART, antiretroviral therapy; IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased in CSF compared to blood

Concentrations significantly ($p<0·05$) decreased in CSF compared to blood

P-values for analysis between groups:

Significant ($p<0·05$) differences between groups are indicated in bold

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary Table 6. Comparison of cerebrospinal fluid (CSF) mediator concentrations between timepoints in TBM patients who A) developed TBM- IRIS and B) those who did not (TBM-non-IRIS)

A) TBM-IRIS (n=16)											
Mediator	**1: TBM diagnosis**		**2: Starting ART**		**3: TBM-IRIS diagnosis**		**4: 2 weeks after TBM-IRIS**		**2 vs 1**	**3 vs 2**	**3 vs 1**
pg/ml †	median	(IQR)	median	(IQR)	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	5102	(808-12860)	248	(156-423)	1572	(622-2554)	666	(168-941)	0·0007	0·0005	0·010
GM-CSF	25	(17-74)	7	(1-15)	12	(5-22)	5	(0-20)	0·0008	0·19	0·0021
IFN-α2	112	(81-127)	96	(81-132)	94	(63-128)	131	(97-153)	0·45	1	0·55
IL-12p40	42	(3-108)	100	(41-184)	168	(78-499)	156	(62-298)	0·012	0·012	0·0004
TNF	248	(129-385)	106	(41-198)	264	(145-475)	206	(157-367)	0·0035	0·0029	0·21
IFN-γ	1256	(695-1710)	159	(36-403)	376	(268-788)	286	(80-577)	0·0009	0·0077	0·019
IL-10	120	(82-189)	79	(52-202)	304	(162-576)	335	(130-580)	0·020	0·0005	0·0010
IL-1β	12	(5-57)	0	(0-0)	3	(0-13)	0	(0-12)	0·0017	0·022	0·0017
IL-6	8816	(1668-10550)	82	(51-386)	772	(366-5152)	372	(114-617)	0·0005	0·0005	0·0025
IL-22	71	(8-154)	40	(2-130)	162	(25-875)	132	(16-498)	0·46	0·0067	0·022
IL-18	228	(0-647)	0	(0-95)	123	(0-2030)	75	(0-449)	0·0049	0·0068	0·59
IL-17A	0·86	(0·34-2·50)	4·06	(1·24-9·00)	7·6	(0·81-14·9)	3·75	(0·52-12·30)	0·010	0·25	0·0066
CXCL1-3	1140	(354-2284)	1177	(487-1636)	1435	(850-2242)	1756	(1079-2943)	0·39	0·14	0·86
CCL2	4524	(2031-8556)	2298	(1307-3827)	2792	(1798-7032)	2540	(2058-5832)	0·025	0·011	0·19
CCL3	118	(72-190)	56	(46-93)	84	(68-124)	95	(50-128)	0·0021	0·028	0·17
CCL4	128	(73-264)	57	(40-65)	108	(67-155)	90	(61-118)	0·0012	0·0009	0·13
CXCL8	3428	(1854-8536)	1571	(722-2545)	3461	(2075-7132)	3491	(1216-6316)	0·021	0·0009	0·94
CXCL10	7901	(6311-8996)	5902	(3005-7282)	6786	(5734-8379)	6668	(4395-7761)	0·0048	0·0066	0·22
HNP 1-3	4879	(3593-5648)	1106	(716-2305)	4406	(2867-6228)	1948	(1254-3350)	0·0009	0·0029	0·29
LL-37	762	(442-1117)	0	(0-311)	719	(314-1323)	0	(0-511)	0·0005	0·0007	0·97

Cathepsin G	12	(2-18)	0	(0-14)	11	(9-14)	9·5	(0-24)	0·070	0·023	0·92
Lipocalin-2	1114	(1046-1192)	877	(679-1013)	1150	(1050-1280)	1191	(889-1295)	0·0005	0·0007	0·39
C5a	1044	(172-1752)	0	(0-952)	1050	(0-10350)	1043	(434-2351)	0·0076	0·0039	0·28
S100A8/A9	45000	(27500-49000)	14000	(0-31750)	33500	(27000-48000)	25500	(17500-38750)	0·0015	0·011	0·90
MMP-1	246	(97-634)	26	(12-46)	73	(34-316)	32	(22-89)	< 0·0001	0·0009	0·21
MMP-2	33550	(20570-41630)	34500	(24550-51040)	49010	(32970-70180)	47240	(28779-83534)	0·17	0·011	0·0007
MMP-3	1442	(744-2006)	829	(365-1219)	1453	(461-2846)	1273	(544-1942)	0·0041	0·0029	0·62
MMP-7	570	(309-914)	230	(194-463)	426	(282-816)	396	(282-613)	0·012	0·041	0·72
MMP-9	60480	(24540-95750)	60810	(33280-104300)	139000	(84410-180400)	165161	(136793-206467)	0·78	0·0077	0·0012
MMP-10	64	(36-85)	24	(16-34)	40	(26-93)	50	(30-83)	0·0008	0·0024	0·16
TIMP-1	514000	(415900-835900)	202200	(152400-298900)	453100	(149700-952100)	289227	(173023-493628)	0·0009	0·012	0·11
TIMP-2	63620	(42400-78370)	62180	(39500-94550)	68380	(52210-91680)	70979	(58889-86093)	0·59	0·59	0·78
MMP-9/TIMP-1	0·10	(0·04-0·22)	0·26	(0·11-0·38)	0·34	(0·09-1·05)	0·55	(0·20-0·91)	0·009	0·053	0·0029

Definitions of abbreviations: ART, antiretroviral therapy; IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased at later compared to earlier timepoint

Concentrations significantly ($p<0·05$) decreased at later compared to earlier timepoint

P-values for analysis between groups:

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

B) TBM-non-IRIS (n=18)									
Mediator	**1: TBM diagnosis**		**2: Starting ART**		**3: 2 weeks after starting ART**		**2 vs 1**	**3 vs 2**	**3 vs 1**
pg/ml †	median	(IQR)	median	(IQR)	median	(IQR)	p-value	p-value	p-value
G-CSF	451	(118-2528)	208	(62-469)	196	(57-640)	0·0020	0·79	0·013
GM-CSF	2	(0-70)	0	(0-6)	0	(0-16)	0·013	-	0·020
IFN-α2	76	(53-104)	66	(54-89)	66	(50-100)	0·27	0·88	0·56
IL-12p40	0	(0-56)	0	(0-58)	0	(0-34)	-	-	-
TNF	36	(13-156)	11	(7-62)	6	(0-63)	0·0090	0·51	0·0025
IFN-γ	18	(4-970)	4	(0-109)	0	(0-105)	0·0061	0·41	0·0041
IL-10	60	(20-214)	14	(6-54)	6	(0-116)	0·0031	0·55	0·058
IL-1β	0	(0-9)	0	(0-1)	0	(0-0)	-	-	-
IL-6	71	(16-3939)	14	(2-282)	10	(0-500)	0·0014	0·81	0·0015
IL-22	0	(0-184)	0	(0-0)	0	(0-0)	-	-	-
IL-18	0	(0-137)	0	(0-0)	0	(0-0)	-	-	-
IL-17A	1·08	(0-1·74)	0·67	(0-1·94)	0·36	(0-4·07)	0·64	0·74	0·65
CXCL1-3	99	(0-806)	0	(0-1048)	0	(0-1079)	0·84	-	0·57
CCL2	1160	(699-7598)	1191	(836-1784)	1005	(606-2274)	0·15	0·91	0·074
CCL3	56	(38-102)	28	(26-53)	32	(28-56)	0·013	0·90	0·012
CCL4	56	(22-114)	22	(10-46)	20	(14-46)	0·0035	0·51	0·0005
CXCL8	264	(132-3910)	116	(64-1225)	91	(43-1839)	0·0079	0·83	0·0069
CXCL10	3152	(1273-7806)	1163	(640-4666)	233	(28-4605)	0·0027	0·16	0·0004
HNP 1-3	1018	(585-3782)	531	(427-870)	633	(417-1568)	0·0027	0·97	0·15
LL-37	428	(0-778)	103	(0-342)	252	(0-353)	0·0081	0·29	0·14
Cathepsin G	8	(0-12)	4	(0-11)	0	(0-10)	0·53	0·96	0·082
Lipocalin-2	845	(314-1135)	242	(125-928)	274	(21-943)	0·0035	0·96	0·012
C5a	0	(0-0)	0	(0-290)	0	(0-204)	-	-	-

S100A8/A9	20500	(0-38250)	0	(0-28500)	0	(0-17500)	0·83	-	0·043
MMP-1	28	(8-167)	7	(0-51)	6	(0-52)	0·17	0·70	0·16
MMP-2	26310	(18670-38790)	27320	(15190-33620)	20800	(13480-35820)	0·24	0·60	0·24
MMP-3	844	(613-2111)	558	(323-964)	632	(371-1006)	0·029	0·22	0·14
MMP-7	209	(172-400)	199	(151-413)	183	(120-414)	0·34	0·24	0·16
MMP-9	9368	(1681-33420)	5943	(2606-32570)	3828	(772-28150)	0·76	0·89	0·90
MMP-10	39	(23-69)	18	(13-23)	16	(14-25)	0·0008	0·49	0·0003
TIMP-1	246100	(99360-484900)	54890	(42140-120800)	57380	(33390-110200)	0·0015	0·28	0·010
TIMP-2	38280	(34320-57320)	35820	(24340-52650)	42990	(24410-52720)	0·074	0·54	0·54
MMP-9/TIMP-1	0·04	(0·01-0·10)	0·10	(0·03-0·25)	0·04	(0·01-0·51)	0·012	1,00	0·067

Definitions of abbreviations: ART, antiretroviral therapy; IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased at later compared to earlier timepoint

Concentrations significantly ($p<0·05$) decreased at later compared to earlier timepoint

P-values for analysis between groups:

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary Table 7. Jaccard similarity indices for testing clusterwise stability of hierarchical clustering analyses of tuberculous meningitis patients (n=34) over time*

Number of clusters		**2**		**3**		**4**	
Timepoint	Resampling method						
TBM diagnosis							
	Subset	0·80	(0·053)	0·73	(0·026)	0·79	(0·037)
	Bootstrap	0·82	(0·065)	0·79	(0·015)	0·84	(0·048)
ART start							
	Subset	0·75	(0·16)	0·81	(0·047)	0·77	(0·18)
	Bootstrap	0·77	(0·14)	0·88	(0·038)	0·78	(0·22)
2 weeks after starting ART †							
	Subset	0·71	(0·10)	0·79	(0·091)	0·71	(0·14)
	Bootstrap	0·73	(0·14)	0·83	(0·12)	0·76	(0·15)

Definitions of abbreviations: TBM, tuberculous meningitis; ART, antiretroviral therapy

* Mean Jaccard index values with standard deviations of clustering analysis (presented in Figure 4) are shown

† For patients who developed TBM-IRIS, the "2 weeks after starting ART" timepoint indicates findings at time of TBM-IRIS

Interpretation of the Jaccard index:

A valid stable cluster should yield a mean Jaccard similarity value of ≥0·75

Highly stable clusters will be >0·85, and values between 0·6 and 0·75 should be taken as indicative of patterns in the data but exactly which points belong to which clusters is doubtful

Supplementary Table 8. Comparison of cerebrospinal fluid (CSF) mediator concentrations between TBM-IRIS, CSF *M. tuberculosis* culture-positive TBM-non-IRIS (Cult pos NI) and CSF *M. tuberculosis* culture-negative TBM-non-IRIS (Cult neg NI) patients at TBM diagnosis

Mediator	TBM-IRIS (n=16)		Cult pos Non-TBM-IRIS (n=6)		Cult neg Non-TBM-IRIS (n=12)		Cult pos NI vs IRIS	Cult pos NI vs Cult neg NI
pg/ml †	median	(IQR)	median	(IQR)	median	(IQR)	p-value	p-value
Neutrophls	38	(11-117)	3	(0-14)	10	(0-43)	0·020	0·37
Lymphocytes	216	(120-419)	120	(20-214)	94	(54-440)	0·083	0·89
G-CSF	5102	(808-12860)	4392	(1573-9708)	260	(84-1203)	0·74	0·0043
GM-CSF	25	(17-74)	76	(21-159)	0	(0-3)	0·20	0·0016
IFN-α2	112	(81-127)	74	(52-110)	82	(54-102)	0·083	0·96
IL-12p40	42	(3-108)	67	(0-106)	0	(0-26)	0·91	0·11
TNF	248	(129-385)	178	(84-257)	29	(10-42)	0·20	0·028
IFN-γ	1256	(695-1710)	1337	(389-2647)	12	(1-20)	0·91	0·027
IL-10	120	(82-189)	175	(76-332)	48	(14-102)	0·48	0·11
IL-1β	12	(5-57)	14	(9-58)	0	(0-0)	0·58	0·0002
IL-6	8816	(1668-10550)	2931	(770-7926)	45	(12-1511)	0·097	0·035
IL-22	71	(8-154)	19	(0-295)	0	(0-147)	0·63	0·49
IL-18	228	(0-647)	64	(0-172)	0	(0-0)	0·15	0·31
IL-17A	0·86	(0·34-2·50)	1·09	(0-1·44)	1·06	(0·14-2·70)	0·58	0·6
CXCL1-3	1140	(354-2284)	1334	(477-2084)	32	(0-134)	0·97	0·013
CCL2	4524	(2031-8556)	7676	(5461-8483)	765	(550-1252)	0·29	0·0043
CCL3	118	(72-190)	96	(54-125)	43	(28-71)	0·29	0·068
CCL4	128	(73-264)	104	(68-127)	37	(20-61)	0·36	0·17
CXCL8	3428	(1854-8536)	5350	(2606-6349)	192	(118-417)	0·80	0·011
CXCL10	7901	(6311-8996)	7978	(5803-8779)	2653	(1248-3479)	0·85	0·068

HNP 1-3	4879	(3593-5648)	2858	(1590-4688)	642	(566-1301)	0·03	0·083
LL-37	762	(442-1117)	597	(0-1174)	400	(0-711)	0·48	0·36
Cathepsin G	12	(2-18)	6	(0-14)	8	(0-13)	0·33	0·92
Lipocalin-2	1114	(1046-1192)	1183	(765-1317)	632	(281-998)	0·85	0·12
C5a	1044	(172-1752)	793	(0-2435)	0	(0-0)	0·91	0·012
S100A8/A9	45000	(27500-49000)	30029	(18231-43575)	4123	(0-34505)	0·20	0·23
MMP-1	246	(97-634)	47·5	(0-216)	27·5	(10-149)	0·025	0·96
MMP-2	33550	(20570-41630)	25017	(17394-29308)	31652	(18021-50055)	0·36	0·37
MMP-3	1442	(744-2006)	844	(611-1907)	830	(585-2192)	0·44	0·89
MMP-7	570	(309-914)	192	(174-239)	215·5	(164-648)	0·001	0·43
MMP-9	60480	(24540-95750)	54726	(20949-87491)	2881	(1323-12475)	0·80	0·0057
MMP-10	64	(36-85)	31·5	(21-67)	39·5	(26-70)	0·10	0·35
TIMP-1	514000	(415900-835900)	280424	(145754-543403)	242091	(92086-472705)	0·043	0·74
TIMP-2	63620	(42400-78370)	47942	(36330-71838)	36974	(31167-48888)	0·25	0·21
MMP-9/TIMP-1	0·1	(0·04-0·22)	0·13	(0·07-0·36)	0·02	(0-0·04)	0·29	0·0032

Definitions of abbreviations: IQR, interquartile range

P-values for analysis between blood and CSF:

Concentrations significantly ($p<0·05$) increased in culture positive TBM-non-IRIS patients compared to comparator group

Concentrations significantly ($p<0·05$) decreased in culture positive TBM-non-IRIS patients compared to comparator group

P-values for analysis between groups:

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Supplementary Table 9. Comparison of cerebrospinal fluid (CSF) mediator concentrations between TBM-IRIS, *CSF M. tuberculosis* culture-positive TBM-non-IRIS (Cult pos NI) and CSF *M. tuberculosis* culture-negative TBM-non-IRIS (Cult neg NI) patients at TBM-IRIS or 2 weeks after starting ART (TBM-non-IRIS)

Mediator	TBM-IRIS (n=16)		Cult pos TBM-non-IRIS (n=6)		Cult neg TBM-non-IRIS (n=12)		Cult pos NI vs IRIS	Cult pos NI vs Cult neg NI
pg/ml †	median	(IQR)	median	(IQR)	median	(IQR)	p-value	p-value
Neutrophils	52	(17-244)	2	(0-11)	0	(0-0)	0·0027	0·045
Lymphocytes	208	(90-363)	54	(6-142)	22	(5-40)	0·011	0·22
G-CSF	1572	(622-2554)	645	(408-1729)	74	(51-231)	0·17	0·0076
GM-CSF	12	(5-22)	19	(4-28)	0	(0-0)	0·77	0·0056
IFN-α2	94	(63-128)	98	(53-142)	58	(37-86)	0·80	0·15
IL-12p40	168	(78-499)	34	(0-221)	0	(0-0)	0·11	0·015
TNF	264	(145-475)	126	(28-214)	0	(0-14)	0·043	0·0038
IFN-γ	376	(268-788)	274	(58-413)	0	(0-2)	0·17	0·0040
IL-10	304	(162-576)	211	(45-374)	0	(0-14)	0·29	0·0020
IL-1β	3	(0-13)	2	(0-9)	0	(0-0)	0·72	0·012
IL-6	772	(366-5152)	522	(122-2183)	3	(0-19)	0·40	0·030
IL-22	162	(25-875)	56	(0-233)	0	(0-0)	0·15	0·012
IL-18	123	(0-2030)	0	(0-331)	0	(0-0)	0·11	-
IL-17A	7·6	(0·81-14·9)	2·54	(0·25-8·81)	0·14	(0-0·96)	0·20	0·16
CXCL1-3	1435	(850-2242)	1137	(643-2316)	0	(0-62)	0·69	0·010
CCL2	2792	(1798-7032)	2332	(1824-5187)	684	(534-1508)	0·80	0·0076
CCL3	84	(68-124)	52	(38-102)	30	(26-35)	0·13	0·024
CCL4	108	(67-155)	50	(30-102)	16	(2-32)	0·02	0·011
CXCL8	3461	(2075-7132)	2753	(1359-4138)	57	(27-102)	0·29	0·0027
CXCL10	6786	(5734-8379)	5548	(3370-7996)	70	(0-516)	0·25	0·0022

HNP 1-3	4406	(2867-6228)	2443	(919-6219)	480	(410-658)	0·22	0·035
LL-37	719	(314-1323)	384	(0-1358)	206	(0-331)	0·32	0·21
Cathepsin G	11	(9-14)	4	(0-25)	0	(0-10)	0·15	0·64
Lipocalin-2	1150	(1050-1280)	1177	(747-1350)	140	(0-395)	0·91	0·0022
C5a	1050	(0-10350)	408	(0-6801)	0	(0-0)	0·45	0·072
S100A8/A9	33500	(27000-48000)	15346	(0-19152)	0	(0-10120)	0·0010	0·20
MMP-1	73	(34-316)	100·5	(8-337)	2	(0-11)	0·74	0·03
MMP-2	49010	(32970-70180)	28930	(14318-44514)	18826	(12295-31445)	0·071	0·43
MMP-3	1453	(461-2846)	856	(564-2273)	458	(297-909)	0·53	0·15
MMP-7	426	(282-816)	288	(150-438)	155	(100-383)	0·18	0·28
MMP-9	139000	(84410-180400)	87968	(19304-268088)	938	(302-9996)	0·48	0·0043
MMP-10	40	(26-93)	16	(11-27)	16	(14-25)	0·039	0·71
TIMP-1	453100	(149700-952100)	91331	(41885-277476)	45729	(26602-91415)	0·017	0·15
TIMP-2	68380	(52210-91680)	29885	(23981-53582)	46000	(24319-55934)	0·014	0·61
MMP-9/TIMP-1	0·34	(0·09-1·05)	1·28	(0·04-2·43)	0·03	(0·01-0·09)	0·44	0·017

Definitions of abbreviations: ART, antiretroviral therapy; IQR, interquartile range

P-values for analysis between groups

Concentrations significantly ($p<0·05$) increased in culture positive TBM-non-IRIS patients compared to comparator group

Concentrations significantly ($p<0·05$) decreased in culture positive TBM-non-IRIS patients compared to comparator group

† Units are picograms per milliliter except for Cathepsin G that is presented as units per milliliter and MMP-9/TIMP-1 that is presented as a ratio

Results shown are mediator concentrations assayed with Luminex multiplex or ELISA

Statistical analyses were not performed if the medians of both comparator groups were zero

Supplementary Table 10. P-values of correlation between cerebrospinal fluid mediators and neutrophil and lymphocyte cell counts in TBM-IRIS and TBM-non-IRIS patients over time †

	TBM diagnosis				Starting ART				2 weeks after starting ART *			
	TBM-IRIS		TBM-non-IRIS		TBM-IRIS		TBM-non-IRIS		TBM-IRIS		TBM-non-IRIS	
	Neutrophils	Lympho-cytes	Neutrophils	Lympho-cytes	Neutrophils	Lympho-cytes	Neutrophils	Lympho-cytes	Neutrophils	Lympho-cytes	Neutrophils	Lympho-cytes
G-CSF	0·71	0·82	0·36	0·41	0·86	0·95	0·03	0·64	0·86	0·61	0·12	0·55
GM-CSF	0·86	0·16	0·68	0·24	0·97	0·20	0·15	0·08	0·95	1,00	0·01	0·04
IFN-α2	0·86	0·87	0·98	0·82	0·86	1,00	0·62	0·28	0·95	1,00	0·35	0·32
IL-12p40	0·95	0·29	0·28	0·14	0·86	0·16	0·05	0·37	0·86	0·50	1,00	0·86
TNF	0·86	0·71	0·36	0·18	0·86	0·22	0·07	0·07	0·86	0·83	0·07	0·08
IFN-γ	0·86	0·24	0·68	0·26	0·86	0·16	0·03	0·07	0·95	0·46	0·03	0·05
IL-10	0·86	1,00	0·30	0·08	0·86	0·69	0·08	0·08	0·95	1,00	0·05	0·06
IL-1β	0·86	0·20	0·93	0·58	0·95	0·57	0·28	0·25	0·86	0·2	0·86	0·84
IL-6	0·86	0·95	0·10	0·22	0·86	0·46	0·03	0·05	0·86	0·24	0·05	0·08
IL-22	1,00	0·41	0·11	0·19	0·86	0·25	0·14	0·08	0·95	0·50	0·17	0·46
IL-18	0·86	0·85	0·17	0·22	0·77	0·25	0·76	0·20	0·93	0·45	0·63	0·49
IL-17A	0·86	0·95	0·06	0·92	0·95	1,00	0·63	0·62	0·86	1,00	0·03	0·05
CXCL1-3	0·86	0·99	0·76	0·55	0·86	0·20	0·03	0·05	0·86	0·83	0·04	0·05
CCL2	0·86	0·25	0·94	0·64	0·86	0·25	0·36	0·64	0·90	0·43	0·03	0·08
CCL3	0·86	0·82	0·22	0·07	0·95	0·99	0·14	0·31	0·86	0·56	0·17	0·28
CCL4	0·86	1,00	0·17	0·05	0·86	0·95	0·17	0·10	0·86	0·87	0·06	0·08
CXCL8	0·86	0·25	0·67	0·37	0·86	0·22	0·03	0·06	0·86	0·82	0·03	0·07
CXCL10	0·86	0·22	0·44	0·32	0·86	0·37	0·05	0·05	0·95	0·50	0·05	0·05
HNP 1-3	0·71	0·93	0·17	0·45	0·71	0·83	0·18	0·97	0·86	1,00	0·12	0·16
LL-37	0·86	0·83	0·10	0·08	0·86	0·19	0·77	0·05	0·95	0·61	0·17	0·07

Cathepsin G	0·86	0·61	0·61	0·21	0·95	0·22	0·44	0·64	0·86	0·20	0·56	0·36
Lipocalin-2	0·86	0·82	0·17	0·37	0·71	0·46	0·04	0·08	0·90	1,00	0·08	0·11
C5a	0·86	0·95	0·91	0·46	0·95	0·28	0·05	0·08	0·95	0·22	0·68	0·64
S100A8/A9	0·91	1,00	0·17	0·30	0·71	0·25	0·03	0·52	0·95	0·98	0·19	0·64
MMP-1	0·86	0·93	0·25	0·21	0·90	0·16	0·08	0·55	0·90	0·16	0·53	0·45
MMP-2	0·97	1,00	0·62	0·08	0·86	0·24	0·62	0·04	0·95	0·50	0·23	0·06
MMP-3	0·86	1,00	0·05	0·2	0·86	0·76	0·76	0·49	0·95	0·61	0·80	0·64
MMP-7	0·86	0·95	0·86	0·23	0·95	0·19	0·68	0·04	0·95	0·16	0·26	0·20
MMP-9	0·86	0·2	0·98	0·59	0·86	0·16	0·03	0·05	0·86	0·95	0·03	0·06
MMP-10	0·95	0·24	0·28	0·28	0·86	0·83	0·87	0·08	0·95	0·99	0·91	0·37
TIMP-1	0·90	0·83	0·07	0·19	0·86	0·20	0·31	0·25	0·86	0·20	0·41	0·32
TIMP-2	0·95	0·87	0·58	0·55	0·95	1,00	0·67	0·23	0·71	0·37	0·29	0·66

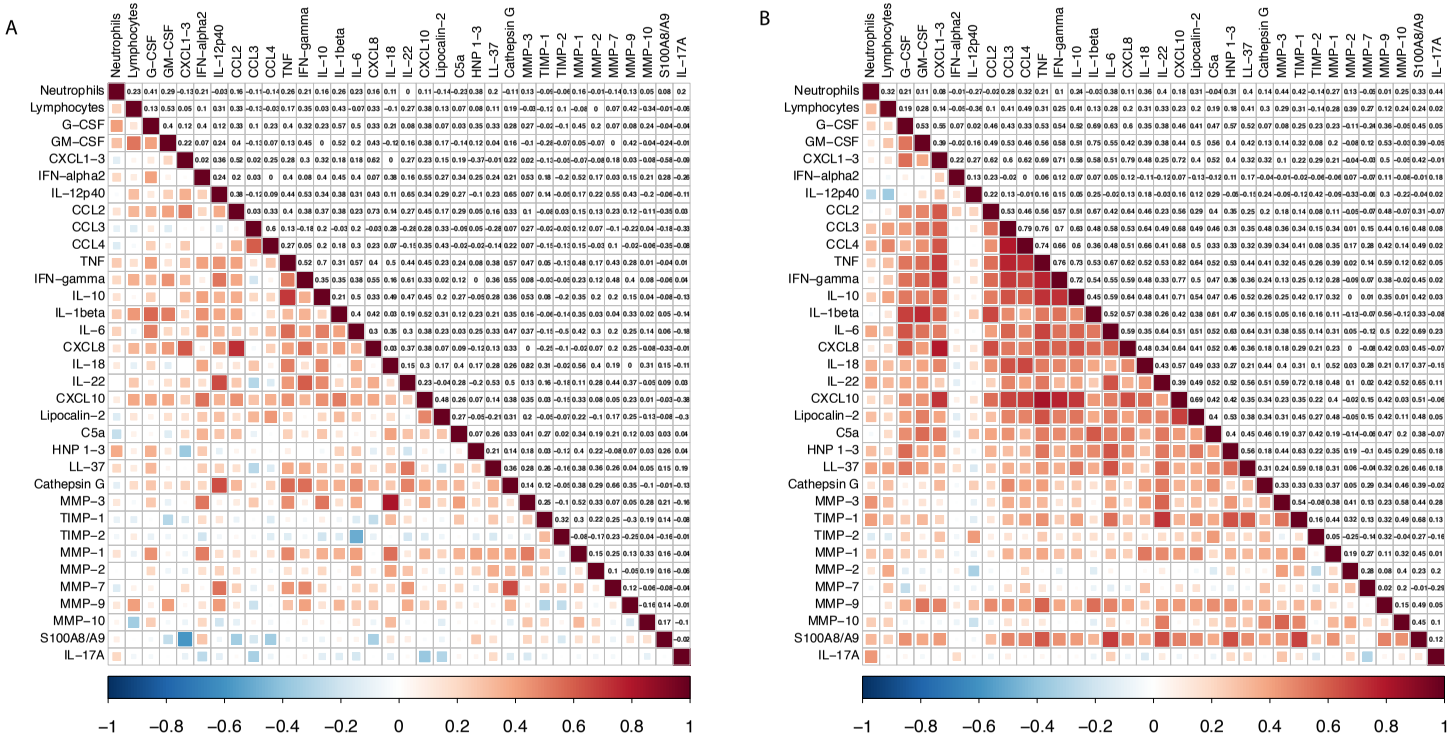
Definitions of abbreviations: TBM, tuberculous meningitis; ART, antiretroviral therapy

Significant correlations ($p<0·05$) are highlighted in red

† P-values adjusted for a false discovery rate (Benjamini and Hochberg) are indicated for each correlation

* For patients who developed TBM-IRIS, the “2 weeks after starting ART” timepoint indicates findings at time of TBM-IRIS

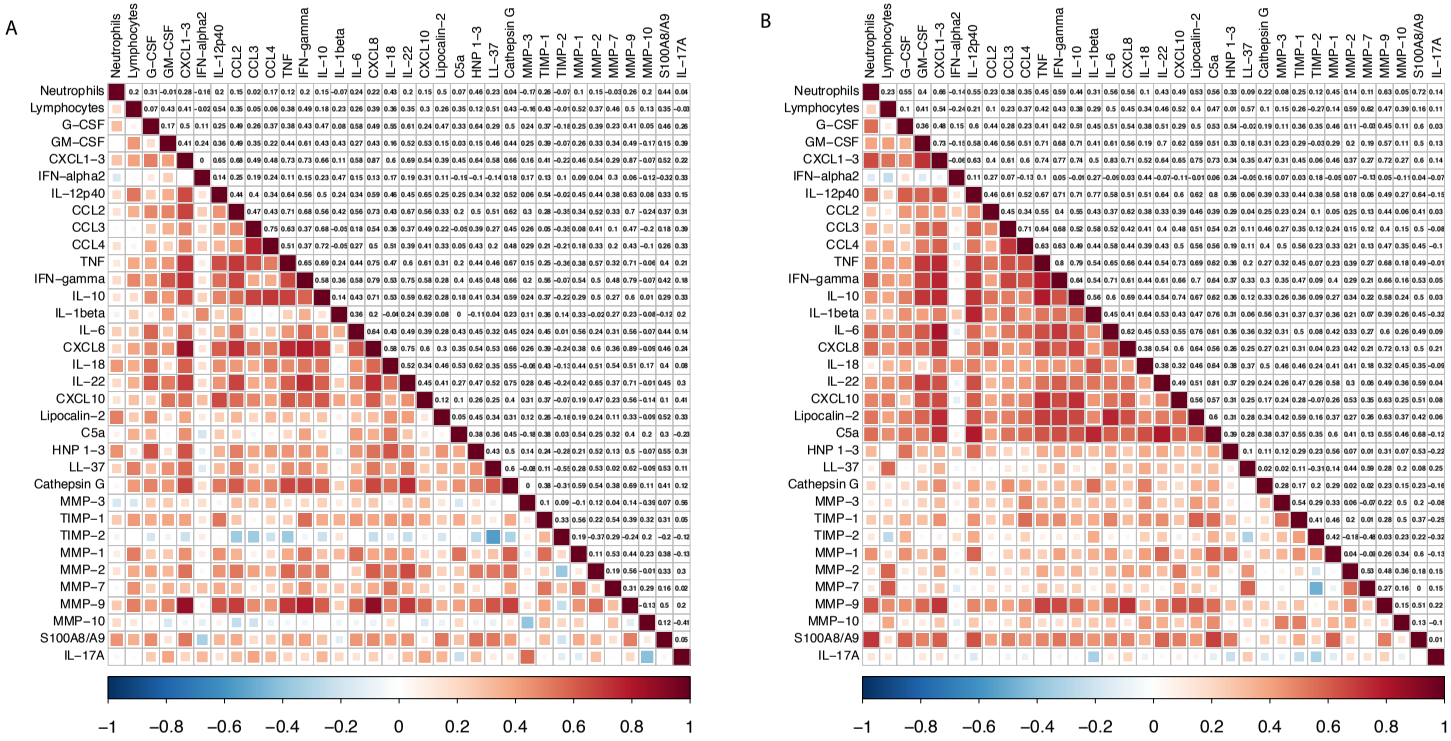
Supplementary Figure 1. Correlation between lymphocytes, neutrophils and mediators measured in CSF of TBM-IRIS (A) and TBM-non-IRIS (B) patients at TBM diagnosis.



Correlations were estimated by Kendall's tau (τ) with the τ values included in the right upper half of the graph. The size and shade of the squares in the left lower half of the graph similarly represent the τ value with increased size and darker shade of red and blue representing a greater absolute magnitude.

Color key: indicates magnitude of the correlation with red representing a positive and blue representing a negative correlation.

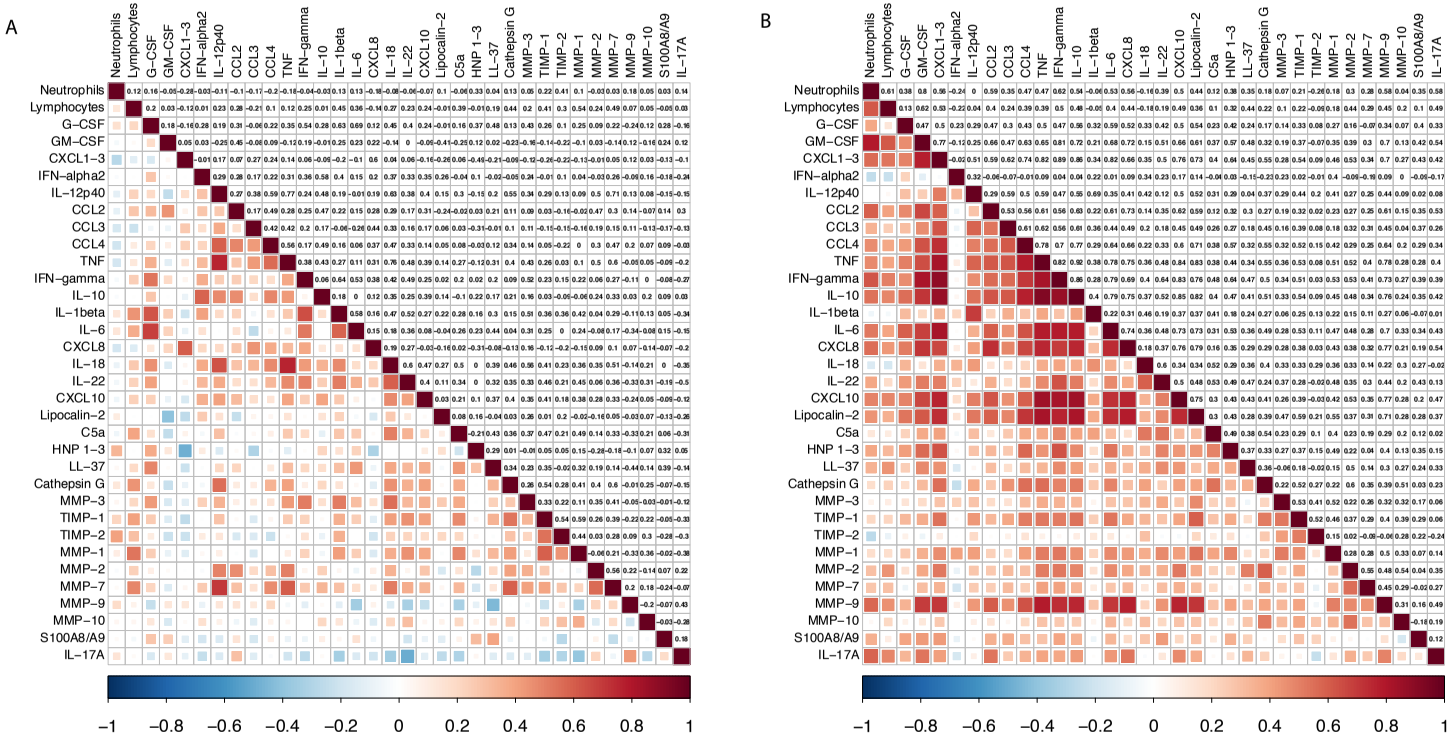
Supplementary Figure 2. Correlation between lymphocytes, neutrophils and mediators measured in CSF of TBM-IRIS (A) and TBM-non-IRIS (B) patients at ART start.



Correlations were estimated by Kendall's tau (τ) with the τ values included in the right upper half of the graph. The size and shade of the squares in the left lower half of the graph similarly represent the τ value with increased size and darker shade of red and blue representing a greater absolute magnitude.

Color key: indicates magnitude of the correlation with red representing a positive and blue representing a negative correlation.

Supplementary Figure 3. Correlation between lymphocytes, neutrophils and mediators measured in CSF of TBM-IRIS (A) and TBM-non-IRIS (B) patients at TBM-IRIS presentation or 2 weeks after ART start (TBM-non-IRIS).



Correlations were estimated by Kendall's tau (τ) with the τ values included in the right upper half of the graph. The size and shade of the squares in the left lower half of the graph similarly represent the τ value with increased size and darker shade of red and blue representing a greater absolute magnitude.

Color key: indicates magnitude of the correlation with red representing a positive and blue representing a negative correlation.

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Chapter 7

Conclusions

Tuberculous meningitis is a devastating disease in HIV-infected patients. Clinicians face numerous challenges in the management of HIV-associated TBM as diagnosing TBM is often difficult, the optimal treatment strategy for TBM is uncertain, and neurological TB-IRIS occurs in a substantial proportion of these patients. A better understanding of HIV-associated TBM is imperative to direct future local and international research with the ultimate aim of improving outcome in affected patients. South Africa is at the epicenter of the TB/HIV syndemic and in Cape Town, GF Jooste Hospital (until its temporary decommissioning in 2013) served those communities that are most affected by these diseases, providing an ideal setting to study HIV-associated TBM.

This thesis reports studies that investigated a continuum of pertinent aspects of HIV-associated TBM. In Chapter 3, a consensus case definition for use in TBM regardless of HIV-status is presented. Chapter 4 reports the disease burden and characteristics of HIV-associated TBM, and the contribution of neurological TB-IRIS to CNS deterioration during the first year of ART, in patients at a referral hospital in Cape Town. In Chapter 5 the first prospective study of TBM-IRIS is described and finally, Chapter 6 examines the immunopathogenesis of TBM-IRIS.

In the first study presented in Chapter 4 (Part 1 of Chapter 4), we report the clinical characteristics, frequency and outcome of definite, probable and possible TBM cases who presented to hospital over a 6-month period. We found that TBM accounted for more than 50% of adult meningitis cases and that 88% of affected patients were HIV-infected. The majority of HIV-

infected TBM cases was severely immunosuppressed (median CD4 count: 79 cells/μL), and was not receiving ART. We further found an increasing proportional contribution of TBM to meningitis cases over time; Jarvis *et al*. reported that during 2006-2008, CM was the most common cause of microbiologically confirmed meningitis (63%), compared to a minority of cases caused by *M. tuberculosis* (28%), at GF Jooste Hospital, whereas during our study period (March 2009-August 2009), TBM (44%) and CM (45%) contributed equally to this group. As discussed in Chapter 4, this proportional shift could be explained by the rapid increase in ART availability during this period, resulting in less patients reaching the severity of immune suppression that associates with CM. Our study emphasizes the poor outcome in TBM in spite of increased access to ART in the referral area; almost 50% of HIV-infected TBM patients were dead at 6-months follow-up and the majority of deaths occurred during the first month of treatment. Risk factors identified for mortality in HIV-associated TBM included low CD4 count, more severe TBM disease (BMRC grade II and III versus I) and not receiving ART during TB treatment. These findings highlight the urgent need to identify and treat HIV infection prior to the development of severe immunosuppression, and the need for evidence-based treatment strategies in TBM.

During the second study reported in Chapter 4 (Part 2 of Chapter 4) all patients who presented with CNS deterioration during the first year of ART over a 1-year period were enrolled, with the exception of those who presented with delirium secondary to a general medical condition. Seventy-five patients met the inclusion criteria which provided an incidence of referral of 23.3 cases [95% CI; 18.3–29.2] per 1000 patient-years at risk. This high incidence rate emphasizes the significant morbidity caused by

CNS disorders in HIV-infected persons, in spite of them receiving ART. This study further provides a comprehensive description of the most common differential diagnoses to consider in patients who present with CNS deterioration after ART initiation in our and other high TB/HIV co-infection settings. TB was the most frequent cause, accounting for 36% of cases, followed by CM that occurred in 24% of cases. Importantly, of patients who presented with CNS TB, the vast majority (16/27 cases) presented with paradoxical TB-IRIS. However, some other reasons for deterioration due to TB related to poor compliance to TB treatment in five, and MDR TB in two, patients respectively. These observations highlight the importance of obtaining a good history, and sending clinical specimens to rule out drug resistance, in neurological TB-IRIS suspects. Neurological TB-IRIS presented with diverse manifestations, including meningitis that was the most frequent manifestation, intracerebral tuberculoma (s), spinal arachnoiditis and a spinal epidural abscess. 15/16 TB-IRIS patients were alive, and outcome for one patient was unknown, at 6-months follow-up. Thirteen of these patients received corticosteroids during the IRIS episode, which might have contributed to their favorable outcome. However, a caveat is that a diagnosis could not be established in nine of 16 patients who presented with intracerebral space occupying lesions and of these nine, six were dead at 6-months follow-up. It is possible that some of these cases had TB-IRIS (manifesting as tuberculomas) and died as a result, and that we therefore underestimated the mortality associated with neurological TB-IRIS.

The studies reported in Chapters 5 and 6 prospectively investigated paradoxical TBM-IRIS in TBM patients. Over a 20-month period, ART-naïve HIV-infected patients who presented with TBM were enrolled. TBM

was diagnosed according to a published case definition and we excluded patients with the most severe TBM disease (i.e. BMRC disease grade III). Patients were started on TB treatment and prednisone and commenced ART two weeks later. We collected clinical specimens at these two timepoints as well as two weeks after ART initiation and at any time that a patient developed TBM-IRIS (which was diagnosed according to the INSHI case definition for paradoxical TB-IRIS modified for meningitis) as well as two weeks after IRIS presentation. Findings were compared between patients who developed TBM-IRIS and those who did not.

As presented in Chapter 5, TBM-IRIS was a frequent complication of ART in TBM patients; 47% (16/34) of patients included in the final analysis developed TBM-IRIS, indicating the importance of anticipating this complication in TBM patients who start ART. A short interval between starting TB treatment and ART is a risk factor for TB-IRIS and our patients commenced ART two weeks after TB treatment initiation. Disseminated disease is a further risk factor for TB-IRIS and all our patients had meningitis. These two factors likely contributed to the high incidence of TBM-IRIS in our study. As discussed in Chapter 2, current South African Department of Health guidelines suggest delaying ART to 4-6 weeks after TB treatment initiation in all TBM patients because of the heightened risk of developing life-threatening neurological TB-IRIS with early ART [1]. Low baseline CD4 count is a strong predictor of TB-IRIS [2, 3]. However, we observed only a trend for lower CD4 counts at ART initiation in patients who developed TBM-IRIS, which could be explained by our small sample size. Of importance is the finding of an association between high baseline CSF neutrophil count and subsequent TBM-IRIS. Although there was a wide range of CSF neutrophil counts both in patients who developed TBM-

IRIS and those who did not, clinicians should be aware of the increased risk of TBM-IRIS when neutrophils are present. Furthermore, the association between neutrophils and subsequent IRIS suggested a pivotal role for the innate system in TBM-IRIS pathogenesis, which directed our further studies presented in Chapter 6. We also found that a high bacillary load predicted TBM-IRIS; *M. tuberculosis* was cultured from the CSF of 15/16 TBM-IRIS patients, compared to 6/18 patients who did not develop TBM-IRIS, at TBM diagnosis. Culture remained positive in seven and two TBM-IRIS patients at two and four weeks on TB treatment, respectively, whereas none of the patients who did not develop TBM-IRIS were culture-positive at these subsequent timepoints. Given these observations, it is plausible that more rapid mycobacterial killing in the CNS early during TB treatment will decrease the probability of subsequent TBM-IRIS.

Corticosteroids did not prevent TBM-IRIS (13/16 IRIS patients were receiving prednisone at TBM-IRIS presentation). Although a possible explanation is that the dose was suboptimal to suppress CNS inflammation adequately, this finding highlights the need for studies to explore other immunomodulatory therapies for the prevention of TBM-IRIS. TBM-IRIS presented with severe, often atypical, features of inflammation such as radiculomyelitis and pachymeningitis. Corticosteroids were started or increased at TBM-IRIS presentation in all patients but regardless, two patients died due to TBM-IRIS. These finding suggests that corticosteroids at a dose of 1.5-2 mg/kg/day may be of benefit in some but not all TBM-IRIS cases. Although 15 of the 16 TBM-IRIS patients experienced symptomatic improvement two weeks after prednisone was increased or restarted at TBM-IRIS presentation, only two of the inflammatory markers measured in CSF decreased significantly at the later timepoint, whilst the others remained unchanged between these

two timepoints. Several reasons could account for this lack of effect on immune mediators in the CNS; firstly, our sample size at two weeks after TBM-IRIS presentation was small (n=10) which may have been inadequate to detect statistically significant differences; secondly, the time between sampling may have been too short to observe changes in CSF which were occurring in the brain; and lastly, we may have failed to include mediators that respond rapidly to corticosteroids in the panel that we measured. Our patients were followed up closely and corticosteroids were increased or started within days of symptom development. In busy public sector facilities, it may happen that affected patients are not routinely advised of the urgency of returning to hospital should any CNS symptoms recur after ART initiation. Patients may further become confused and for that reason fail to seek medical help. The mortality associated with neurological TB-IRIS may therefore be higher in routine settings, compared to what we observed. A vital component of the management of HIV-associated TBM patients due to start ART is a comprehensive discussion with the patient, and preferably a family member, about the risk of developing TBM-IRIS, the typical symptoms that could be expected and the need to return to hospital should any of these develop or worsen.

The immunopathogenesis of TB-IRIS is incompletely understood and we therefore analyzed mediators of diverse cellular origins and functions in the CSF and blood of TBM-IRIS and TBM-non-IRIS patients. Furthermore, considering our finding of the association between neutrophils and subsequent TB-IRIS, we included a panel of neutrophil-associated mediators in our CSF experiments. TBM-IRIS was associated with a broad inflammatory response compared to patients who did not develop TBM-IRIS at TBM diagnosis. This finding is contrary to that found in CM-IRIS,

where an attenuated inflammatory response at CM presentation predicted those who subsequently developed CM-IRIS. These differences suggest that more than one mechanism underlies pathological immunity in IRIS.

We further observed that after ART initiation, both TBM-IRIS as well as CSF *M. tuberculosis* culture-positive TBM-non-IRIS patients, developed recurrent CNS inflammation. These findings suggest that a high CSF bacillary load predisposes to a recurrent inflammatory response that manifests as IRIS in most, but not all patients. If not considered, this spectrum of inflammation observed after ART initiation may complicate the interpretation of findings of clinical studies investigating the immunopathogenesis of TB-IRIS. Importantly, neutrophils and their product S100A8/A9 were increased in TBM-IRIS patients compared to initially culture-positive TBM-non-IRIS patients at two weeks after ART initiation. This suggests a key-contributing role for neutrophils to the recurrent inflammatory response that manifests as clinical deterioration in patients with TBM-IRIS.

With regards to predictive markers for TBM-IRIS, in Chapter 5 we report the predictive value of the combination of CSF TNF and IFN-γ for TBM-IRIS in TBM patients when five cytokines implicated in TB-IRIS pathogenesis were included in a multivariate model. However, subsequent statistical analysis that combined all mediators detected in CSF revealed that, due to high inter-individual variation and the high number of analytes included in analysis, further exploration by multivariate methods was flawed. Initial studies focused on the role of T-cells, whilst recent studies are increasingly investigating the role of the innate immune system, in TB-IRIS immunopathogenesis [4]. Our findings of increased mediators of the innate as well as the adaptive immune system in TBM-IRIS patients imply that

both systems contribute to TB-IRIS pathogenesis.

Impact of the studies in the field

In spite of promising recent advances in rapid diagnostic tests, currently available laboratory methods lack adequate sensitivity to allow their use as definitive diagnostic tools and the majority of TBM diagnoses are still based on supportive clinical findings [5]. As discussed in Chapter 3, published clinical criteria for microbiologically unconfirmed TBM were not standardized prior to 2010 and this methodological heterogeneity complicated comparisons between research findings from different settings. The consensus clinical case definition for TBM presented in Chapter 3 is the first published case definition for use in clinical research that aims to be applicable to all TBM patients, regardless or age, HIV-status or resource availability. Since its publication, several studies have applied the case definition in various settings. Haldar *et al*. describes the utility of ELISA and qPCR for the detection of selected *M. tuberculosis* proteins and DNA for the diagnosis of TBM [6]. In their study, children (n=532) with TBM and unknown HIV status were classified as definite, probable, possible and not TBM cases according to the consensus case definition. A test combination of qPCR with two of the protein ELISAs (GlcB and HspX) accurately detected all TBM samples with a specificity of 90%. These results provide support for the utility of the consensus case definition in children with TBM. In a retrospective study, Hristea *et al*. classified children and adults (n=55) with definite TBM (i.e. either CSF culture or PCR positive for *M. tuberculosis*) according to the probable and possible TBM categories of the consensus case definition [7]. Four of these patients (7%) could not be classified as either probable or possible TBM cases. The authors suggest that the retrospective nature of

their study and the unavailability of some data required for the classification, might have contributed to some misclassifications. However, these results underscore the importance of further studies to validate the consensus case definition in diverse settings.

Neurological TB accounts for ~1 % of notified TB cases in resource-rich settings [8, 9], but due to poor epidemiological data the incidence in resource-poor settings, such as South Africa, is uncertain. Knowledge of the magnitude of the disease burden in our setting and the outcome of those affected is a crucial first step to determine the need for local interventional trials that aim to improve outcome in TBM. Our findings of the major contribution of *M. tuberculosis* as a cause of meningitis, and the poor outcome of affected patients, in our high TB/HIV setting, underscore the feasibility in terms of patient numbers, and need for, randomized controlled trials to investigate optimal TBM treatment strategies in Cape Town.

Prior to 2010 only one case series that described paradoxical neurological TB-IRIS existed [10, 11]. In these previous studies, we enrolled patients at neurological TB-IRIS presentation and their clinical and investigative findings and outcome were reported. The studies presented in Chapter 5 and 6 of this thesis are the only studies prior to and since 2010 to prospectively describe the clinical, laboratory and immunological characteristics of paradoxical TBM-IRIS from the time of TBM diagnosis. Furthermore, these are the first studies thus far to describe the immunological profiles of TB-IRIS at the site-of-disease. Our findings therefore not only contribute to knowledge in the field of TBM-IRIS per se, but also inform on TB-IRIS pathogenesis in general.

Research priorities in HIV-associated TBM

The current mortality rate associated with HIV-associated TBM is unacceptably high, thus a pressing research priority in this field is the identification of better evidence-based treatment strategies. Improving outcome in HIV-associated TBM may require a multistep approach targeting several stages of management, including the initial phase of TBM treatment and the initial phase of ART.

As discussed in Chapter 2, drug regimens used in the treatment of TBM are based on the principals of pulmonary TB treatment rather than being informed by evidence from randomized controlled trials in TBM. The majority of deaths in TBM occur within the first month of TB treatment and suboptimal CNS TB drug concentrations may contribute to this poor outcome. The first trial to compare two antimicrobial regimens in the treatment of TBM in which mortality is the primary endpoint, is ongoing in Vietnam [12]. In this randomized controlled trial, standard TBM treatment is compared with standard treatment plus an increased dose of oral rifampicin (increased from 10mg/kg/day to 15 mg/kg/day) and the addition of oral levofloxacin (20 mg/kg/day) during the intensive phase of TB therapy. Levofloxacin has high early bactericidal activity [13], is safe and well tolerated [14] and shows good CSF penetration [15] and is therefore an attractive treatment option for TBM. Furthermore, as discussed in Chapter 2, the standard dose of rifampicin may be inadequate in neurological TB patients. Results are awaited, but even if the intensified regimen is associated with improved outcome, administering drugs intravenously may provide an additional mortality benefit, particularly in HIV-infected patients. Malabsorption may contribute to decreased systemic drug bioavailability

in HIV-infected persons due to enteric infections such as cryptosporidiosis and HIV-associated enteropathy [16]. TBM patients also often present critically ill, vomit and receive drugs through a nasogastric tube. All these factors may further associate with reduced TB drug concentrations if drugs are administered enterally. The randomized controlled trial by Ruslami *et al*. [17] suggested a mortality benefit in patients who received higher-than-normal dose intravenous rifampicin during the early treatment of TBM, compared to oral standard dose rifampicin. However, the study design was not sufficiently robust (considering that mortality was not the primary endpoint) for results to change treatment policy globally, hence larger treatment trials to explore intravenous strategies further are warranted.

Recent studies suggest that host genetic variability may influence the response to adjunctive corticosteroid treatment in TBM [18]. Leukotriene A4 hydrolase (LTA4H) controls the balance of pro- and anti-inflammatory eicosanoids; an excess of LTA4H results in increased TNF and excessive inflammation, whilst a deficiency of LTA4H results in decreased TNF and attenuated inflammation. In both zebrafish and TBM patients, a single nucleotide polymorphism (SNP) in the LTA4H promoter (rs17525495) was associated with hyperinflammatory (TT homozygous) and hypoinflammatory (CC homozygous) phenotypes. Heterozygosity (CT) for the SNP resulted in an intermediate inflammatory phenotype. Survival analysis of the TBM patients revealed that homozygosity (both TT and CC) was associated with the highest mortality. In a subgroup of patients who received dexamethasone, an associated survival benefit was observed in those with the hyperinflammatory phenotype, whilst being detrimental in those with the hypoinflammatory phenotype. These findings raise important questions of our current generalized approach with regards to adjunctive

corticosteroids in TBM. As the authors suggest, it may be advantageous to use opposite immunomodulatory strategies depending on a patient's LTA4H genotype, with those with the TT genotype receiving immunosuppressive therapy, whilst those with the CC genotype receiving drugs that augment immune responses. However, only Vietnamese patients were included in these studies and the results need to be confirmed in different ethnic populations. Furthermore, due to high costs, limited availability and the time it may take for results to become available, such genetic testing is not currently feasible in resource-poor settings.

An ironic contribution to poor outcome in HIV-associated TBM is neurological TB-IRIS, which typically occurs within weeks after ART initiation. Both TB treatment and ART are life-saving in TB/HIV co-infected patients but in patients with TBM the combination of these two therapies may result in recurrent CNS inflammation and, as a consequence, death. Neither prevention nor treatment trials, which are imperative to inform optimal management strategies in HIV-associated TBM, have been performed for neurological TBM-IRIS. Trials to compare corticosteroids with placebo for the treatment of neurological TB-IRIS would be unethical, given the suggested benefit of corticosteroids in this context. However, trials could potentially compare the safety and benefit of different corticosteroid doses and routes of administration (intravenous versus oral), neither of which are standardized in neurological TB-IRIS treatment. There is a paucity of data in the literature regarding the roles of other drugs in the treatment of TB-IRIS; only case reports of the benefit of immunomodulatory agents such as thalidomide and leukotriene antagonists exist [19]. Thalidomide is not consistently associated with improvement in neurological TB-IRIS cases [20], and its use is further limited by its strong association with teratogenicity

and potential toxicities, such as peripheral neuropathy. The leukotriene antagonist montelukast has a favorable side effect profile compared to corticosteroids, making it a good candidate for prevention and treatment trials in neurological TB-IRIS. TNF blockers such as infliximab and adalimumab are further potential treatment options as infliximab has shown benefit in steroid-resistant paradoxical CNS TB reactions in HIV-uninfected patients [21], and adalimumab has been used with success in a case of CM-IRIS [22]. However, a serious concern in HIV-infected TBM patients, most of whom are severely immunosuppressed, is the increased risk of severe infections that is associated with TNF blocker use, particularly when considering the long half lives (10-20 days) of these agents. Given the limited evidence for the benefit of these alternative immunomodulatory drugs in neurological TB-IRIS, and considering the potential serious adverse events associated with many of these agents, it may be advantageous to first perform studies to assess their immune modulatory effects in animal models of TBM prior to conducting human trials in neurological TB-IRIS.

A better understanding of the immunopathogenesis of TB-IRIS is crucial to identifying future drug candidates for patients both at risk of, and affected by, neurological TB-IRIS. Studies that explore the cellular sources of inflammatory mediators in the CNS of these patients may shed light on the sequence of events that ultimately result in TB-IRIS and thus inform on crucial steps in the dysregulated immune pathway (s) that might be amenable to drug intervention. To this effect, research should include *in vivo* studies to determine types and proportions of CSF white blood cells and their contribution to cytokine production by flow cytometric methods in patients with TBM and TBM-IRIS, as well as *in vitro* studies of *M. tuberculosis*-induced inflammatory mediator production by cultured human CNS cells

such as microglia, astrocytes and neurons. Future research should also continue the quest to identify thus far elusive diagnostic biomarkers for TB-IRIS, which will likely contribute significantly to improved management and outcomes of patients with neurological TB-IRIS.

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