Diagnostic evaluation of smear negative tuberculosis in a resource poor setting

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Diagnostic Evaluation of Smear Negative Tuberculosis in a Resource Poor Setting

Mala George Otieno

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Abbreviations

AFB	Acid Fast Bacilli
AUC	Area Under Curve
BMI	Body Mass Index
CT	Computer Tomography
CXR	Chest X-Ray
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Treatment-short Course
DST	Drug Susceptibility Testing
FDCs	Fixed Drug Combinations
FGDs	Focus Group Discussions
HIV	Human Immunodeficiency Virus
HRCT-scan	High Resolution Computer Tomography-scan
IFN- γ	Interferon-gamma
ISTC	International Standards for Tuberculosis Care
LMICs	Low-and Middle-Income Countries
MDR-TB	Multidrug Resistant-TB
MGIT	Mycobacterium Growth Indicator Tube
MTB/RIF	New molecular test for detecting the presence of <i>Mycobacterium TB</i>
NO	Nitric Oxide
ORs	Odds Ratios
PCR	Polymerase Chain Reaction
QFT test	Immunological test for detecting the presence of <i>Mycobacterium TB</i>
RHZ	Rifampicin, Isoniazid and Pyrazinamide
RHZE	Rifampicin, Isoniazid, Pyrazinamide and Ethambutanol
RNS	Reactive Nitrogen Species
ROC curve	Receiver Operating Characteristic curve
TB	Tuberculosis
WHO	World Health Organization

Chapter 1

General introduction

1.1 INTRODUCTION AND RESEARCH SCOPE

The 22 high-burden Tuberculosis (TB) countries account for 95% of world's TB cases and 98% of TB related deaths [1]. Only 14 of these countries reached the 85% target for treatment success among all new cases in 2010 [2]. This despite the availability of highly effective TB therapy that could cure 90% of cases, and an intensive worldwide effort to improve access to the diagnostic and treatment interventions embodied in the directly observed treatment-short course (DOTS) strategy. Apart from that, in Ethiopia smear-positive case detection rate remains at 34%, far less than the World Health Organization (WHO) estimate of 70% [3]. The regional disaggregation showed highest case detection rate in urban administrations (Harari 95%, Dire Dawa 81% and Addis Ababa 63%); whereas, Somali, Amhara and Tigray regions performed low at 19%, 23%, and 26% respectively. Furthermore, the performance of the diagnostic and treatment guideline is far from good in real life [4], as it is often negatively influenced by local factors and practice related context [5,6]. Consequently, the relative importance of the different characteristics of a diagnostic test depends upon the setting in which the test is to be performed and the intended use of the results. Hence, the focus for TB control has moved towards reaching successful treatment of all cases of TB through offering a standard of care consistent with TB guidelines [7]. However, recent studies have reported multiple pitfalls in the diagnosis and treatment of TB patients, even in regions where TB might be considered a rarity [8-10]. These pitfalls echo a fundamental principle of TB control espoused by Piot more than 40 years ago, that a substantial proportion of TB cases occur among individuals who fail to access high-quality diagnostic and treatment services [11]. Therefore, this research will seek to offer real-life examples of what is needed to customize TB diagnostic guidelines to local contexts of healthcare in order to improve compliance.

1.2 BACKGROUND

1.2.1 Global quality of TB care

TB remains a major global health problem. In 2012 an estimated 8.6 million people developed the disease, and 1.3 million people died [12]. Great progress has been made in combating TB. The 2015 Millennium Development Goal of halting and beginning to reverse the incidence of TB has been achieved, and a target of 50% reduction in mortality compared with 1990 is on track. However, in some parts of the world, like Africa, this target may not be reached, and the global elimination target set for 2050 does not currently seem within reach, because TB incidence is declining far too slowly. Current TB control strategies in many countries are crucially dependent on care that is provided to persons who have symptoms of TB and those who have the disease. When TB is not

considered and appropriately evaluated in persons with early symptoms, the resulting delay promotes further transmission of infection and more severe disease [13, 14].

Thus, individual care of people with TB symptoms or disease is the key and, at the same time, the vulnerable point of current public health actions for TB control. Substantial variations in the clinical approach of persons with TB symptoms or disease have been observed within and among countries, in the public and private health care sector, and among clinicians in the same health facility [15, 16]. These variations can have important implications for TB control. While many national TB control programs have progressively adopted the recommendations for TB care of the World Health Organization (WHO), private practitioners, non-governmental caregivers, and academics often have perceived national TB control program guidelines as inferior or not applicable to their settings. To address these concerns and ensure maximum adherence to scientific evidence, an international coalition of organizations led by the WHO and the American Thoracic Society published the first edition of the International Standards for TB Care (ISTC) in 2006. The ISTC aims to unify approaches to clinical care of persons with TB symptoms or disease, irrespective of the country or setting in which care is taking place [17].

1.2.2 TB burden in Ethiopia

Ethiopia is one of the world's high TB, TB/HIV and multi-drug resistant TB (MDR-TB) burden countries. Prevalence and incidence of TB are 210 and 224 per 100 000 persons respectively [18]. The estimated TB case detection rate (percentage of detected cases among the estimated number of incident cases) in Ethiopia has been consistently low; the WHO estimate is 64% for all forms. According to the same WHO report, there were 200 000 prevalent TB cases in Ethiopia in 2013 (211 per 100 000). TB mortality is estimated at 30 000 deaths (excluding deaths among HIV positive TB cases) or 32 deaths per 100 000 per year. In a recent Ethiopian national population based survey in 2011, the prevalence of bacteriologically-confirmed TB (smear and/or culture positive) for persons aged 15 years and above was 277 per 100,000 [19]. The prevalence of smear positive pulmonary TB was 108 per 100000, or 63 per 100000, including children. The population-based TB survey concluded that TB burden in Ethiopia was lower than previously thought, which may indicate better program performance. However, a high proportion of TB among young persons' suggests that TB is circulating in the community and that there is a need for more efforts to limit the spread of TB disease [20].

Both men and women are equally vulnerable to TB in their productive ages of 20-30 years. Gender differences in health-seeking behaviour might contribute to differences in delay in TB diagnosis, treatment interruption, gender based TB stigma and discrimination [21]. Low detection rate and treatment for TB among women have been seen due to delay in accessing care, poor compliance to treatment, and belief in alternate treatments [21]. The reporting system does not disaggregate data by sex, but a recent study

found no difference in diagnostic delay and treatment by sex [21]. The delay in males was higher but not statistically different.

1.2.3 TB diagnostics

In its Global TB Report 2013, the WHO highlighted detection of missed cases as a priority action to reach 2015 global targets [22]. The report notes “TB remains unique among the major infectious diseases in lacking accurate and rapid point-of-care tests, largely due to insufficient progress in biomarker discovery for TB diagnosis. The most pressing priority in TB diagnostics research today is the development of a simple, low-cost, instrument-free rapid test of suspected patients”. Currently, the diagnosis relies on demonstration of the presence of bacteria in clinical specimens by serial sputum smear microscopy and culture. When applied to resource-limited settings, these methods suffer from significant limitations. Serial sputum smear microscopy requires the patient to make repeated trips to the clinic, which often is cost-prohibitive for the patient, resulting in the inability to properly diagnose and treat the patient. In addition, TB diagnostic tests are either too expensive, too time consuming (24 hour or more), or require specialized equipment, expertise, and power [23–25]. Furthermore, diagnostic tests have low sensitivities (50% in the sputum smear test), or are unreliable when used on HIV-positive patients or children [26–28].

1.2.4 Providers adherence to diagnostic guidelines

Substantial effort has been devoted to improving physician compliance with evidence-based guidelines. Physicians are under increasing pressure to comply with evidence-based guidelines and to achieve high performance measures. Nevertheless, compliance with so-called best practices is far from ideal [29–31]. The barriers to guideline compliance have been categorized into three domains: physician knowledge (lack of awareness, lack of familiarity, or oversight), physician attitudes (lack of agreement; lack of self-efficacy that is, the belief that a physician can perform guideline recommendations; lack of outcome expectancy that is, skepticism that complying with the guideline would help patients; or the inertia of previous practice), and external barriers [32].

Studies of the quality of TB evaluation (i.e. diagnostic workup of patients with symptoms suggestive of TB) in high burden countries have generally shown poor adherence to international or national guidelines. ISTC, Edition 3 TB CARE I, The Hague, 2014, which has been endorsed by nearly all TB programs worldwide, recommended that at a minimum, all patients in high burden countries with cough of at least 2 weeks’ duration should have at least two sputum smears examined for acid-fast bacilli (AFB) and be treated for TB if sputum AFB is smear-positive. Previous studies found that providers are not following guidelines for TB diagnosis and treatment, both from the public and private sector in many other high burden countries [33–39]. While previous research,

including quantitative [40-42], qualitative [43-49] and mixed methods approaches [50], has assessed barriers patients face in accessing primary care centers that provide TB diagnostic services, less is known about barriers providers in these settings face in adhering to guidelines for evaluating patients for TB. The two studies evaluating provider perspectives have focused on their perceptions about patient barriers to accessing TB care [43, 51]. Less well understood are the determinants of provider adherence to TB guidelines. In particular, there is increasing recognition that guideline implementation is heavily dependent on provider behaviour and in order to improve the quality of care, understanding and subsequently changing provider behaviour is required.

1.2.5 Pathway to better diagnostics

Poor diagnosis remains a major obstacle to global TB control. In most high-burden countries, TB is still diagnosed using tools such as direct sputum microscopy and chest radiographs. Although a large number of systematic reviews have been published on TB diagnostics, almost all focus on test accuracy (i.e. sensitivity and specificity) [52]. Evidence on test accuracy is essential, but policy development requires more than that. Along with data on test accuracy, there is need to consider user-important as well as patient-important outcomes [53, 54]. User-important outcomes consist of practical concerns for the usability of a test in real-practice situations. Although these generally do not require fundamentally different strategies to evaluate, it is important that they are assessed under implementation or real-live settings. Wherein a study shows that implementing a diagnostic test in a given situation results in clinically relevant improvements in patient care and patient outcomes. For TB diagnostics, this might mean an increased number of patients detected and receiving appropriate treatment, fewer patients defaulting from the diagnostic pathway due to reduced numbers of patient visits, or more patients cured due to accurate detection of drug resistance [44, 52]. These types of evidence must be taken into account, along with test accuracy and reliability, when policy makers or programs are evaluating a diagnostic test or procedure for recommendation or widespread use [50]. Systematic reviews of diagnostics should make an effort to summarize data on these outcomes in addition to accuracy, appraise the quality of available evidence, and explore the uncertainty regarding the often assumed values and preferences of patients associated with these tests.

1.2.6 Predictive model for diagnosis of TB

Predictive models for the diagnosis of TB provide a useful framework for systematization of the diagnostic approach and are able to standardize data collection from clinicians [55]. Numerous decisions are made by care providers, on the basis of an estimated probability that a specific disease or condition is present (diagnostic setting) in an individual. In the diagnostic setting, the probability that a particular disease is present

can be used, for example, to inform the referral of patients for further testing, to initiate treatment directly, or to reassure patients that a serious cause for their symptoms is unlikely. In diagnostic settings, probability estimates are commonly based on combining information from multiple predictors observed or measured from an individual [56, 57]. Information from a single predictor is often insufficient to provide reliable estimates of diagnostic probabilities or risks [58]. In virtually all medical domains, diagnostic multi-variable (risk) prediction models are being developed, validated, updated, and implemented with the aim to assist doctors and individuals in estimating probabilities and potentially influence their decision making. These models could be used to identify patients at very high risk of TB that may require further diagnostic tests after the results of a negative smear test. Besides, further research is necessary to determine the usefulness of these prediction models to establish the exact causes of false smear negative TB (patients that have TB, but have a negative smear test), that are still unknown. These models are suited to resource-poor high TB burden settings, where the available diagnostic test is perceived to be poor and some variables may have lost their predictive value.

1.2.7 Clinical need for biomarkers of extent of tuberculosis

Biomarkers are measurable characteristics that indicate normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention [59]. It has been suggested that volatile metabolites from *Mycobacterium tuberculosis* (*M. tuberculosis*) organisms may be exploited to diagnose TB rapidly and could be used as biomarkers of cure and relapse [60]. However, the metabolite pattern found in individuals will vary greatly depending on the time of the day when the blood or urine sample is taken, and food, fluid or drug intake before sample collection, etc. For clinical trials, standardization of patients will become crucial. Although no published results exist for TB biomarker discovery using advanced technological platforms, metabolomics have been used to analyse several other conditions [61] and there is good reason to believe that similar approaches might be of benefit in TB biomarker discovery. Hosts as well as mycobacterial metabolites might be able to provide insight into host–pathogen specific interactions as well as disease state and treatment response and might be able to predict final outcome. This is an area of much interest and may lead to a practical, non-invasive way of diagnosing TB at point of care. However, there is no evidence yet that these markers can predict microbiological and treatment outcome.

1.2.8 Nitric oxide in TB

Nitric oxide (NO) is a signaling molecule with several well-known biological functions including vasodilation, neurotransmission and immune response [62]. Several animal and macrophage experiments have shown that NO and related reactive nitrogen spe-

cies (RNS) constitute a major host defense mechanism against intracellular pathogens including *M. tuberculosis*, in both the acute and the latent phases of infection [63, 64]. It has also been shown that RNS are actively produced in human TB, although their relative importance is controversial [65–70]. Thus, uncertainties exist for the use of NO to diagnose TB prior to initiation of therapy, the most important being the degree to which this biomarker can differentiate recent or progressive from latent infection [71]. Inability to distinguish these two states will greatly reduce its potential to add value to clinical decision-making in TB-endemic regions, where a majority of adults would be expected to have been exposed to *M. tuberculosis*. There are many studies that suggest that the magnitude of the immune response reflects the magnitude of the bacterial load and two longitudinal studies in untreated contacts suggest that the highest responders have the highest risk of TB [72,73], but these studies are relatively small and lack detailed sequential testing routines. Thus the kinetics and duration of the differences are unknown; however, the fact that the two studies produced similar results in very different populations and environments (Ethiopia and Germany) is encouraging. Studies to address the questions of remote versus recent infection, predictive value and optimization of the assays, should prove very valuable.

1.3 OBJECTIVES AND OUTLINE OF THE THESIS

The studies presented in this thesis focus on the quality of diagnostic care provided to suspected TB patients and centre on four principle investigations. The central aim of the studies presented in **Chapters 2 and 3** was to improve understating of the current quality of diagnostic care and reasons for not following the guidelines. To achieve this, in **Chapter 2** we first determined the quality of diagnostic care offered to patients with smear-negative TB compared with smear-positive cases. We primarily evaluated clinical measures commended by ISTC which represent activities that TB care providers perform mostly themselves and are consistent with the national Ethiopian TB guideline. While, in **Chapter 3** we explored health care providers' practice-related and context-specific reasons for non-compliance to diagnostic procedures at health facilities in Ethiopia. Our findings may offer real-life examples of what is needed to customize TB guidelines to local contexts of health care in order to improve compliance. We also examined whether a barrier is actual or perceived, as this may also affect the type of intervention needed to overcome it. The overarching aim of the study presented in **Chapter 4** is to develop strategies to diagnose TB in patients whose initial test is negative for smears. These cases are mostly culture-negative TB and their diagnosis is problematic. Therefore, a prospective study was designed to determine the predictors of culture-negative TB among smear negative TB cases. Finally, in the Global TB Report 2014, the WHO declared that "one of the most urgently needed tests is a rapid biomarker-based test that can diagnose TB". Ideally, such TB biomarker should be able to detect the earliest

and most difficult to diagnose TB cases, and should be suitable for use in resource-limited settings. Although previous studies show that the presence of NO and its metabolites are potential screening biomarkers for TB, its relative importance is debated. Therefore, there is very limited evidence on NO in direct diagnosis of TB and in addition to lack of clinical studies describing the level of salivary NO in suspected TB patients. So, in **Chapter 5**, we evaluated the role of salivary NO in TB diagnosis.

These above aims raise the following study objectives:

1. To determine quality of diagnosis and monitoring of treatment response of patients with smear-negative TB compared with smear-positive cases in Ethiopia.
2. To explore TB service providers' reasons for not complying with TB treatment guidelines.
3. To evaluate the performance of predictors for estimating the probability of tuberculosis (TB) (1) among all clinical suspects and (2) among smear negative cases.
4. To assess the diagnostic value of the level salivary NO metabolites with microbiological outcome (infectiousness), and its association with the severity of the disease (commonly seen as wasting) in TB suspects.

Finally, we consider the main findings, methodological issues and general conclusion in a **general discussion** chapter. We conclude with a brief **summary** of the overall findings of each study presented in this thesis and implications, both for the theoretical development of research on TB care and specific public policies for professional practice.

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Chapter 1

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Chapter 2

Quality of diagnosis and monitoring of tuberculosis in Northern Ethiopia: medical records-based retrospective study

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ABSTRACT

Objectives: To determine quality of diagnosis and monitoring of treatment response of patients with smear-negative pulmonary tuberculosis (TB) compared with smear-positive cases in Ethiopia.

Methods: A retrospective analysis of medical records of newly diagnosed pulmonary TB cases that were registered for taking anti-TB medication and had completed treatment between 2010 and 2012. We evaluated the percentage of cases that were managed according to the International Standards of Tuberculosis Care (ISTC) and compared smear-negative with smear-positive cases.

Results: We analyzed 1168 cases of which 742 (64%) were sputum smear-negative cases. Chest radiography examination at diagnosis and microbiological testing at the end of the intensive phase of treatment was performed in a smaller proportion than in smear-positive TB cases (70% vs. 79%, P value <0.001) and (70% vs. 95%, P value <0.001), respectively.

Conclusions: Clinical actions recommended in the ISTC are of greatest importance in minimizing pitfalls in care of smear-negative TB yet were performed less often in smear-negative than smear-positive TB cases.

INTRODUCTION

Despite available treatment that could cure 90% of cases, there were 8.7 million tuberculosis (TB) cases and 1.4 million deaths from TB worldwide in 2011 [1]. Moreover, of the 22 high-burden TB countries, only 14 reached the 85% target for treatment success among all new cases in 2010 [1].

The focus for TB control has moved towards reaching successful treatment of all cases of TB through offering a standard of care consistent with TB guidelines [2]. To achieve this, the ISTC provides a widely accepted protocol of care that all providers should follow in managing patients who have suspected TB.

The degree to which clinical actions specific for smear-negative TB are performed is largely unknown, owing to its relatively low public health priority [3] and there is an assumption that the prognosis for smear-negative cases is better than for smear-positive cases [4, 5]. We hypothesized those clinical measures related to care for smear-negative TB patients may be poorly followed compared to smear-positive TB cases.

To understand the current diagnosis and monitoring of treatment response of TB, this study determined the proportion of TB cases in which clinical measures were followed according to ISTC 5 and 10 [2], in both smear-negative and smear-positive cases, diagnosed and treated in four different hospitals. A critical analysis of current clinical actions against these agreed standards, could lead to the identification of the most prevalent gaps in TB care. Accordingly, if measures are taken to bridge these adherence gaps in performed clinical actions, usual care will become more effective, for both smear-negative and smear-positive TB.

METHODS

Study design

We conducted a retrospective review of medical records of four hospitals in Ethiopia. All adults aged 18 years and above, newly diagnosed pulmonary TB cases that were registered for taking anti-TB medication and had completed treatment from September 2010 through December 2012, were included in the study. Patients were excluded if they: (1) had a previous history of TB; (2) had extra-pulmonary TB; (3) had no clinical data about treatment completion; (4) were documented to have a concurrent HIV infection, as there are differences in necessary clinical actions for these patients; and (5) if what was recorded in the patient's chart, TB treatment logbook and laboratory logbook was contradictory. All patients diagnosed with TB were also routinely tested for HIV. Currently the prevalence of HIV in Ethiopia is estimated to be 2.1% and 41% of TB patients are estimated to have co-infection with HIV [6, 7].

ISTC 5 on diagnosis

Sputum smear microscopy is critical in the diagnosis of TB. Suspected patients are treated for smear-positive TB if the test is positive on admission. If the test is negative, but there is a strong suspicion of TB based on clinical symptoms, diagnosis of TB may be made by other means. According to ISTC 5, the following actions should be performed after a patient has an initial smear-negative test: (1) repeat the sputum test; (2) start antibiotic treatment; and (3) perform a Chest x-ray radiography (CXR). Thereafter, patients suspected of having TB (e.g. compatible radiograph and clinical symptoms) should be treated as smear-negative TB patients. Patients who are not treated for TB should receive abroad based antibiotic to treat other possible bacterial infections. The antibiotic course should be completed.

ISTC 10 on monitoring treatment response

In addition to TB diagnosis, sputum smear microscopy has further use in monitoring treatment response. In both smear-negative and smear-positive, the treatment response should be monitored at the time of completion of the initial phase of treatment (2 months). If the sputum smear is positive at this stage, sputum smears should be examined again at 3 months, and if still positive, culture and drug susceptibility testing should be performed. However, the costs and complexities of routine cultures for TB as well as the delay in receiving results limit the widespread use of sputum culture. New diagnostic strategies using the more sensitive polymerase chain reaction (PCR) amplification of *Mycobacterium tuberculosis* deoxyribonucleic acid (DNA) have recently been introduced, but these may be too expensive for wide-spread use in resource-limited settings [8, 9]. Patients whose sputum smear microscopy was negative before treatment as well as after 2 months need no further sputum monitoring and should be monitored clinically, for which body weight is a useful progress indicator, because this should increase with successful treatment.

Study setting

Patients were recruited from four of the six hospitals in the Tigray region in Ethiopia. In these hospitals the Ethiopian TB guidelines are used for diagnosis and monitoring of treatment response. The ISTC recommendations are cross-referenced into the Ethiopian guidelines [10]. However, the former differ from the latter in that they recommend which clinical measures should be followed, whereas the Ethiopian guidelines describe the sequence in which the measures should be accomplished.

TB treatment consists of two phases, the intensive and continuation phases. During the first, ambulatory patients who live in communities far from the TB clinic are supplied with drugs and then referred to a health facility near their place of residence for

Directly Observed Therapy under Supervision (DOTS) by a TB service provider on a daily basis. Patients living close to the TB clinic go to the hospital where their TB was diagnosed for DOTS every morning. TB patients are only admitted to hospital if they are very ill and are discharged when they can walk by them-selves; there is no isolation for those with positive sputum smears.

In the continuation phase, all patients are seen at the TB clinic every month on an ambulatory basis, irrespective of where DOTS was administered during the intensive phase. Monitored treatment response, all medications given, the bacteriologic response, and adverse reactions, are all recorded. Treatment is provided free of charge to all TB patients in Ethiopia.

TB data records and data collection

The register is maintained at local treatment units and comprises a list of all persons who have been diagnosed with TB. It contains entries for demographic and clinical characteristics including status of patients follow-up, laboratory tests results and treatment outcome. Occasionally the healthcare worker in charge of a TB unit does evaluate practices recommended by national guidelines and the ISTC, and reminds local staff to document these practices on the clinical encounter form. These clinical actions are cross-checked to evaluate completeness, accuracy and promptness of record-keeping, and for the purpose of programme auditing.

Table1. Patient profile at diagnosis

	Proportion of variables by hospital settings					P-value
	<i>Hospital 1</i>	<i>Hospital 2</i>	<i>Hospital 3</i>	<i>Hospital 4</i>	<i>Total</i>	
Baseline Characteristics	<i>n=306</i>	<i>n=279</i>	<i>n=283</i>	<i>n=300</i>	<i>n=1168</i>	
Age (years; mean (SD))	38.8 (15.9)	39.2 (17.1)	35.4 (14.1)	36.1 (15.3)	37.4 (15.7)	0.017
Male gender (n (%))	170 (56%)	156 (56%)	153 (54%)	165 (55%)	644 (55%)	0.981
Mean weight (kg (SD))	45.8 (8.0)	45.9 (7.5)	44.7 (9.1)	46.7 (8.8)	45.8 (8.4)	0.077
Smear-negative TB (n (%))	234 (77%)	169 (61%)	171 (60%)	168 (56%)	742 (64%)	< .001
Smear-positive TB (n (%))	72 (23%)	110 (39%)	112 (40%)	132 (44%)	426 (36%)	< .001
Symptoms compatible with TB (n (%))						
Cough	178 (58%)	279 (100%)	236 (83%)	273 (91%)	966 (83%)	< .001
Haemoptysis	5 (2%)	11 (4%)	64 (23%)	67 (22%)	147 (13%)	< .001
Night Sweating	260 (85%)	276 (99%)	163 (58%)	206 (69%)	905 (78%)	< .001
Auxiliary Temperature >37.8°C	242 (79%)	13 (5%)	50 (18%)	118 (39%)	423 (36%)	< .001

SD standard deviation

A structured data collection checklist was designed and tested before data collection. A team of four registered nurses working in TB clinics attended a 1-day workshop about the structured data sheet provided by the principal investigator, and were trained for data collection. Data were extracted from TB clinic registries based on paper patient charts, paper TB treatment logbooks and paper laboratory logbooks. From patient charts we collected demographic characteristics and clinical features (symptoms, CXR findings and prescription of broad-spectrum antibiotics). Laboratory results (smear results, type of TB) and clinical monitoring of body weight were collected from TB treatment logbooks. We counter-checked TB treatment logbooks and laboratory logbooks to confirm if the sputum smear test was performed.

Data analysis

Data were analyzed using SPSS version 19 (SPSS, Inc., Chicago, IL, USA). Descriptive analyses were employed to calculate the proportion of TB cases that were diagnosed and monitored according to ISTC protocol. Differences between smear-negative and smear-positive cases were analyzed by applying Pearson's Chi-square tests for dichotomous outcomes and Mann-Whitney U tests for continuous outcomes. For testing differences between the four different hospitals, we used Pearson's Chi-square and Kruskal-Wallis tests.

Personal identifiers were not collected and data were analysed anonymously. The study protocol was reviewed and approved by the Institutional Review Board of the Research and community service of College of Health Sciences of Mekelle University (ERC 0304/2010). A letter of permission to review medical records was obtained from the office of the Director of Ayder Referral Hospital.

RESULTS

Patient profile at diagnosis

We selected 1168 medical records of newly diagnosed pulmonary TB cases that were registered for taking anti-TB medication and had completed treatment from September 2010 to September 2012 (Table 1). In total, 742 (64%) were smear-negative and 426 (36%) smear-positive. Patients' age at diagnosis was in the range of 18–86 years with a mean of 37 years and 644 (55%) were male. Almost all patients' weights were measured at diagnosis with a remarkably low mean of 45.8kg. The most common recorded symptoms compatible with TB were cough (83%), night sweating (78%), fever (36%) and hemoptysis (13%), but there were significant differences between the different hospitals.

Performed clinical actions

Microbiological testing by sputum smear microscopy was always performed (100%) at diagnosis and at the end of the continuation phase of treatment (Table 2). However, microbiological testing at the end of the intensive phase of treatment, to monitor response to treatment, was performed in a higher proportion in smear-positive than smear-negative cases (96% vs. 70%, P value <0.001). A significant difference (P value <0.001) in the proportion of patients who received CXR examination at diagnosis was observed between the hospitals. Interestingly, CXR examination was performed at diagnosis less often in smear-negative than in smear-positive cases (70% vs. 79%, P value <0.001). In Hospital 1, only half of smear-negative cases were evaluated by CXR examination compared to 100% in smear-positive TB cases (P value <0.001). Broad-spectrum antibiotics were never prescribed. Weight was always measured at diagnosis in all hospitals. However, weight monitoring at the end of the intensive was averagely performed less often in smear-negative than smear-positive TB cases (27% vs. 52%, P value <0.001). However, monitoring of weight at the end of the continuation phase was rarely done across all settings except in Hospital 4.

DISCUSSION

Our findings show that a higher proportion of clinical actions were omitted during diagnosis and monitoring of treatment response in smear-negative compared with smear-positive TB cases, despite the fact that the clinical actions were especially important for the former. We also observed large differences between hospitals regarding ISTC adherence.

Performing CXR examination at diagnosis in smear-negative TB cases was only performed in 70% of cases, and significantly less often in smear-negative than in smear-positive cases. This is a determining factor especially in the diagnosis of smear-negative TB where diagnostic evaluation is challenging, even when all appropriate investigations have been conducted [11]. Besides, clinical monitoring of body weight at the end of the intensive phase, which is an important indicator of treatment response in smear-negative cases, was performed less often than in smear-positive TB cases. Hence, it is difficult to understand how smear-negative TB patients were monitored clinically.

Microbiological testing at the end of the intensive phase was on average done less in smear-negative than smear-positive TB cases. It is also difficult to understand how patients whose sputum smear microscopy was negative at the start of treatment, were re-checked at the end of the intensive phase either to rule out non-response to treatment or an error at the time of initial diagnosis (i.e. a true smear-positive patient was misdiagnosed as smear-negative). It is surprising that there was little use of such easily measured and inexpensive markers such as sputum and weight monitoring.

The overall performed clinical measures also differed significantly between the hospitals despite the available basic infrastructure to provide TB services being of comparable quality. Significant differences in performance of CXR examination at diagnosis, microbiological testing at the end of intensive phase and monitoring of body weight were observed between hospitals. For example, Hospital 4 almost fully met the desired standard in diagnosis and monitoring treatment response in all TB cases, while Hospital 3 performed poorly. Although we did not evaluate infrastructural differences between hospitals, a study in a similar resource poor settings reported no relationship between quality of structural facilities and quality of TB care in hospitals [12]. However, healthcare providers' expertise in management of TB was linked to quality of care [13]. Therefore, dissimilarities in performed clinical actions in our study are related to findings by WHO and others [14-16] that have shown that clinicians often deviate from internationally recommended TB management. Strategies that increased clinicians' adherence to set standards in some hospitals need to be identified and introduced to the less adherent hospitals in order to improve the quality of TB care.

Relevance for healthcare

The performance of the WHO's diagnostic algorithm is far from good in real life [17], since it is often negatively influenced by local factors and practice-related context [14,18]. Evaluating quality of TB care may be more realistic in routine care, if the overall performed clinical actions are assessed instead of assessing the sequence of actions in the diagnostic algorithm [19]. In our study, we assessed the overall performed clinical actions in the targeted standards which are of greatest need in minimizing pitfalls in diagnosis and monitoring response of treatment in smear-negative TB patients and reported that they were done less often in smear-negative than smear-positive TB cases. Our findings add that care offered to smear-negative cases continues to be deviant from the standards, compared to smear-positive TB cases. This could be due to the assumption that the prognosis for smear-negative cases is better than for smear-positive cases. However, this assumption can only be valid if microscopy procedures are followed completely and reliably [20-22]. It has regrettably been shown that in many low-income settings, laboratory procedures are not optimal [14, 23, and 24]. Therefore, there is a need to explore the reasons behind poor standardized clinical work-up in smear-negative compared to smear-positive TB cases.

Table 2. Percentages of TB cases in which clinical actions were performed by settings

Performed Clinical actions (n (%))	Smear-negative PTB (SNPTB)					Smear-positive PTB (SPPTB)					P-value difference between SNPTB and SPPTB	
	Hospital 1				P-value between Hospitals	Hospital 2				P-value between Hospitals		
	n=234	n=169	n=171	Hospital 3 n=168		Total n=742	n=72	n=110	n=112			Hospital 4 n=132
Microbiologic testing at diagnosis	100	100	100	100	100	N/A	100	100	100	100	100	N/A
Microbiologic testing at end intensive phase	65	62	53	100	70	<.001	100	94	93	97	96	0.078
Microbiologic testing at end continuation phase	100	100	100	100	100	N/A	100	100	100	100	100	N/A
Chest X-ray examination at diagnosis	50	100	52	85	70	<.001	100	81	42	96	79	<.001
Prescription of broad spectrum antibiotics	0	0	0	0	0	N/A	0	0	0	0	0	N/A
Weight measured at diagnosis	100	99	90	100	98	<.001	100	100	89	98	96	0.277
Weight measured at end intensive phase	1	19	0	99	27	<.001	69	46	2	92	52	<.001
Weight measured at end of treatment	0	5	0	97	23	<.001	64	32	0	85	45	<.001

Strengths and limitations

Our study primarily reviewed clinical measures recommended by ISTC which represent activities that TB care providers perform mostly themselves and are consistent with the national Ethiopian TB guideline. The main limitation of our study was that absence of data did not always signify the absence of services. It is possible that, services may have been provided to patient (e.g. prescription of broad spectrum antibiotics), but not documented. Since incomplete records were taken as non-adherence, adherence could have been larger in reality. Besides, we were limited in the number of clinical measures we could evaluate because our analysis relied on TB cases with complete medical records. However our findings from analysis of the best cases possible, with complete medical records, echo a fundamental principle of TB control espoused by Piot more than 40years ago, that a substantial proportion of TB cases occur among individuals who fail to access high quality diagnostic and treatment services [25].

CONCLUSIONS

Performing clinical measures recommended by ISTC is of the greatest importance in optimizing care for smear-negative TB. Recommended actions were unfortunately performed less often in smear-negative than smear-positive TB cases. We perceived this to be a habitual bias by TB healthcare providers, who apparently still consider managing smear-positive cases to be the most effective strategy to curb the TB epidemic. This treatment adherence gap would be significantly closed if strategies that increase compliance to ISTC are identified in hospitals that best meet the desired standards. There is also a need to explore qualitatively the reasons and hidden assumptions behind poor standardized clinical work-up in smear-negative as compared to smear-positive TB cases.

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Chapter 3

Why tuberculosis service providers do not follow treatment guideline in Ethiopia: a qualitative study

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ABSTRACT

Aim: Little is known about the reasons why tuberculosis (TB) service providers do not follow TB treatment guidelines. A better understanding of their real-world experiences associated with non-compliance with guideline may highlight opportunities for enhanced TB care. Whether the problem is actual or perceived may also affect the type of intervention needed to overcome the barrier. This study aimed to explore TB service providers' reasons for not complying with TB treatment guidelines.

Method: A descriptive qualitative design was used. Focus group discussions were held with 39 service providers involved in TB care. The study was carried out in the Tigray region of Ethiopia, in four of the six zonal hospitals. Data were analyzed using content analysis.

Results: The main reasons for non-compliance with TB treatment guidelines were: (1)insufficient diagnostic modalities, limiting the capacity to correctly diagnose TB and provide the right regimen; (2) ambiguity in guideline recommendations especially for specific subgroups of patients, making it difficult to prioritize them to the right regimen; and (3) poor documentation concerning referred patients, with no appropriate examination and difficulty obtaining information from previously treated patients.

Conclusion: Non-compliance may reflect valid questions about the applicability of guideline recommendations in the local context of health care. Special consideration should be given to previously treated patients, as an important factor influencing guideline compliance. Guideline compliance may also be improved by a better strategy to retest those patients whose initial tests are negative in health care contexts where TB is frequently missed.

INTRODUCTION

The primary aims of tuberculosis (TB) control programmes are early diagnosis and prompt treatment of infectious cases in order to limit transmission [1]. The World Health Organization (WHO) has developed specific outcome measures to evaluate TB control programmes. To achieve the intended outcome, the WHO has recommended diagnosis and treatment guidelines for the implementation of national tuberculosis programmes for each country [2, 3]. Treatment outcomes are recorded internationally, and targets of 70% case detection and 85% cure in smear-positive pulmonary TB have been set [4]. However, these broad outcome measures do not provide detailed insight into the pathways of clinical care, nor do they identify reasons for missing the targets.

Recent studies have reported multiple pitfalls in the diagnosis and treatment of TB patients, even in regions where TB might be considered a rarity [5–7]. Clinicians and patients have identified suboptimal diagnostic processes [8], uncertainty about appropriate clinical practice in the treatment of specific subgroups of patients, lack of integration and coordination of health services [9], as well as excessively prolonged prescription of anti-TB drugs [10]. This despite the availability of highly effective TB therapy and an intensive worldwide effort to improve access to the diagnostic and treatment interventions embodied in the directly observed treatment-short course (DOTS) strategy. This pitfalls echoes a fundamental principle of TB control espoused by Piot more than 40 years ago, that a substantial proportion of TB cases occur among individuals who fail to access high-quality diagnostic and treatment services [11].

Little information is available to explain why TB service providers do not follow TB guidelines in regions where TB prevalence is high. Studies to document quality deficits in TB care in these regions have taken the form of surveys using closed questions [12, 13]. In addition, surveys of barriers have relied on the providers' opinions and may not accurately reflect how problematic a barrier actually is, and the surveys may not have captured all the relevant barriers. Furthermore, studies that attempted to explore physician compliance with guidelines have focused on general practitioners' knowledge and physicians' attitude, rather than practice-related and context-specific reasons for not following guidelines [12, 13]. In this study, we explored reasons given by TB service providers for their non-compliance with TB guidelines. The objective was to explore health care providers' practice-related and context-specific reasons for not following the guidelines, since a better understanding of their real-world experiences associated with non-compliance with guidelines may reveal opportunities for enhanced TB care. We also examined whether a barrier is actual or perceived, as this may also affect the type of intervention needed to overcome it.

METHODS

Study design

This study used a descriptive qualitative design to explore TB service providers' practice-related and context-specific reasons for not complying with TB treatment guidelines. Semi-structured interviews were held with 39 service providers involved in TB care. Data were analyzed using content analysis.

Study setting

The study was carried out in the Tigray region of Ethiopia, in four of the six zonal hospitals, ranging in size from 36 to 500 beds. In these hospitals, TB is diagnosed and registered, and anti-TB treatment is started. The Ethiopia TB guideline is used for the diagnosis, treatment and monitoring of treatment response in adult patients who have, or are suspected of having, TB. Sputum smear microscopy is the main critical element in the diagnosis of active cases of TB. TB treatment consists of two phases, the intensive and continuation phases. During the intensive phase of treatment, patients who live in communities far away from the TB clinic are supplied with drugs and then referred to a health facility near their place of residence for DOTS by a TB service provider on a daily basis. Patients living close to the TB clinic go to the hospital where their TB was diagnosed for DOTS every morning. In the continuation phase of treatment, all patients are seen at the TB clinic every month on an ambulatory basis, irrespective of where DOTS was administered during the intensive phase. Response to treatment is monitored and recorded, including all medications given, bacteriologic response and adverse reactions. Treatment is provided free of charge to all TB patients in Ethiopia.

TB treatment guideline

Suspected TB patients with positive sputum smear are treated for TB. While suspected TB patients with negative sputum smears are managed according to a diagnostic algorithm that includes treatment with broad spectrum antibiotic and chest X-ray examination to determine whether or not the patient has smear-negative TB [3, 14]. Patients having chest X-ray findings compatible with TB are treated for smear-negative TB. Other suspected patients not treated for TB receive a broad-spectrum antibiotic to treat bacterial infection. Response to antibiotics is assessed within 3–5 days into treatment, and, if there is no improvement, anti-TB treatment is initiated.

Patients are prescribed anti-TB regimens based on their previous TB treatment history: as new patients and previously treated patients. New patients are treated with 'new patient regimen', while previously treated patients receive a retreatment regimen containing first-line drugs [3, 14]. All patients treated for TB are evaluated for treatment

response. TB treatment is considered failed if the specimen obtained at the end of the third month is still smear-positive; hence sputum culture and drug susceptibility testing (DST) should be performed. TB patients whose treatment has failed or other patient groups with high likelihood of multidrug-resistant TB (MDR-TB) should be started on an empirical MDR regimen. TB patients returning after defaulting or relapsing from their first treatment course may receive the retreatment regimen containing first-line drugs if country-specific data show low or medium levels of MDR in these patients or if such data are unavailable. Furthermore, in settings where DST results are not yet routinely available to guide the management of these individual patients, the empirical regimens will continue throughout the course of treatment.

Participants

A total of 39 TB service providers participated. They were six health officers, 12 nurses, six laboratory technicians, five internists and 10 general practitioners. Included were participants who were either involved in TB guideline implementation or provided services to TB patients in government-run hospitals. Participants were directly involved in the diagnosis and treatment of TB patients, with the exception of the laboratory technicians. Excluded were health care professionals who were not involved in providing care to TB patients. In order to get a broad view of the topic under study, we selected a range of professionals with different perspectives, using purposive sampling [15]. We made sure that the sample included males and females, young/inexperienced and older/experienced professionals and specialists working in the hospitals, to foster discussion among participants.

Ethical considerations

Ethical approval for the study was obtained from Tigray Bureau of Health, College of Health Sciences, Mekelle University and four health institutions. The aim of the study was explained to the participants, who gave informed written consent.

Data collection

Data collection took place between June and August 2011. A total of six focus group discussions (FGDs) were held, each with six to eight people participating. The FGDs took place at a location that was accessible to all participants. All FGDs were led by an experienced moderator with an understanding of the topic under study, who was a trained health worker and a native speaker of the local/official language of the study area. A questioning route for the FGDs [16] was prepared, with open-ended questions, probes and prompts. The questions were based on information from the current literature (sensitizing concepts) [17], and had been reviewed by stakeholders and adapted accord-

ingly. The topics were quality deficits, compliance with TB guidelines and information transfer. The topics in the questioning route were presented one by one, allowing sufficient discussion of each topic. Since data collection and analysis happened simultaneously, the questions were adjusted according to the findings of previous data collection rounds, and more questions were added or modified as we proceeded through the research process to obtain more in-depth information about the topic. For example, we added questions about adherence to DOTS principles. Data were collected in three rounds, with two FGDs conducted in each round. Each FGD lasted 2 hours. The FGDs were tape recorded and field notes were written down in a notebook by an assistant moderator. Data were transcribed verbatim in Amharic language and translated into English.

Data analysis

Data analysis used the qualitative content analysis approach based on the constant comparison method [17]. Codes and categories emerged inductively from the data through careful examination by the researcher. First, the transcripts from the FGDs and the field notes were read to get a general impression, after which the data were coded using open coding. The open codes were compared and contrasted and categories were defined. These categories were again compared and contrasted, and correct categories and subcategories emerged. This was done within and across the data sets. The ongoing data analysis took place throughout the study. After the first two FGDs with professionals, the research team read the translations and discussed emerging categories and subcategories, and decided which issues needed further elaboration. This procedure was followed in three analytical sessions, one after each round of FGDs [15]. We reached data saturation after four FGDs and used the remaining two FGDs to confirm the findings.

Trustworthiness

To ensure the reliability of the study data, we used investigator and methodological triangulation [18]. Data triangulation was secured by including different professional groups (health officers, nurses, laboratory workers, etc.). Investigator triangulation was applied by involving two different investigators in the data collection and analysis process. Methodological triangulation was ensured by gathering different types of data, such as FGD transcripts and field notes. Furthermore, to improve the transferability of the findings to different settings and contexts, a detailed description was prepared of the setting, sampling, sample size, inclusion and exclusion criteria, interview procedure and topics. This should help readers decide whether the findings of this study are transferable to their own context [18].

RESULTS

Five categories of factors influencing TB service providers' compliance with the guidelines emerged. They were listed in order of the most to the least important reason for non-compliance with guidelines as insufficient diagnostic modalities, ambiguity in guideline recommendations, poor documentation, health service situation and professional social context, respectively.

Insufficient diagnostic modalities

The TB service providers reported that the national TB programme guideline recommendations were insufficiently helpful in situations where there was a lack of diagnostic modalities. They believed that the major cause of non-adherence to the guidelines was having sputum smear microscopy as the only critical modality for the diagnosis of active TB cases.

We have very deficient diagnostic modalities; we cannot do sputum culture or blood culture (. . .) the diagnostic guidelines are not adaptable to the situation on the ground.

Respondents also reported that they doubted the results of sputum smear microscopy tests. In their practice, they observed that the sputum smear microscopy test gave false-negative or false-positive results even in patients with clear clinical symptoms. Consequently, TB service providers had adopted a practice of requesting both sputum smear microscopy test and chest X-ray examination simultaneously at the beginning of the diagnostic process. This is, however, not in accordance with the diagnostic algorithm, and results in overreliance on chest X-ray radiography for diagnosis.

To diagnose pulmonary TB we can do only sputum smear microscopy test, which usually gives negative results in our set-up (. . .) If those pulmonary TB suspects that might be positive are told that they are negative, this is the worst thing for the transmission of TB. For this reason, we do order both the sputum smear microscopy and chest X-ray together because the sputum microscopy test usually gives us a negative result.

Ambiguity in guideline recommendations

TB service providers experienced ambiguity in prioritizing previously treated patients who had relapsed, failed or defaulted treatment. Prioritization is important to provide the right regimen for these patients as recommended in the guidelines. Guidelines recommend that patients should be categorized for retreatment according to their likelihood of developing drug resistance and the availability of alternative diagnostic

modalities. Respondents reported that they could only prioritize these patients for re-treatment based on the sputum smear microscopy examination, which was of poor quality. Thus, their clinical judgment was not supported by these recommendations in everyday practice.

We do not have faith in the sputum smear microscopy test, and if there is no culture service, I should be allowed to use my clinical judgment not only in smear-negative patients, but also in categorizing previously treated patients, but the guide-line does not allow this. I mean the guideline should support what we have.

Respondents also claimed that guidelines were not sufficiently relevant for specific subgroups of patients, particularly patients who developed drug-induced toxicity or patients with special requirements such as those with TB meningitis. A source of particular concern was the difficulty of choosing the appropriate treatment regimen if the preferred combination of anti-TB drugs is unavailable. The preferred presentation of most anti-TB drugs was lacking because they were donated to hospitals as fixed drug combinations (FDCs). Consequently, respondents reported that guideline recommendations did not reflect the best practice in their situation, where only FDCs were available in unsuitable combinations.

Drugs may not be available even in fixed doses preparations; let alone the single drugs. Then we are blamed for not sticking to the guideline recommendations and not sticking to the right regimens, but they don't supply the drugs. We don't have any other option, so how should we treat these patients? What we do have currently is Rifampicin, Isoniazid, Pyrazinamide and Ethambutanol (RHZE) instead of RHZ and we are still treating the patient with RHZE and streptomycin like a re-treatment case. This is a retreatment regimen. 'What should I do'? Tell me what to do when the right presentation of FDCs is unavailable? How can I remove the Ethambutanol from the RHZE?

Poor documentation

Poor referral linkage was a theme repeatedly reported by respondents in all FGDs. The TB service providers did not have the necessary information about which tasks had been completed, and they also lacked information on why certain interventions had already been applied to the patients referred to them. Respondents reported that patients were referred without a systematic clinical history, examination and appropriate investigation. Hence, they experienced difficulties in the care of such patients due to a loss of transparency in rational clinical decision-making.

Most of the time patients come to you without proper referral papers or with no clear evidence why the patient was started on anti-TB drugs and the evidence is

difficult to trace back. First of all, we don't know how the patient was diagnosed with TB and we rely on the patients to tell us whether their sputum was negative or positive, but most patients wouldn't know. This is what I usually see as a deficit in the quality of TB care.

The TB service providers also reported key information being lacking from patient records. They expressed their frustration about the TB management of previously treated patients, where they knew little about their clinical history. In most cases, these patients can hardly provide any accurate past and present treatment information. Many respondents were unable to trace the information about diagnosis and medication previously taken by patients.

We usually face these difficulties with the care of patients who have been treated with anti-TB drugs previously. We don't know what type of TB they've been having and what kind of medication they've been taking, because these patients don't know anything about their disease condition. We face a dilemma on how to manage the patient because the whole history is missing, and this is common in many big hospitals in the country, not only in ours.

Health service situation

The TB service providers observed that almost all patients were faced with limited access to health service, due to geographical or economic disadvantages. In addition, patients were obliged to make multiple visits to TB clinics often over a considerable period of time. Such patients warrant making exceptions to some components of ideal care. The respondents were concerned that without exceptions to guideline recommendations, such patients might be at significantly higher risk of missing care due to loss to follow-up.

Most patients come from far away to the TB clinic, and are poor peasant farmers. So we cannot strictly follow the guideline recommendations (especially the diagnostic algorithm and DOTS principle) because patients will be lost to follow-up and may either go to another place or may not come at all next time.

Respondents expressed negative expectations about DOTS principles. In particular, they considered DOTS to be ineffective in the prevention of drug resistance. As a result, anti-TB drugs were distributed at the bedside to inpatients without direct observation, while outpatients were provided with the anti-TB drugs for weeks without any professional supervision.

Do we really believe DOTS works to prevent the development of MDR-TB, which is actually emerging and is rising alarmingly? I think that the DOTS principles are

a difficult ideology to practice but that there's currently no other option than DOTS to prevent the development of drug-resistant TB.

Professional social context

Respondents mentioned that medical professionals in government-run and privately run hospitals had little interest in following national guideline recommendations, especially where they perceived them to conflict with their own interests.

In private practice there is a tendency not to stick to the diagnostic algorithm. If I tell a patient you have bronchitis or something else, which is not TB, and I give the patient only amoxicillin and then advise them to come back after a month or after two weeks for re-evaluation, then it's not uncommon to see the same patient with an anti-TB drug the next day, in one of the private clinics. Even in government-run hospital settings this follow-up may not be perfect, because I may not be there and then the patient is kind of lost in between, this is my concern. In a rural healthcare setting it may be easier to do this, where I'm the only one who follows the same patient and can eventually follow the guidelines. But the issue here is, if I follow the guidelines and give the patient antibiotics; other healthcare providers may start anti-TB when I am no longer involved with the same patient.

In addition, junior health care providers reported that influential colleagues deviated from the guidelines based on their expert knowledge or individual treatment preferences.

With regard to the categorization of pediatric patients in particular, most of them are categorized as category three because of a lack of diagnostic modalities and the influence of seniors.

DISCUSSION

Summary of main findings

The aim of this study was to explore Ethiopian TB service providers' practice-related and context-specific reasons for not complying with TB treatment guidelines. Major reasons for non-compliance were insufficient diagnostic modalities, ambiguity in guideline recommendations, poor documentation, health service situation and professional social context.

The TB service providers in this study had developed their own routine and style of diagnostic evaluation. They had adopted a practice of requesting both a sputum smear

microscopy test and a chest X-ray examination at the beginning of the diagnostic process. This finding is similar to that of a study in India, where poor quality of the sputum smear microscopy test was also considered to be the reason for non-compliance with diagnostic guidelines [19]. These authors also observed a tendency to over-rely on chest X-ray for the diagnosis of pulmonary TB. A specific aspect in our setting was that respondents had developed negative expectations about the results of sputum smear microscopy tests. This contextual factor, which is unrelated to the actual performance of this diagnostic tool, substantially influenced their compliance with the guidelines.

Several barriers hampering the diagnosis and treatment of specific subgroups of patients have been described previously, especially in a regions where TB is considered a rarity [7]. In that study, the most common reasons for non-compliance involved issues of older patients and immigrant populations. TB was often considered difficult to ascertain in elderly patients, and the authors feared drug-induced toxicity in these patients. The most critical problem in immigrant patients was the lack of information on drug resistance patterns [7]. Consequently, clinical decision making about the initial treatment regimen for immigrant patients was perceived as a great challenge [6].

In our study, the most problematic subgroup was that of previously treated patients who had relapsed or failed or defaulted on treatment. It was difficult to prioritize these patients for the retreatment regimens available. Correctly diagnosing TB and providing the right regimen for these patients was a major problem, due to insufficient diagnostic modalities, lack of information on previous medical history and unavailability of information on drug resistance patterns. The consequences were numerous. Respondents observed diagnostic errors that exposed patients to inappropriate anti-TB drugs, leading to drug-induced toxicity.

The question arises if non-compliance is justified in these cases. Our findings suggest that non-compliance may reflect valid questions about the applicability of guideline recommendation to this specific subgroup of patients. Prescription of non-standard medication regimens has been reported in other studies [20, 21], and our findings confirm that this practice continues. The most commonly reported reasons for not using recommended regimens were doubts about the efficacy of the regimen for the recommended period, and the perceived risk of toxicity [20, 21]. Our findings do not enable us to confirm these reasons, because the use of inappropriate regimens was associated with either a lack of separate medicines or unavailability of FDCs in the preferred presentation. Priority was given to average patients with no complications, and fewer investments were being made to improve the quality of TB care for patients that do not fall into the category of average patients. But as Vijan pointed out, 'Not all patients are the same. Treating them as such not only minimizes autonomy, but it is also a recipe for inefficiency' [22].

Most previous studies have acknowledged that unsupervised treatment was taking place under DOTS. DOTS was perceived to be useful only for a few average patients, suggesting a mismatch between patient needs and the existing strategy [23]. Geograph-

ic and economic disadvantages were the most common reasons for non-compliance with DOTS in our study, as has also been found in other studies [24–26]. Surprisingly, unsupervised treatment was provided by our respondents because of negative expectations about a core principle of DOTS that is effective prevention of the development of drug resistance. The rise of MDR-TB in our setting [27] signals a failure to adhere to the DOTS strategy. Although randomized controlled trials of DOTS have shown no benefit in terms of adherence to medication [28–30], it is still believed to be the best strategy available for TB control [31].

Strengths and limitations of our study

The strengths of this study were that it included a range of different TB service providers from a number of different health care settings. This allowed us to identify divergent perceptions and experiences, assumptions and beliefs, and to explore what actually happens at the patients' bedside. It is also important to note that almost all the respondents claimed to have expert knowledge and were willing to comply with the guidelines, although we made no attempt to evaluate their knowledge of and attitude towards the guidelines. The purposive sampling may have led to the selection of respondents who were optimistic. Finally, the findings of this study should be interpreted against the background of its setting (with poorly developed infrastructure).

Implication for future research and practice

TB guidelines may not improve the quality of care when available diagnostic tests are, or are perceived to be, poor. Our findings indicate that the degree of trust that TB service providers had in the diagnostic test results had a great impact on compliance with the guidelines. Thus, addressing their negative perception of sputum smear microscopy may be just as important as scaling up the diagnostic test. Furthermore, there was dilemma among TB service providers about relying only on sputum microscopy in active case finding. The underlying conflict was a lack of alternative diagnostic modalities when the initial test result does not correspond with the patient's clinical presentation. A better strategy to retest those patients whose initial tests are negative in settings where TB is frequently missed may improve compliance with the guideline.

A recent review of the TB guidelines showed that the overall methodological quality of the guideline was low [32]. Several domains of the guidelines, such as applicability to specific local situations, contributed to their poor performance [32]. Our findings offer real-life examples of what is needed to customize TB guidelines to local contexts of health care in order to improve compliance. We recommend developing intervention strategies that are not tailored only to average patients. Consideration should be given to specific subgroups as an important influence on guideline compliance.

CONCLUSION

Non-compliance may reflect valid questions about the applicability of guideline recommendations to the local context of health care. Special consideration should be given to previously treated patients, as an important influence on compliance with guidelines. Guideline compliance may also be improved by a better strategy to retest those patients whose initial tests are negative in regions where TB is frequently missed.

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Chapter 4

Predicting tuberculosis in clinical suspects and among smear negative cases, with routinely available diagnostic tools in Ethiopia

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Submitted

ABSTRACT

Objectives: To evaluate the performance of predictors for estimating the probability of tuberculosis (TB) (1) among all clinical suspects and (2) among smear negative cases.

Methods: From September to November 2013, a total of 426 consecutive patients, admitted to two hospitals in Tigray Ethiopia, with suspicion of TB, were evaluated by sputum smear microscopy and chest radiography in accordance with the Ethiopian guidelines of the National Tuberculosis and Leprosy Program. The performance of predictors for estimating the probability of TB in clinical suspects and smear negative TB cases were based on the results of culture tests.

Results: Among all cases the strongest predictive variables both in univariate and multivariate analysis of culture-positive TB, were a positive smear test (OR 172; CI 23.23-1273.54) and having chest radiography lesions compatible with TB (OR 10.401; CI; 5.862-18.454). However, among smear negative cases almost all variables showed no significant difference between culture-positive and culture-negative TB. The regression model had a good predictive performance, for identifying culture-positive TB among patients (AUC 0.84) with clinical suspicion, but it was rather poor in patients with a negative smear result (AUC 0.64). Combining all the predictors in the model compared to only the independent significant variables did not really improve its performance to identify culture-positive (AUC 0.84 to 0.87) and culture-negative (AUC 0.64 to 0.69) TB.

Conclusions: Our findings suggest that neither the hallmark symptoms nor radiographic findings are invariably associated with culture-negative compared to culture-positive TB, among smear negative suspects. Therefore, predictive models based on clinical variables will not be useful to discriminate patients with culture negative from culture-positive TB among smears negative cases.

INTRODUCTION

Pulmonary tuberculosis (TB) with culture-negative, but positive clinical and chest radiographic findings is often encountered in routine practice in low-income countries. These cases are mostly also smear-negatives and their diagnoses have been problematic, which has been a matter of concern in recent decades [1, 2]. The clinical form of presentation is insidious and early diagnosis is often difficult if TB is not advanced [3]. Without a standardized clinical work up, the rate of diagnostic error has been estimated as high as 35% to 52% [4-6]. However, in settings with adequate resources, allowing for comprehensive diagnostic work-up and the exclusion of other respiratory diseases, a substantial proportion of the cases is diagnosed as culture-negative TB [7, 8]. Despite the considerable incidence of culture-negative TB, little is known about its clinical manifestations compared to culture-positive TB among smear negative cases.

Recognizing and diagnosing culture-negative TB is clinically challenging. The Nguyen et al. and a Hong Kong based study in the early 1980s [9, 10], found a lower frequency of cavitation in culture-negative pulmonary TB compared with culture-positive subjects. These findings indicate that, culture-negative pulmonary TB is an early disease state with low mycobacterial burden. Nguyen et al. and others [9, 11-13] observed a reduced frequency of clinical symptoms and radiographic lesions in cases with culture-negative TB, but their comparison groups (culture-positive) had both smear negative and smear positive cases. Inclusion of smear positive cases may potentially lead to different findings compared with a situation where the analysis is restricted only to smear negative TB. Therefore, we hypothesize that culture-negative TB cases may present with no significant differences in clinical and radiographic abnormalities when compared to those with culture-positive among smear-negative cases.

In addition, while reviewing deaths due to respiratory infections in Africa, several authors [14-19] found that missed TB diagnoses were likely due to less symptomatic states of TB premortem. Therefore, if bacteriological confirmation is needed before initiating the anti-TB treatment as per the Ethiopian TB guideline [20], it will result in far too many culture-negative TB cases left untreated, as there is no gold standard test for the diagnosis [7]. Moreover, the clinical definitions in existing guidelines are rather vague [21-23] and do not allow to classify patients according to their probability of TB. While application of predictive modelling in patients with clinical suspicion of TB has been described before, these studies usually combined only independent significant predictive variables into the decision models [24-28]. In addition, information from a single predictor is often insufficient to provide reliable estimates of diagnostic probabilities or risks [29-30]. In particular for pragmatic reasons, clinicians in Ethiopia use all available information for each patient to determine whether TB is indeed present or absent. Therefore, in this study, we evaluated the performance of predictors for estimating the probability of TB when all versus only significant variables are considered; (1) among all clinical suspects and (2) among smear negative cases.

METHODS

Study setting, population and data collection

This study was conducted at the Aydar and Mekelle Hospitals, 500 and 350 inpatient beds public sector referral hospitals respectively, that serve approximately 18 million people in Northern Ethiopia. From September to November 2013, a total of 426 consecutive patients, admitted to these hospitals, with suspicion of TB, were evaluated in accordance with the Ethiopian guidelines of the National Tuberculosis and Leprosy Program [17]. The study included all patients aged ≥ 18 years, with a history of cough of >3 weeks, night sweats, fever for 1 month, weight loss and/or loss of appetite. All suspects were asked to produce two sputum samples (spot and early morning sample), used for culture testing. Patients unable to produce sputum and unwilling to give written informed consent were excluded from the study. Patients were also excluded from the study if they had discontinued anti-TB therapy in the past 3 months. Furthermore, we collected data in four main domains 1) patient history, 2) physical examination, 3) chest radiograph, and 4) sputum smear results. Personal identifiers were not collected and data were analyzed anonymously. The study protocol was reviewed and approved by the Institutional Review Board of the Research and community service of College of Health Sciences of Mekelle University (ERC 0304/2010).

Diagnosis of culture-positive TB was based on *Mycobacterium tuberculosis* growth in at least one of the first three sputum cultures. Smear-negative TB was defined as no acid fast bacillus (AFB) identified in the initial three sputum smears, while smear-positive TB was defined as at least one positive AFB smear. Culture-negative TB was defined as clinical and/or radiographic presentation consistent with TB, three initial mycobacterial sputum cultures negative, and no evidence of other respiratory disease.

Only early morning sputum specimens submitted to the hospital TB laboratory as part of routine sputum smear microscopy test was used for culture examinations. Sputum specimens were decontaminated on arrival at the reference laboratory using 2% sodium hydroxide and 0.5% N-acetyl-cysteine for 25 min, then neutralized to pH 7, concentrated by centrifugation (3000xg for 15 min) and inoculated into a single Mycobacterium Growth Indicator (MGIT) Tube 960 (MGIT, Becton Dickinson Microbiology Systems, Sparks, MD, USA) [32]. Smears were made from isolates obtained from the MGIT tubes, stained by the Ziehl Neelsen staining method, and examined under 100 x magnifications using a light microscope for the presence of AFB. The growth on AFB positive MGIT tubes were further inoculated into two Lowenstein-Jensen slants, one containing sodium pyruvate. The cultures were examined twice a week and their rate of growth and colonial morphologies recorded.

The chest radiography lesions were categorized in terms of the involved lung field and the pattern of involvement. The involved field was categorized as upper and lower lung fields; left and right side of lung affected with lesions. The radiographic pattern was

categorized as normal, or abnormal with lesions consistent with TB [31]. “Normal” pattern was defined as the absence of any abnormal lesion on chest radiography. A targeted physical examination was performed for height, weight, axillary or oral temperature, pulse rate, respiration rate and blood pressure. Chest radiography was read by local site investigators.

Statistical analysis

Univariate comparisons were performed using the χ^2 test and Fisher’s exact test for categorical variables and Student’s t-test for continuous variables where appropriate. In this study, active TB was determined by a culture-positive result. Receiver operating characteristic (ROC) analysis was used to compare the accuracy of each predictive variable compared to culture results. The area under the ROC curve (AUC) was used as a measure of diagnostic accuracy. Under common practice, AUCs of 0.60–0.69, 0.70–0.79, 0.80–0.89 and 0.90–1.0 were considered to correspond respectively to ‘poor’, ‘fair’, ‘good’ and ‘excellent’ diagnostic accuracy.

Unconditional logistic regression models were used to discriminate the outcome of culture results in clinical suspects and among smear negative cases and generate odds ratios (OR) and 95% confidence intervals (CI) as estimates of effect size. Independent variables were utilized in logistic regression models in two ways: continuous and binary. Binary values were determined by median values. Best cutoff values were chosen for those continuous variables with values that discriminate culture-negative from culture-positive TB among smear-negative TB cases using ROC. For both continuous and binary independent variables, following initial models that included all variables, a second model that excluded nonstatistically significant variables ($P > 0.05$) was run. The Hosmer–Lemeshow test was used to assess the fit of the logistic regression model. Estimates of sensitivity, specificity, and AUC were determined by final model fit. Standard errors for the AUC were calculated using the Mann–Whitney method. All statistical analyses were performed using SAS ver. 9.2 (SAS Institute, Cary, NC, USA). Statistical significance was at two-tailed $P < 0.05$.

RESULTS

Among the 426 enrolled cases, 255 (60%) had an abnormal chest radiograph, of which 192 (75%) had lesions compatible with TB (Figure 1). 46 (24%) patients with chest radiography compatible with TB had positive results on sputum smear microscopy test and were therefore diagnosed as smear positive TB. Of these, 43 (98%) were confirmed culture-positive cases. The remaining 146 (76%) patients had negative sputum smears, while having a positive chest radiography and were therefore considered to have smear negative TB. However, 78 (64%) of these patients were culture-negative TB cases. None

of the patients with normal CXR had a positive smear test. A total of 72 patients without culture results were excluded from final analysis (2 with positive smears and 33 with negative smears among patients with abnormal CXR and 37 with negative smears among patients with normal CXR).

Figure 1. Participant flow diagram

*Total culture positive cases (n=105); +Total culture negative cases (n=249); Total excluded cases (because culture test not done) (n=72)

Predictive variables of culture-positive TB cases among patients with clinical suspicion are given in Table 1. The results of the univariate analysis revealed that patients previously treated with anti-TB drugs (OR 2.72; CI: 1.57-4.69), were more likely to have culture-confirmed TB than never treated patients. In addition, upper lung fields (OR 4.76; CI: 2.52-8.99) and having bilateral lesions (OR 5.58; CI: 2.85-10.91) seemed to be related to culture-positive TB. However, lesions present in the lower lung fields and on either side of the lungs, was less significantly related. The strongest predictive variables both in univariate and multivariate analysis of culture-positive TB, were a positive smear test (OR 172; CI 23.23-1273.54) and having chest radiography lesions compatible with TB (OR 10.40; CI 5.86-18.45). A prediction model based on only the independent significant predictors (sputum smear microscopy and chest radiography) had a good performance by ROC analysis (AUC 0.84) for diagnosing culture-positive TB (Figure 2). Combining all predictors in the model compared to only the independent significant variables did not really improve its performance to identify culture-positive TB (AUC 0.84 to 0.87). The discriminating ability of the model neither showed much difference to rightly classify culture positive TB (AUC rose from 82 to 85).

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Table 1: Predictive risk factors of culture-positive compared to culture-negative TB among all clinical suspects with culture results (n=354).

				Model 1		
Predictive variables	Culture-positive for TB (n=105)	Culture-negative for TB (n=249)	Univariate Odds Ratio (95% CI)	β(SE)	Multivariate Odds Ratio (95% CI)	P Value
Sex						
Female	40(25%)	119(75%)	0.67(0.42-1.07)	-0.489(0.35)	0.613(0.31-1.23)	0.167
Male	65(33%)	130(67%)	1.0		1.0	
						0.423
Age						
45+	38(27%)	104(73%)	0.89(0.47-1.67)	-0.007(0.557)	0.993(0.33-2.96)	0.990
25-44	46(33%)	94(67%)	1.19(0.64-2.21)	0.449(0.55)	1.567(0.54-4.59)	0.413
0-24	21(29%)	51(71%)	1.0		1.0	
						0.605
BMI						
BMI <16	15(26%)	42(74%)	0.98(0.50-1.93)	-0.451(0.47)	0.637(0.26-1.59)	0.335
BMI 16>18.50	43(35%)	78(65%)	1.51(0.92-2.50)	-0.004(0.390)	0.996(0.46-2.1)	0.992
BMI 18.5+	47(27%)	129(73%)	1.0		1.0	
History of anti-TB						
Previously treated	33(47%)	37(53%)	2.72(1.57-4.69)	0.495(0.39)	1.641(0.77-3.52)	0.203
Never treated	65(25%)	198(75%)	1.0		1.0	
						0.998
Duration of cough						
13 weeks and above	25(36%)	44(64%)	1.50(0.83-2.69)	0.025(0.44)	1.03(0.43-2.43)	0.955
7-12weeks	27(29%)	65(71%)	1.09(0.63-1.90)	0.022(0.40)	1.02(0.47-2.24)	0.957
1-6 weeks	52(28%)	137(72%)	1.0		1.0	
						0.643
Lung fields						
Lower fields	20(36%)	35(64%)	1.97(1.05-3.68)	0.411(0.471)	1.509(0.60-3.80)	0.382
Upper fields	29(58%)	21(42%)	4.76 (2.52-8.99)	0.351(0.520)	1.420(0.51-3.94)	0.500
Unspecified	56(23%)	193(77%)	1.0		1.0	
						0.742
Lung sides						
Left	20(39%)	32(61%)	2.84(1.46-5.54)	0.168(0.54)	1.182(0.41-3.41)	0.757
Bilateral	27(55%)	22(45%)	5.58(2.85-10.91)	0.577(0.54)	1.780(0.62-5.11)	0.284
Right	23(39%)	36(61%)	2.90(1.50-5.50)	0.164(0.54)	1.178(0.41-3.42)	0.764
Unspecified	35(18%)	159(82%)	1.0		1.0	
Smears results						
Positive smears	43(98%)	1(2%)	172.00(23.23-1273.54)	4.352(1.05)	77,668(9.89-609.68)	0.001
Negative smears	62(20%)	248(80%)	1.0		1.0	
CXR findings						
Compatible with TB	87(52%)	79(48%)	10.401 (5.86-18.45)	1.660(0.43)	5.261(2,280-12,141)	0.001
Not Compatible with TB	18(10%)	170(90%)	1.0		1.0	

BMI Body Mass Index, CXR Chest x-ray

Figure 2. Receiver operator curve characteristics for the prediction of culture positive TB from culture-negative among clinical suspects (n=354).

Almost all variables showed no significant difference between culture-positive and culture-negative TB among smear negative cases (Table 2). Female sex (OR 0.33; CI: 0.15-0.71), was the only independent negative predictor of culture-positive TB in both analyses, and could be due to less severe lung lesions compared to males. The ROC curve for female sex as the only independent predictor compared to combinations of all predictors (Figure 3), both reported poor clinical performance of the model (AUC from 0.64 to 0.69).

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Table 2: Predictive risk factors of culture-negative compared to culture-positive among smear negative TB cases (n=146)

Predictive variables	Culture-negative TB (n=78)	Culture-positive TB (n=44)	Univariate Odds Ratio (95% CI)	Model 1		P Value
				β(S.E)	Multivariate Odds Ratio (95% CI)	
Sex						
Female	46(77%)	14(23%)	0.33(0.15-0.71)	-1.13(0.40)	0.325(0.15-0.71)	0.005
Male	32(52%)	30(38%)	1.0			
Age (group)						2.67
45+	43(67%)	21(33%)	0.78(0.23-2.68)	0.74(0.91)	2.10(0.36-12.43)	0.414
25-44	27(60%)	18(40%)	1.07(0.30-3.79)	1.30(0.93)	3.67(0.59-22.78)	0.163
0-24	8(62%)	5(38%)	1.0			
Body Mass Index (BMI)						0.721
BMI <16	23(74%)	8(26%)	0.55(0.21-1.45)	-0.43(0.540)	0.650(0.22-1.88)	0.462
BMI 16>18.50	22(60%)	15(40%)	1.07(0.46-2.52)	-0.05(0.52)	0.952(0.35-2.61)	0.924
BMI 18.5+	33(61%)	21(39%)	1.0			
History of anti-TB						
Previously treated	21(57%)	16(43%)	1.58(0.71-3.53)	0.26(0.48)	1.294(0.51-3.29)	0.588
Never treated	54(68%)	26(32%)	1.0			
Duration of cough						0.909
13 weeks and above	22(65%)	12(35%)	0.92(0.37-2.27)	0.22(0.53)	1.249(0.44-3.53)	0.674
7-12weeks	23(66%)	12(34%)	0.88(0.36-2.16)	0.15(0.52)	1.162(0.42-3.25)	0.774
1-6weeks	32(63%)	19(37%)	1.0			
Lung fields with lesions						0.521
Lower fields	22(65%)	12(35%)	1.02(0.42-2.44)	0.47(0.56)	1.605(0.53-4.84)	0.401
Upper fields	15(60%)	10(40%)	1.24(0.48-3.22)	0.64(0.60)	1.899(0.58-6.19)	0.288
Unspecified	41(65%)	22(35%)	1.0			
Lung sides with lesions						0.693
Left	20(67%)	10(33%)	0.91(0.32-2.62)	-0.65(0.70)	0.523(0.13-2.06)	0.353
Bilateral	17(61%)	11(39%)	1.18(0.41-3.38)	0.08(0.65)	1.087(0.30-3.90)	0.898
Right	21(64%)	12(36%)	1.04(0.37-2.89)	-0.25(0.67)	0.777(0.21-2.87)	0.705
Unspecified	20(64%)	11(36%)	1.0			

NB: 24 cases of smear negative were excluded from analysis because of missed culture test results

Figure 3. Receiver operator curve characteristics for the prediction of culture-negative from culture-positive among smear-negative TB cases (n=146).

DISCUSSION

Our study was designed to evaluate the performance of predictors for estimating the probability of TB in patients suspected of TB and in smear negative cases. 98% of smear positive TB were confirmed by culture while 64% of smear negatives were not. The smear test and chest radiography remain the strongest predictors for culture identified TB. Our findings suggest that neither the hallmark symptoms nor radiographic findings are invariably associated with culture-positive compared to culture-negative TB, among smear negative suspects.

The high rates of culture unconfirmed TB in our study confirms the WHO report 2007 and implies that diagnosing smear-negative TB remains problematic even with access to culture and early radiography [32]. A similar phenomenon of culture positivity in smear negative cases has been reported by others where it ranges between 24-62% [33-35] in different geographical locations. Other studies from high HIV prevalence

settings using combined clinical and bacteriological case-definitions and not restricted to smear-negative suspects have reported between 10% and 45% of patients as culture-negative [36–38]. Furthermore, Nguyen *et al.* and a Hong Kong based study in the early 1980s [9, 10], indicate that culture-negative pulmonary TB is an early disease state with low mycobacterial burden. In addition, a US study of HIV-negative culture-positive patients estimated that negative smears are obtained from 60%–80% of patients with minimal disease, but from only 5%–10% of patients with extensive cavitory lesions [39]. Consequently, we conclude that predictive models based on clinical variables are not useful to discriminate patients with culture negative from culture-positive TB among smears negative cases. This finding suggests that additional indicators are required for predicting TB in patients with a smear and culture negative result.

Many investigators have examined the predictive value of individual signs and symptoms in assessing the risk of TB in clinical suspects [24–28]. Our findings confirm that independent significant variables (positive sputum smear and chest radiography compatible with TB) remain critical elements in the prediction of culture positive TB among patients with clinical suspicion. Scott *et al.* found that chest radiograph findings consistent with TB was the best available predictor of TB [40], while, Knirsch *et al.* were successful in using a computer protocol that interpreted chest radiographs to identify high-risk patients for TB [41]. Our study confirms previous reports that identified upper lobe lesions as independent predictive variables of culture-positive TB among clinical suspects [42, 43]. Our findings are also consistent with others who found lesions on lower lung fields were not really related to culture positive TB [44, 45]. Previous studies [46] have also confirmed the reliability of smear microscopy as a proxy for culture in the classification of TB cases. However, the wide confidence interval of positive sputum smear results may indicate the variability in the sensitivity for microscopy [47, 48]. For example, large variations were reported in the percentage of sputum smear-positive patients among all patients with TB notified to the WHO by sub-Saharan African National Tuberculosis Programs: in 1999, reports ranged from 9% in Eritrea to 80% in Benin [49]. This variability may depend on the population tested, technique and skills in getting a good sputum sample, preparation, and reading. Quality assurance is therefore important.

Among smear negative cases only gender was an independent predictor of culture-positive TB. The male predominance in pulmonary TB is well recognized. This has also been shown in reviews in Malaysia, Ireland, Britain and United States [50–54]. So far no undisputed explanation has been forwarded for this finding, thus supporting our hypothesis that among smear-negative cases, culture-negative TB may present itself with no differences in clinical and radiographic abnormalities compared to those with culture-positive TB. Previous studies for the clinical diagnosis of smear negative TB showed also no consistent predictors in the clinical presentation except expectoration, which was identified as a negative predictor [55–57]. Nguyen *et al.*, found a lower frequency of cavitation in culture-negative TB compared with culture-positive subjects [9, 10], but it should be emphasized that the differences reported in these previous studies are due to including

both smear positive and smear negative cases in the groups that were compared with culture negative TB cases. If only smear negative cases would have been used, the results of Nguyen would probably be the same as our results. It is imperative that a high index of suspicion be maintained in order not to miss such cases. This is especially so if the same patient had persistent complaints but without a definitive diagnosis. Hence, a continuing awareness of the clinical spectrum of culture negative TB remains important.

Our study has some limitations. First, the usefulness of our approach is probably limited to African settings with poor performance of sputum smear microscopy and clinically suspected patients relying on chest radiography. The differences in quality of sputum smear microscopy, are likely to have negative impact on the power of other predictors. Second, as there is no gold standard test for the diagnosis of culture negative TB, our study was limited by the lack of absolute certainty that all cases definitely had TB. In particular for pragmatic reasons, clinicians in Ethiopia use all available information for each patient to determine whether TB is indeed present or absent. Accordingly, more insight into clinician's considerations of TB even in case of a negative smear and culture tests results is needed.

Our findings suggest that neither hallmark symptoms nor radiographic findings are invariably associated with culture negative compared to culture positive TB, among smear negative TB cases. It emphasizes that medical providers should not expect a clinical presentation with the characteristic symptoms of advanced TB in order to diagnose and initiate treatment in culture-negative TB. Such awareness is critical, as it is possible to treat culture negative TB, described as an early disease state, based on its likelihood of progression despite the low mycobacterial burden (reviewed in [58]). However, culture negative TB may be more recognized in settings with sufficient resources for extensive work-up to exhaustively exclude other respiratory diseases. For example, Nguyen et al. observed that computer tomography (CT) scans were significantly more frequently performed in culture-negative compared to culture-positive subjects, likely because chest CT scans can reveal abnormalities not seen on chest radiography and can thus aid in diagnosis of culture-negative TB. A prospective consensus diagnostic evaluation study, should be designed to evaluate the added value and ease at which clinical diagnosis of culture-negative TB is made from other possible diagnoses by CT-scan. Since, previous study found that, the clinical judgment of infectious diseases specialists has been shown to be more accurate than prediction models [22].

CONCLUSION

Our findings suggest that neither the hallmark symptoms nor radiographic findings are invariably associated with culture-negative compared to culture-positive TB, among smear negative suspects. Therefore, we found that a predictive models based on clinical variables will not be useful to discriminate patients with culture negative from culture-positive TB among smears negative cases.

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Chapter 5

Exploratory analyses of the level of salivary Nitric Oxide (NO) metabolites and microbiological outcome in suspected Tuberculosis patients

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Submitted

ABSTRACT

Objectives: To examine the diagnostic value of the level salivary NO metabolites in order to assess microbiological outcome (infectiousness), and its association with the severity of the disease (commonly seen as wasting) in TB suspects.

Methods: In this exploratory study, we compared the salivary NO levels in confirmed and unconfirmed TB patients by sputum smear microscopy, chest radiography and molecular GeneXpert tests. Measurements of salivary NO were performed in 90 suspected TB patients. Estimation of salivary NO was by the measurement of nitrite (NO_2), employing the Griess reaction according to the method of Green et al. [21]. The nitrite levels in the saliva were determined from the universal color change, constructed from the known standard concentration.

Results: We found that, there is a significantly higher proportion of low salivary NO levels ($P < 0.019$) in patients positive for smear (smear positive TB) than among negatives for smears. Surprisingly, among smear negative cases, we did not observe a significant difference in salivary NO levels (0.086) between the TB-cases and non-TB-cases. Interestingly, in our exploratory study no significant correlation between BMI and NO levels was found.

Conclusions: The results of our findings support our hypothesis that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mycobacterium Tuberculosis* infection, are more likely to be associated with increased risk of acquiring TB. Interestingly, in our exploratory study no significant correlation between BMI was found on the groups compared and with regards to the levels of salivary NO. Hence we could not support our second hypothesis that levels of salivary NO could be associated with the severity of the TB.

INTRODUCTION

The diagnosis of tuberculosis (TB) in resource-poor endemic regions where 95% of cases and 98% of TB deaths [1] occur is challenging. In these settings, diagnosis still depends on sputum smear microscopy [2], even though accuracy is suboptimal and fails to diagnose one-third to half of all TB patients [3]. In addition, cases are diagnosed based upon passive case finding, and it is likely that a large proportion of these patients will be misdiagnosed.

In the Global TB Report 2014, the World Health Organization (WHO) declared that “one of the most urgently needed tests is a rapid biomarker-based test that can diagnose TB” [4]. Ideally, such TB biomarker should be able to detect the earliest and most difficult to diagnose TB cases, and should be suitable for use in resource-limited settings. Previous studies show that the presence of nitric oxide (NO) and its metabolites are potential screening biomarkers for TB [5, 6], but its relative importance is debated [7, 8]. Patients with TB were reported to have lower levels of exhaled NO and urinary NO metabolites, compared to healthy controls [6, 9, and 10]. However, there is very limited evidence on NO in direct diagnosis of TB [11].

Human mouth represents the environment with a constant supply of NO metabolites from L-arginine. The production of a stable salivary NO metabolites, may be affected during nutritional deficiencies, a common condition in TB suspects [12, 13]. Several studies reported that patients with active TB are more likely to experience wasting, and have a lower body mass index (BMI=kg/m²) than healthy individuals [14-17]. Therefore, it is hypothesized that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mycobacterium Tuberculosis* infection, are more likely to have severe (commonly seen as wasting in TB suspects) and more infectious forms of TB.

NO can be measured directly in the exhaled air [18-20] as well as by the stable urinary metabolites, nitrite and nitrate [19]. In recent years there has been much interest to investigate the level and especially the role of urinary and exhaled air NO in relation to host defense in human TB [20, 21]. However, there is a lack of clinical studies describing the level of salivary NO in suspected TB patients in the diagnostic phase. Therefore, in this study, the diagnostic value of the level salivary NO metabolites was examined to assess microbiological outcome (infectiousness), and its association with the severity of the disease (commonly seen as wasting) in TB suspects.

METHODS

Study population and methods

In this prospective study, we compared the salivary NO levels in confirmed and unconfirmed suspected TB patients by sputum smear microscopy, chest radiography and molecular GeneXpert tests. Measurements of salivary NO were performed at the Kilifi County Hospital and Malindi District Hospital, which serve as both referral centers and primary-care facilities in the coastal region of Kenya. Kilifi County Hospital is a 172-bed hospital on the eastern coast of Kenya, serving an area of 3870km² with a population of approximately 500,000 people [22]. Whereas, the Malindi District Hospital is a 208-bed hospital situated on the same coast 60km north of Kilifi town [23].

The study protocol was reviewed and approved by the Ethical Review Committee of Pwani University (ERC/MSc/026/2015), and authorization to conduct this study at the hospitals was given by the Kilifi County Research Ethical Review Committee. Patients were asked to read and sign the informed consent form for the collection of samples and subsequent analysis. These were available in both English and Swahili languages, and an interpreter was available if required. Personal identifiers were not collected and data were analysed anonymously.

All consenting patients aged ≥ 18 years, consecutively presenting themselves at the hospital with symptoms compatible with TB, were considered eligible for inclusion. The patients were screened for TB in accordance with the guidelines for Management of Tuberculosis and Leprosy in Kenya [24], between 23 June and 22 August 2015. Exclusion criteria were current anti-TB treatment. All participants were interviewed by treating clinicians using a pre-coded structured questionnaire on demographics, TB signs and symptoms and factors potentially affecting salivary NO levels, i.e., smoking history, recent alcohol consumption, history of antibiotic treatment, antibacterial mouthwash, HIV status and recent consumption of nitrate-rich vegetables and caffeine-rich tea.

NO estimation in the serum and saliva

After the interview, salivary NO levels were analyzed using commercially available NO Test Strips (manufactured by Berkeley Test, USA). The NO Test Strip is a simple, non-invasive procedure that can be performed by any individual in remote setting with no established diagnostic methods. Estimation of salivary NO was by the measurement of nitrite (NO₂), employing the Griess reaction according to the method of Green et al. [25]. The strip is administered on the tongue of the patient for no longer than 5 seconds, for the absorption pad to saturate. The nitrite levels in the saliva were determined from the universal color change (Figure 1) constructed from the known standard concentration.

Figure 1. Color change seen depending upon patients' salivary NO level.

Principle of the Griess reaction

The Griess reaction involves formation of a chromophore during the reaction of nitrite with sulfanilamide and heterocyclic amine of naphthylene-ethylene-diamine (Griess reagent) under low pH conditions. During this reaction, acidified nitrite undergoes diazotization with sulfanilamide to form a diazonium salt. This diazonium salt then couples to N-(1-Naphthyl) ethylenediamine to form a magenta-colored azo dye with a characteristic absorption spectrum. The only stable product formed by spontaneous autoxidation of NO in oxygenated solutions is NO₂.

The chemistry of the Berkeley Test Nitric Oxide Test Strips involves the conversion of NO₂ to a deep purple azo compound, producing a readily observable colour change that enables accurate determination of NO concentration [25]. These strips can detect between 21 and 869 μM of NO₂ ($\pm 15\mu\text{M}$). NO levels have been categorized as low, normal and high, which correlates to specific concentrations of >21, 108-217 and 434-869 μM , respectively [26]. Typical NO levels in human saliva range from 10 to 1000 μM , therefore these strips are sufficient to detect the NO levels present in most samples [27].

Data analysis

Data were analyzed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Descriptive categorical variables are summarized as proportions, whereas continuous variables as means $\pm$ standard deviations. The χ^2 test was used to determine the difference in proportions. Analysis of differences between diagnostic outcome and levels of NO was performed using the χ^2 test and Fisher's exact test for categorical variables and Student's t test for continuous variables where appropriate. The salivary NO levels were stratified for known influencing factors which had a distinctly different distribution between the patient groups. All tests with a p-value less than 0.05 are considered statistically significant.

RESULTS

We recruited 90 suspected TB patients during a two-month period from June to August 2015 (Figure 2), of which, 24 (27%) were diagnosed as smear-positive TB. The remaining 66 (73%) suspected TB patients had negative smear results, of which 41 (46%) subsequently received a diagnosis by chest radiography as smear-negative TB if the lesions were highly consistent for TB. However, in only 14 (52%) of the smear negative TB cases, TB diagnosis was confirmed by the molecular Xpert MTB/RIF test (new molecular test for detecting the presence of TB bacteria).

Figure 2. Patient flowchart in the diagnostic process and the point at which NO test strips may be used to measure salivary NO levels.

Clinical parameters and salivary NO levels between different groups of patients are given in Table 1. Almost all variables showed no significant difference between patients positive (smear-negative TB) compared to negative (non-TB cases) for chest x-ray lesions compatible with TB. However, a significant difference in age was observed between patients positive for smear compared to negative for smear TB. In addition, the level of salivary NO in cases positive for smear (smear positive TB cases) was significantly ($P<0.019$) lower than among negative for smears. Although we did not observe a significant difference in salivary NO levels ($P=0.086$) between patients smear-negative TB compared to non-TB cases, but there was a larger proportion of patients with high levels of NO in the smear-negative TB group. Furthermore, no significant correlation between BMI was found on all the groups compared.

Table 1. Clinical parameters and salivary Nitric oxide levels between different groups of patients

	Smear sputum microscopy results			Chest X-ray findings		
	Smear positive TB (n=24)	Not detected by smears (n=66)	P value	Compatible with TB (Smear negative TB) (n=44)	Not compatible with TB (No TB) (n=21)	P value
Sex (Male)	16(67%)	31(47%)	0.098	20(46%)	10(48%)	1.000
Age	33.9(12.2)	42.7(17.2)	0.023	42.4(16.7)	43.2(19.2)	0.879
Body mass index	19.8(2.8)	21.0(4.4)	0.228	20.8(3.5)	20.8(4.7)	0.978
HIV status						
Positive	7(29%)	20(30%)	0.230	12(27%)	6(29%)	0.794
Negative	17(71%)	39(59%)		28(64%)	12(57%)	
Unknown	-	7(11%)		4(9%)	3(14%)	
Salivary NO levels:						
Low	15(63%)	25(38%)	0.019	18(41%)	10(48%)	0.086
Normal	8(33%)	20(30%)		11(25%)	9(43%)	
High	1(4%)	21(32%)		15(34%)	2(9%)	

HIV Human Immunodeficiency Virus, *NO* nitric oxide

The relationship between associated factors that may impair the salivary NO levels in suspected TB patients is presented in table 2. No effect of the nitrate/arginine-rich diet consumption was found to affect the salivary NO levels, and the same goes for known confounders like alcohol, recent antibiotic treatment or antibacterial mouthwash use. The analysis yielded ($P<0.012$) gender, and ($P<0.041$) smoking as significant factors that impaired the level of salivary NO in suspected TB patient.

Table 2. Relationship between associated factors and NO levels in patients suspected of having TB

	NO levels			
	Low n=40 (%)	Normal n=28(%)	High n=22(%)	P value
Gender				
Male	18(45)	21(75)	8(36)	0.012
Female	22(55)	7(25)	14(64)	
Age	41(18.1)	40.3(13.3)	39.2(17.4)	0.922
Body Mass Index	20.7(4.4)	20.3(3.4)	21.2(4.2)	0.735
History of anti-TB				
New	40(100)	26(93)	19(86)	0.073
Relapse	-	2(7)	3(14)	
Alcohol status				
Alcohol	4(10)	4(14)	2(9)	0.808
No Alcohol	36(90)	24(86)	20(91)	
Smoking status				
Smoking	2(5)	7(25)	2(9)	0.041
Non-smoking	38(95)	21(75)	20(91)	
Diet consumption				
Nitrate-/Arginine-rich diet	35(88)	24(86)	18(82)	0.830
No Nitrate-/Arginine-rich diet	5(12)	4(14)	4(9)	
Antibiotic status				
Antibiotic treatment	27(68)	18(64)	9(41)	0.106
No Antibiotic treatment	13(32)	10(36)	13(59)	
HIV status				
Positive	10(25)	11(39)	6(27)	0.776
Negative	27(68)	15(54)	14(64)	
Unknown	3(7)	2(7)	2(9)	

DISCUSSION

The results of our findings support our hypothesis that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mycobacterium Tuberculosis* infection, are more likely to be associated with increased risk of acquiring TB. Thus, we found that, there is a significantly higher proportion of low salivary NO levels in patients positive for smear (smear positive TB) than among negative for smears. Surprisingly, among smear negative cases, we did not observe a significant difference in salivary NO levels (0.086) between the TB-cases and non-TB-cases. Similarly, a previous study also found no difference in NO levels between positive and negative chest radiography findings at treatment initiation [6]. Although our exploratory analyses of salivary

NO levels were restricted only to TB suspects, our findings are similar to Idh et al., [6] who observed an association between a low level of exhaled NO with smear positive TB compared to control healthy subjects. It should be emphasized that previously reported differences may potentially introduce bias due to comparing TB cases and healthy controls than if the analysis is restricted only to TB suspects.

Weight is a well-recognized measure of TB severity and treatment response [28]. Several studies reported that patients with active TB are more likely to experience wasting, and have a lower BMI (BMI=kg/m²) than healthy individuals [14-17]. This may lead to deficiencies of important nutrients, such as the amino acid L-arginine, which is essential for NO production. Interestingly, in our exploratory study no significant correlation between BMI and NO levels was found. Hence we could not support our second hypothesis that levels of salivary NO could be associated with BMI and thus indirectly with the severity of the TB. This finding is in line with the study of Idh et al., [6] in which they neither found an association between BMI and NO levels at treatment initiation. However, larger clinical studies, including long term clinical follow up will be essential to test this hypothesis thoroughly.

The present investigation is limited in some aspects, such as the small sample size. Thus, further longitudinal studies must be undertaken involving a larger number of subjects to evaluate the role of salivary NO in the screening of suspected TB patients. In addition, our analysis yielded ($P<0.012$) gender and ($P<0.041$) smoking as significant factors to influence on the level of salivary NO in TB patients. Thus, these factors may have obscured TB-related differences in salivary NO levels among TB patients. The results of our study cannot explain the reason underlying the difference in NO levels between males and females. However, it has been speculated that males might not be as susceptible as females in the production of NO [29-31], due to low endothelial responsiveness to the circulating adipocytokines produced by visceral fat tissue. However, previous studies reported higher NO production in female than males, despite the controversy over the differences [32, 33]. Finally, a possible explanation for the effects of smoking on NO levels may be the oxidative burden, a process that stimulates the production of NO [34, 35].

A series of reviews has shown that biomarkers such as adenosine deaminase, interferon-gamma (IFN- γ) [36-39] and NO have the potential for screening TB suspects [5, 6]. These biomarkers, especially salivary NO, are easy to measure and inexpensive. However, there is the potential downside that these biomarkers are not necessarily TB-specific, a weakness that may be addressed by the identification of biosignatures-multiple signals driven by specific host-pathogen interaction [40, 41]. Therefore, this makes the determination of a single host biomarker's sensitivity and specificity challenging. So far only a few studies have integrated combinations of markers to predict treatment outcome [42]. Consequently, uncertainties exist for the use of single biomarkers to diagnose infection status prior to initiation of therapy, and differentiate recent or progressive infection from and distant or latent infection. Inability to distin-

guish these two states will greatly reduce the potential for these tests to add value to clinical decision-making in TB-endemic regions, where a majority of adults would be expected to have been exposed to *Mycobacterium tuberculosis*. This is particularly important where laboratory services are being overwhelmed with demand for smear microscopy. Despite these limitations, a better prospective study should be designed to confirm our findings in various populations of suspected TB patients in order to determine if and how salivary NO strip test may be routinely used by admitting physician to improve the adequacy of current diagnostic tests.

CONCLUSION

The results of our findings support our hypothesis that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mycobacterium Tuberculosis* infection, are more likely to be associated with increased risk of acquiring TB. Our findings and previous studies suggest that the use of NO metabolites as a biomarkers may have great value for screening of smear positive TB. Interestingly, in our exploratory study no significant correlation between BMI was found on the groups compared and with regards to the levels of salivary NO. Hence we could not support our second hypothesis that levels of salivary NO could be associated with the severity of the TB.

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Chapter 6

General Discussion

INTRODUCTION

In this chapter, the main findings with regards to the research questions are summarized and general conclusions based on the findings of the studies presented in this thesis are described. Furthermore, the strength and limitations of this thesis are considered and suggestions for further research into quality of TB diagnostic care are presented. This chapter concludes with recommendations for three categories of stakeholders: TB service providers, program managers and policy makers.

The main research finding is that culture-negative TB cannot be discriminated from culture-positive TB among smear negative suspects. Thus supporting our hypothesis that culture-negative TB may present with no differences in clinical and radiographic abnormalities compared to those with culture-positive TB among smear-negative cases. Our findings further emphasize that in settings like ours with higher TB prevalence, there is a low threshold for starting antituberculous therapy, especially in patients with radiographic lesions compatible with TB, despite negative culture results. However, we confirmed that independent significant variables (positive sputum smear and chest radiography compatible with TB) remain critical elements in the prediction of culture positive TB among patients with clinical suspicion. Furthermore, the results of our findings support our hypothesis that patients unable to respond with an effective production of salivary NO metabolites in response to *Mycobacterium Tuberculosis* (*Mtb*) infection, are more likely to be associated with increased risk of acquiring TB. Our findings and previous studies suggest that the use of NO metabolites as a biomarkers may have great value for screening of smear positive TB. Finally, our study indicates that the degree of trust that TB service providers have in the diagnostic test results have a great impact on compliance with guidelines. However, a specific aspect of our findings was that TB service providers had developed negative expectations about the results of sputum smear microscopy tests.

Our findings suggest that neither the hallmark symptoms nor radiographic findings are invariably associated with culture-positive compared to culture-negative TB, among smear negative suspects. This confirms the findings of a previous study which reported that diagnosing smear-negative TB remains problematic even with access to culture and early radiography [1]. Considering that, other studies using combined clinical and bacteriological case-definitions reported between 10% and 45% of patients as culture-negative TB [2-4]. Most importantly, high rates of late diagnoses and low rates of culture confirmed TB were reported [1]. This is particularly common in real practice due to a number of obstacles intrinsic to the clinical setting [5]. First, in the majority of patients with a negative smear- and culture result, their illness is commonly in its early stage, when 'red flag' symptoms are yet to evolve and bacteriological confirmation is often difficult if TB is not advanced [6-8]. Second, the time to detection and speciation of cultures, may be up to 7 weeks, particularly when the burden of the mycobacteria is very low, as is often the case in smear and culture-negative TB [9]. All of these factors

considered, emphasizes that medical providers should not expect a clinical presentation with the characteristic symptoms of advanced TB in order to diagnose and initiate treatment in culture-negative TB. Such awareness is critical, as it is possible to treat culture negative TB, described as an early disease state, based on its likelihood of progression despite the low mycobacterial burden (reviewed in [10]). This is particularly important to policy-makers and from medical personnel to volunteer health workers, given that in resource-limited settings TB is often diagnosed by non-physicians, who have low TB knowledge and little experience [11].

To improve the accuracy of diagnostic work-up several new diagnostic tests have been suggested [12-16]. Ideally, a single reference test to classify culture negative TB is preferred. However, such a single and error-free test, also known as a reference or “gold” standard, is not available [17]. This is problematic, as errors in the final disease classification can seriously bias the results from correct diagnosis [17, 18]. In addition, clinical definitions of culture-negative TB in existing guidelines are rather vague [19-21] and do not allow to classify patients according to their probability of TB. Our findings confirm that independent significant variables (positive sputum smear and chest radiography compatible with TB) remain critical elements in the prediction of culture positive TB among patients with clinical suspicion. Our finding is in agreement with a previous study [22] that confirmed the reliability of smear microscopy as a proxy for culture in the classification of TB cases. Therefore, in countries with a high prevalence of TB, the specificity of smear microscopy may thus be superior to that of culture. This may be true even for the diagnosis of TB [23], since acid-fast bacillus (AFB) demonstrated in direct sputum smears would then almost invariably represent *M. tuberculosis*, even in areas with a high burden of HIV [24]. By contrast, in countries with a low prevalence of TB, culture (or alternative techniques of species identification) will often be indispensable to the differentiation of TB from other mycobacterial diseases. As prevalence falls, clinicians will be less likely to suspect TB, and will be less likely to be experts on recognizing TB, so that even a late culture result will be useful. Although, other sensitive rapid diagnostics have shown great promise when evaluated against culture [25], these tests still need to be evaluated for their usefulness in diagnosing culture negative TB. Diagnostic barriers will not be completely resolved until rapid diagnostic methods with sensitivity approaching 100% become widely available [26].

The Xpert MTB/RIF test (new molecular test for detecting the presence of *Mycobacterium TB*) for diagnosing TB increases the number of true cases diagnosed, and reduces the number of false-positive cases [27, 28] and is the preferred diagnostic test for use after screening with chest radiography. This screening should be considered for risk groups in which it is critical not to miss TB, such as cases with smear and culture negative results. However, at a 1% prevalence the positive predictive value is 78% with an Xpert MTB/RIF test for diagnosing TB, while at 0.5% prevalence it is 64%. [28]. At a prevalence less than 0.5%, using chest radiography to screen for abnormalities suggestive of active TB followed by an Xpert MTB/RIF test yields 20% false-positive cases (posi-

tive predictive value = 79% at 0.5% prevalence) unless further diagnostic verification is done. Hence, culture or careful diagnostic assessment, or both, are necessary for persons who are positive by Xpert MTB/RIF testing, especially when the TB prevalence is less than 0.5%. Immunodiagnosics of TB has also been shown to be useful in predicting active smear negative TB, especially in low incidence settings of TB [29-31]. Furthermore, an immunological test (such as the QFT test) is expected to be a stronger predictor of pulmonary TB because it is less likely to be affected by non-tuberculous mycobacteria infection and Bacillus Calmette–Gue’rin vaccination compared to the tuberculin skin test [31]. However, the QFT test is unable to differentiate between latent and active TB infection. Because of this, it is of limited value, although it is a significant predictor in the clinical diagnosis of active pulmonary TB.

WHO estimates that about one third of all incident cases of active TB are not properly diagnosed or receive care of questionable quality outside of national TB programs [32]. What seems clear from our study is that TB suspects without an initially confirmed diagnosis of TB are poorly served by current systems. Thus, echo a fundamental principle of TB control espoused by Piot more than 40 years ago, that a substantial proportion of TB cases occur among individuals who fail to access high quality diagnostic and treatment services [33, 34]. On the other hand, since early 2000, all surveys of TB prevalence done in countries with reasonably well performing national TB programs, have consistently demonstrated that the majority of undiagnosed cases of TB are sputum-smear negative. They have also demonstrated that more than 50% of those with prevalent bacteriologically confirmed TB do not report symptoms that correspond to the commonly used criteria for suspecting disease and prompting diagnostic investigation [35-37]. In our studies we showed that a higher proportion of clinical actions were omitted during diagnosis and monitoring of treatment response in smear-negative compared with smear-positive TB cases, despite the fact that the clinical actions are especially important for the former. Our findings add that care offered to smear-negative cases continues to be deviant from the standards, compared to smear-positive TB cases. However, this could be reduced if programme managers and policymakers, pay more attention to clinical measures related to care for smear-negative TB patients that are poorly followed compared to smear-positive TB cases. Therefore, we suggest that future quality improvement initiatives should focus on the role of real-time, clinic-level monitoring of smear negative TB suspect evaluation practices, in order to capture individual data on clinical measures related to care of smear-negative TB from every patient evaluated. Tools for electronic data collection, including mobile telephone based applications, are becoming readily available and can facilitate real-time monitoring and evaluation of TB care at the level of individual clinics. This approach deserves preference over existing monitoring and evaluation systems, since the system captures individual data on symptoms of TB from every patient evaluated in primary health clinics, permitting direct calculation of the numbers at risk for and suspected of TB, and longitudinal measurement of the quality of TB evaluation services for an individual clinic or provider. In a study by Davis *et al*, electronic monitoring

and evaluation proved to be feasible, and this study demonstrated that modest improvements in individual clinic performance in assessing patients suspected of TB were associated with increases in TB case detection [38].

Screening is particularly appropriate to find TB patients who are non-symptomatic or only have vague symptoms that are unlikely to be recognized by the person with the disease during early stages [39]. Thus, the critical question is whether culture-negative TB suspects can be detected and treated early enough. Our finding is in agreement with a previous study that reported that predictive models based on clinical variables may not be useful to discriminate culture-negative TB, because of the variable presentations of symptoms [7]. Screening for active TB is one of several possible interventions that can improve early detection of all forms of TB, but providing better access to diagnostic tests that are more sensitive than smear microscopy is the first essential step. From the above studies it is not possible to confidently conclude whether predictive models contributed additional cases or only identified cases that would have been detected through passive case finding. For such an evaluation, a controlled design comparing areas that use or do not use such predictive models, is required. In addition, when there is little empirical evidence, mathematical modelling can help create scenarios to assess the possible impacts, but these scenarios require verification through clinical trials [40, 41]. However, without reliable parameters for the natural course of culture-negative TB, such models should be interpreted with caution.

It has been suggested that volatile metabolites from *Mtb* organisms may be exploited to diagnose TB rapidly and could be used as biomarkers of cure and relapse [42]. This is an area of much interest and may lead to a practical, non-invasive way of diagnosing TB at site of care. However, there is a lack of clinical studies describing the level of salivary NO in suspected TB patients in the diagnostic phase. Human mouth represents the environment with a constant supply of NO metabolites from L-arginine. The production of salivary NO metabolites, may be affected during nutritional deficiencies, a common condition in TB suspects [43, 44]. The results of our findings support our hypothesis that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mtb* infection are more likely to have TB. Our findings and previous studies suggest that the use of NO metabolites as a biomarker may have great value for screening of smear positive TB. However, there is the potential downside that this single biomarker is not necessarily TB-specific, a weakness that may be addressed by the identification of biosignatures-multiple signals driven by specific host-pathogen interaction [45, 46]. Multiplex assessments have proven to have significant prognostic/diagnostic value in cancer [47, 48], HIV infection [49] and malaria [50]. Several recent studies suggest the same is true in TB, implying that highly multiplexed assays may be superior to single immune biomarkers. Furthermore, uncertainties exist for the use of these tests to diagnose infection status prior to initiation of therapy, the most important being the degree to which these tests can differentiate recent or progressive infection from a distant or latent infection. Inability to distinguish these two states will greatly reduce

the potential for these tests to add value to clinical decision-making in TB-endemic regions, where a majority of adults would be expected to have been exposed to *Mtb*. There are many studies that suggest that the magnitude of the immune response reflects the magnitude of the bacterial load and two longitudinal studies in untreated contacts suggest that the highest responders are at greatly elevated risk of TB [51, 52], but these studies are relatively small and lack detailed sequential testing routines. Thus the kinetics and duration of the differences are unknown; however, the fact that the two studies produced similar results in very different populations and environments (Ethiopia and Germany) is encouraging. Studies to address the questions of remote versus recent infection, predictive value and optimization of the assays, and their usefulness in HIV infected patients should prove very valuable.

We must consider a few methodological issues when interpreting the results of our studies. First, in this study we evaluated the performance of predictors for estimating the probability of culture-negative TB when all versus only significant variables were considered among smear negative cases. Our approach is different from previous studies that based their analysis on combining only independent significant predictive variables into a decision model [53-57]. Information from a single predictor is often insufficient to provide reliable estimates of diagnostic probabilities or risks [58, 59]. However, we did not follow-up predictive variables (or clusters of variables) of culture-negative TB, progressing over time that may be useful in diagnostic decision making. Culture-negative TB patients often present early in the course of an illness when typical symptoms and signs are absent [60]. Therefore, as the condition evolves, clinicians may rely more on the assimilation of information gained over a period of time ('dynamic evidence', e.g. the addition of new features, the persistence or changes in the characteristics of previous problems) rather than the traditional static information we obtained in our study at one point [61]. Second, it is clear from our study, that we did not evaluate how often and to what extent uncertain diagnostic outcome is shared with patients during consultations. Failure to communicate uncertainty effectively can lead to patients failing to return until they are approaching death [62].

Operational research has been increasingly recognized as vital to the strengthening of health programmes. For example, the expanded Stop TB Strategy [63] explicitly includes operational research as one of the key components for successful TB programmes. A strong connection exists between good monitoring and evaluation of infectious-disease programmes and operational research [64]. Since, good quality data on cases and treatment outcomes can be used to do operational research, which in turn can help to improve the routine data collected in the field. Operational research means that the search for knowledge on interventions, strategies, or tools can enhance the quality, effectiveness, or coverage of programmes in which the research is being done [64]. The importance of operational research is its ability to address and solve local problems in delivering quality health services. A necessary starting point is to identify the obstacles to providing high quality services, analyse why these obstacles occur and

to adopt policies and practices to overcome them [65]. As such, operational research is important to initially obtain more data to build on knowledge already acquired [6-8] and then to work out how best to integrate TB suspect follow-up into routine care. If we do act to improve the follow-up and management of TB suspects with smear and culture negative results, how will we do this and how will we monitor it? Firstly, if the diagnosis is uncertain, that uncertainty should be communicated to the patient (or parent/carer), so that they are empowered to re-consult if necessary. In addition, if there is a recognized risk of deterioration or complications developing then a safety-net advice should include the specific clinical features (including red flags) that the patient (or parent/carer) should look out for. Secondly, we suggest that future research into quality of diagnostic care of culture-negative TB, should better adopt a study design that closely reflects its natural history. For example, three to four weekly re-assessments (if possible combined with symptom diaries) of patients would not only provide more useful 'dynamic' clinical information, but would also enable the study to assess more reliably the association between the clinical information and the eventual diagnosis. This pragmatic approach would be ideal, since clinicians in Ethiopia use all available information for each patient to determine whether TB is indeed present or absent in case of negative smear and/or culture test results [66]. Besides, there are a number of 'test of time' strategies, [67] which allow clinicians to deal with uncertain diagnostic outcome more effectively. Often these strategies 'buy time' for the condition to either resolve or declare itself more floridly and leads to explicit identification of patients with 'undifferentiated presentations' that can all help target antibiotics to those most likely to benefit. For example, safety-netting has been proposed as a method of reducing the risk of missing serious disease in patients with an 'uncertain' diagnostic outcome [68]. Research designed around the explicit identification of patients with 'undifferentiated presentations' would facilitate the evaluation of different strategies for programme managers to policy-makers and from medical personnel to handle this subgroup in clinical practice [69]. This would impact significantly on general practice, providing clinicians with a set of validated tools to confidently handle uncertain diagnostic outcome.

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Chapter 6

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Summary

The studies presented in this thesis were focused on the quality of diagnostic care provided to suspected tuberculosis (TB) patients and centre on four principle investigations. The central aim of the studies presented in **Chapters 2 and 3** was to gain understanding of the current quality of TB care and reasons for not following the guidelines. The overarching aim of the study presented in **Chapter 4** is to develop strategies to diagnose TB in patients whose initial tests are negative for smears. A prospective study to determine the predictors of culture-negative TB among smear negative TB cases is described. Finally, in **Chapter 5** we explore the level of salivary NO and its metabolites as a potential biomarker to detect the earliest and most difficult to diagnose TB cases. In the final chapter of this thesis, **Chapter 6**, we presented the overall major findings, methodological considerations, and general conclusion of the studies.

In **Chapter 1** of this thesis, we summarize the result of a literature review related to research questions regarding the quality of diagnostic care provided to suspected TB patients. In its Global TB Report 2013, the WHO highlighted detection of missed cases as a priority action to reach 2015 global targets. The estimated TB case detection rate (percentage of detected cases among the estimated number of incident cases) in Ethiopia has been consistently low; the WHO estimate is 64% for all forms. In a recent Ethiopian national population based survey in 2011, the prevalence of bacteriologically-confirmed TB (smear and/or culture positive) for persons aged 15 years and above was 277 per 100 000. Besides, reports from previous studies, showing a high proportion of TB among young persons, suggests that TB is circulating in the community and that there is a need for more efforts to limit the spread of TB disease.

Current TB control strategies in many countries depend on care that is provided to persons who have symptoms of TB and/or those who have the disease. In most high-burden countries, TB is still diagnosed using tools such as direct sputum microscopy and chest radiographs. When applied to resource-limited settings, these methods suffer from significant limitations; accuracy is suboptimal and fails to diagnose one-third to half of all TB patients. To improve the accuracy of diagnostic work-up several new diagnostic tests have been suggested. However, no single parameter has gained undisputed acceptance. Predictive models for the diagnosis of TB provide a useful framework for systematization of the diagnostic approach and are able to standardize data collection from clinicians. These models could be used to identify patients at very high risk of TB that may require further diagnostic tests after the results of a negative smear test. Besides, further research is necessary to determine the usefulness of these prediction models to establish the exact causes of false smear negative TB (patients that have TB, but have a negative smear test). Furthermore, substantial effort has been devoted to improving physician compliance with evidence-based guidelines. Studies of the quality of TB evaluation (i.e. diagnostic workup of patients with symptoms suggestive of TB) in high burden countries have generally shown poor adherence to international or national guidelines. While previous research has assessed barriers patients face in accessing primary care centers that provide TB diagnostic services, less is known about barriers

providers in these settings face in adhering to guidelines for evaluating patients for TB. We identified diagnostic guideline adherence and professional practice, as relevant elements of the under-emphasized theme in quality of TB care.

The aim of the study reported in **Chapter 2** was to determine quality of diagnosis and monitoring of treatment response of patients with smear-negative TB compared with smear-positive cases. A retrospective review of medical records of newly diagnosed TB cases was performed and we descriptively analysed the proportion of TB cases managed according to ISTC protocol. Our findings show poor standardized clinical care offered to smear-negative TB cases compared to smear-positive TB cases. This could be due to the assumption that the prognosis for smear-negative cases is better than for smear-positive cases. However, this assumption can only be valid if microscopy procedures are followed completely and reliably. Therefore, there is a need to explore the reasons behind poor standardized clinical work-up in smear-negative compared to smear-positive TB cases.

The research aim of the second study, described in **Chapter 3**, was to explore TB service providers' reasons for not complying with TB treatment guidelines at health facilities in Ethiopia. A descriptive qualitative design was used. Focus group discussions (FGD) were held with 39 service providers involved in TB care. A questioning route for the FGDs was prepared, with open-ended questions, probes and prompts. The questions were based on information from the current literature (sensitizing concepts), and had been reviewed by stakeholders and adapted accordingly. The topics were quality deficits, compliance with TB guidelines and information transfer. For the data analysis we used the qualitative content analysis approach based on the constant comparison method. Codes and categories emerged inductively from the data through careful examination by the researcher. The main reasons for non-compliance with TB treatment guidelines were (1) insufficient diagnostic modalities, limiting the capacity to correctly diagnose TB and provide the right regimen; (2) ambiguity in guideline recommendations especially for specific sub-groups of patients, making it difficult to prioritize them to the right regimen; and (3) poor documentation concerning referred patients, with no appropriate examination and difficulty obtaining information from previously treated patients. Our findings offer real-life examples of what is needed to customize TB guidelines to local contexts of healthcare in order to improve compliance. We recommend developing intervention strategies that are not only tailored to average patients. Consideration should be given to specific sub-groups as an important influence on guideline compliance.

Despite the considerable incidence of culture-negative TB, little is known about this specific group's clinical manifestations compared to culture-positive TB among smear negative cases. In **Chapter 4**, we hypothesize that culture-negative TB cases may present with no significant differences in clinical and radiographic abnormalities when compared to those cases with a culture-positive and smear-negative result. A prospective evaluation study was designed to determine the predictive ability of routine diagnostic tests and clinical symptoms. While application of predictive modelling in patients

with clinical suspicion of TB has been described before, previous studies based their analysis on combining only independent significant predictive variables into a decision model. Therefore, in this study, we evaluated the performance of predictive models when all and only significant variables were considered for estimating the probability of TB; (1) among all clinical suspects and (2) among smear negative cases. Employing all variables and only independent significant ones into a regression model, failed to discriminate between culture-negative and culture-positive among smear-negative TB cases, thereby supporting our hypothesis that among smear-negative cases, culture-negative TB may present with no differences in clinical and radiographic abnormalities compared to those with culture-positive TB. This finding emphasizes that medical providers should neither rely on culture as the gold standard tests for TB, nor expect a clinical presentation with the symptoms characteristic of advanced TB in order to diagnose and initiate treatment in culture-negative TB. Furthermore, our findings suggest that independent significant variables (positive sputum smear and chest radiography compatible with TB) remain critical elements in prediction of culture-positive TB among patients with clinical suspicion.

In the Global TB Report 2014, the WHO declared that “one of the most urgently needed tests is a rapid biomarker-based test that can diagnose TB”. Ideally, such TB biomarker should be able to detect the earliest and most difficult to diagnose TB cases, and should be suitable for use in resource-limited settings. A series of reviews has shown that biomarkers such as NO have the potential for screening TB suspects. Human mouth represents the environment with a constant supply of NO metabolites from L-arginine. The production of stable salivary NO metabolites, may be affected during nutritional deficiencies, a common condition in TB suspects. Several studies reported that patients with active TB are more likely to experience wasting, and have a lower body mass index (BMI) than healthy individuals. Therefore, it is hypothesized that patients unable to respond with an effective production of salivary NO metabolites in response to *Mtb* infection, are more likely to have severe (commonly seen as wasting in TB suspects) and more infectious forms of TB. In **Chapter 5**, an exploratory study to compare the salivary NO levels in confirmed and unconfirmed suspected TB patients by sputum smear microscopy, chest radiography and molecular GeneXpert tests, is summarized. The objective of the study was to examine the diagnostic value of the level of salivary NO metabolites, in order to assess microbiological outcome (infectiousness), and its association with the severity of the disease (commonly seen as wasting) in TB suspects. The results of our findings support our hypothesis that patients unable to respond with an effective production of salivary NO metabolites in response to *Mtb* infection, are more likely to be associated with increased risk of acquiring TB. Interestingly, the results could not support our second hypothesis that levels of salivary NO could be associated with the severity of the TB. This finding is also in line with the study of Idh et al., in which they neither found an association between BMI and NO levels at

treatment initiation. However, larger clinical studies, including long term clinical follow up will be essential to test this hypothesis thoroughly.

The findings in this studies presented in this thesis suggest a number of avenues for further research and specific public policy implications for professional practice. Our findings indicate that the degree of trust that TB service providers had in the diagnostic test results had a great impact on clinician's compliance with the guidelines. Thus, addressing their negative perception of sputum smear microscopy may be just as important as scaling up the diagnostic test. Consideration should be given to specific sub-groups (smear and culture negative TB suspects) as an important influence on guideline compliance. However, in our study a critical question was whether smear and culture negative patients can be detected early by predictive modelling. Our finding is in agreement with a previous study that reported that predictive models based on clinical variables may not be useful to discriminate culture-negative TB, because of the variable presentations of symptoms. These symptoms are somewhat ubiquitous in a clinical setting, thereby non-specific, and could possibly indicate a multitude of other illnesses. Although the coexistence of illness in smear and culture negative TB is problematic in itself, a fundamental challenge arises when clinical responses to this uncertainty are guided by the single disease paradigm that dominates the existing health care system. This paradigm leads to 'incomplete assessment of diagnostic outcome and failure to modify the clinical approach accordingly'. One possible direction for further research is the use of a diagnostic panel, i.e. a group of experts who assesses the results from all available relevant patient data to reach a final diagnosis in each patient. However, few studies have systematically examined the accuracy of TB diagnosis by such a panel and the type of diagnostic evidence available. Besides, approaches that combine clinical judgment based on all available information with additional value of new diagnostic tests have been proposed, but rarely addressed. Furthermore, we suggest that future quality improvement initiatives should focus on the role for real-time, clinic-level monitoring of smear negative TB suspect evaluation practices, in order to capture individual data on clinical measures related to care of smear-negative TB from every patient evaluated.

Valorisation

VALORISATION: IMPLICATION FOR FUTURE RESEARCH AND PRACTICE

In this section, I consider possible implications, both for the theoretical development of research on quality of TB diagnostic care and the specific public policy implications for professional practice in patients with suspected TB in resource poor setting. The findings in this study suggest a number of avenues for further research. Firstly, I recognize that by situating this research in Ethiopia context, the generalizability of my findings outside the resource constrained setting is limited. Although recent WHO report and others have shown that clinicians often deviate from internationally recommended TB management, our findings add that care offered to smear-negative cases continues to be deviant from the standards, compared to smear-positive TB cases. This then raises the question of the perceived habitual bias by TB healthcare providers, who apparently still consider managing smear-positive cases to be the most effective strategy to curb the TB epidemic. This treatment adherence gap would be significantly closed if strategies that increase compliance to ISTC are identified by programme managers and policymakers in hospitals that best meet the desired standards. Therefore, we suggest that future quality improvement initiatives should focus on the role for real-time, clinic-level monitoring of smear negative TB suspect evaluation practices, in order to capture individual data on clinical measures related to care of smear-negative TB from every patient evaluated.

Less well understood are the determinants of provider adherence to TB treatment guidelines. In particular, there is increasing recognition that guideline implementation is heavily dependent on provider behaviour and in order to improve the quality of diagnostic care, understanding and subsequently changing provider behaviour is required. Our findings indicate that the degree of trust that TB service providers had in the diagnostic test results had a great impact on compliance with the guidelines. Thus, addressing their negative perception of sputum smear microscopy may be just as important as scaling up the diagnostic test. Furthermore, there was dilemma among TB service providers about relying only on sputum microscopy in passive case finding. The underlying conflict was a lack of alternative diagnostic modalities when the initial test result does not correspond with the patient's clinical presentation. A better strategy to retest those patients whose initial tests are negative in settings where TB is frequently missed may improve compliance with the guideline.

A recent review of the TB guidelines showed that the overall methodological quality of the guideline was low. Several domains of the guidelines, such as applicability to specific local situations, contributed to their poor performance. Our findings offer real-life examples of what is needed to customize TB guidelines to local contexts of healthcare in order to improve compliance. We recommend, more studies in other regions and across a wider health facility spectrum to generalize a whole picture of non-adherence in Ethiopia and develop appropriate strategies that influence their compliance with the guidelines. With the important limitation of this study described above in

mind, further studies are now needed to develop appropriate interventions aimed at addressing the critical factors suggested by this study, and to test these systematically. Consideration should be given to specific sub-groups (smear and culture negative TB suspects) as an important influence on guideline compliance.

Numerous decisions are made by care providers, on the basis of an estimated probability that a specific disease or condition is present in an individual. In the diagnostic setting, the probability estimates are commonly based on combining information from multiple predictors observed or measured from an individual. While application of predictive modelling in patients with clinical suspicion of TB has been described before, these studies usually combined only independent significant predictive variables into the decision models. In addition, information from a single predictor is often insufficient to provide reliable estimates of diagnostic probabilities or risks. However, in this study the critical question is whether patients with smear and culture negative result can be detected and treated early by predictive model. Our finding is in agreement with a previous study that reported that predictive models based on clinical variables may not be useful to discriminate culture-negative TB, because of the variable presentations of symptoms. Therefore, screening for active TB is one of several possible interventions that can improve early detection of all forms of TB, but providing better access to diagnostic tests that are more sensitive than smear microscopy is the first essential step. In addition, sound training in threshold principles and providing tools to correctly assess patients with smear and culture negative results might help in making a better diagnostic decision in resource poor settings.

There is an absolute need to find biomarkers that can distinguish between individuals infected having the highest risk of developing TB (susceptibility) and those who have the highest probability of resisting development of the disease (protection). This would significantly help the control programme to break the chain of transmission of the disease in the long run. We are also in need of validated biomarkers that can be translated as point-of-care assays for early diagnosis and treatment of active TB patients in high-incidence countries. The results of our findings support our hypothesis that, patients unable to respond with an effective production of salivary NO metabolites in response to *Mtb* infection, are more likely to be associated with increased risk of acquiring TB. Our findings and previous studies suggest that the use of NO metabolites as a biomarker may have great value for screening of smear positive TB. Will it be possible to learn from this naturally induced host response and better understand those mechanisms that control infection and exploit their value as potential indicators of protection? There are many studies that suggest that the magnitude of the immune response reflects the magnitude of the bacterial load and two longitudinal studies in untreated contacts suggest that the highest responders are at greatly elevated risk of TB, but these studies are relatively small and lack detailed sequential testing routines. Thus the kinetics and duration of the differences are unknown; however, the fact that the two studies produced similar results in very different populations and environments (Ethio-

pia and Germany) is encouraging. Studies to address the questions of remote versus recent infection, predictive value and optimization of the assays, and their usefulness in HIV infected patients should prove very valuable.

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With many thanks!!!!!!

Mala George

Biography

GEORGE MALA OTIENO

1. Personal details

Date of Birth: 15th June 1974
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2. Professional qualification

2011- 2017 PhD Candidate at University of Maastricht, department of Family Medicine and Primary Healthcare
Degree Expected: PhD (spring 2017)
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Funded by: Nuffic, PhD fellowships program

2000-2005: The University of Nairobi, Department of Biochemistry
Degree: MSc. Biochemistry
Thesis Title: Elution, purification and partial characterization of IgG bound to normal and pathological human placentae.

1995-2000: Egerton University- Kenya, Faculty of Sciences
Degree: BSc. (Biochemistry/Zoology)

3. Professional appointments

Date	Department	Organisation	Position
Nov 2004 – August 2006	Dept. of Biochemistry	University of Nairobi	Graduate Assistant
Sept 2006 – To Date	Dept. of Medical Biochemistry	Mekelle University	Lecturer
Jan 2011 – 2017	Dept. of Family Medicine	Maastricht University	PhD candidate

4. University Teaching Experience (Mekelle University-Ethiopia 2006-Date)

Department	Programs	Courses offered	Special modules offered
Depts. of Health's and Medicine	MB.Ch B I, BDS I, Medical B.Pharm I, BScN I, BSc Public health (Regular programs)	Medical Biochemistry	Molecular medicine, Biochemical pharmacology, Nutritional Biochemistry, Neurochemistry, Clinical Immunology
Depts. of Nursing	Cataract, Neonatal and Emergency, Nursing (Special programs)	Medical Biochemistry	Biochemistry of the eye, In born errors of metabolism, Acid-Base balance, electrolyte and Paediatric Biochemistry
Dept of Public Health	MSc. Public Health	Medical Biochemistry & research supervision	Nutritional Biochemistry, research supervision in TB diagnostic and quality of care
Dept of Dentistry	MSc. Orthodontics	Medical Biochemistry	Molecular genetics (Principles of Orofacial and Malocclusion)

Other responsibilities

1. Head of Clinical Biochemistry course unit (2006-2013)
2. Mentor for medical students (mentoring students throughout the first year of their bachelor in medicine)
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Current Research Interest

1. Endocrine disruptors in reproductive health and metabolism

2. The role of biomarker for screening and diagnostics of suspected tuberculosis (TB) patients and relation to treatment outcome.
3. Impact of cholesterol and cathelicidin LL-37 levels on Host Immunity to Mycobacterium Tuberculosis and Response to Treatment
4. The protective role of glucose-6-phosphate dehydrogenase (G-6-PDH) activity against risk factors of cardiovascular diseases

6. List of publications and submitted Manuscripts

1. Omwandho C. A, Gruessner E.E, Falconer J, Tumbo-Oeri A.G, Mala G, Mecha E, Tinnerberg H.R, Roberts T.K 2006. Ovine placental eluate immunoglobulins recognize isologous and third party acid treated trophoblast microvesicles antigens in Vitro. *Journals of South African Veterinary Association* 77(1):24-27.
2. Mala G, Spigt M, Gidding LG, Blanco R and Dinant GJ. Quality of diagnosis and monitoring of tuberculosis in Northern Ethiopia: medical records-based retrospective study: *Tropical Doctor* 2015; 45(4):214-20.
3. Mala G, Moser A, Dinant GJ, and Spigt M. Why tuberculosis service providers do not follow treatment guideline in Ethiopia: a qualitative study. *J Eval Clin Pract* 2013.
4. Mala G, Dinant GJ, Berhe S, Kiti LA and Spigt M. Predicting smear- and culture-negative tuberculosis with routinely available diagnostic tools in Ethiopia. Submitted Manuscript
5. Mala G, Spigt M, Berhe S, Tesfay H and Dinant GJ. Dealing with diagnostic uncertainty in suspected Tuberculosis (TB) patients with a negative smear-and culture results: A consensus diagnostic evaluation study. Submitted Manuscript
6. Parsons B J, Mala G, Dinant GJ, Ngeny C and Spigt M. Exploratory analyses of the level of salivary Nitric Oxide (NO) metabolites and microbiological outcome in suspected Tuberculosis (TB) patients. Submitted Manuscript

7. Referees

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