Determinants of tuberculosis in Lambaréné and barriers towards successful antituberculous treatment

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Abbreviations

AIDS	-	Acquired immune deficiency syndrome
AM	-	Amikacin
ART	-	Antiretroviral treatment
aOR	-	Adjusted odds ratio
BELE	-	Base d'épidémiologie
BCG	-	Bacille Calmette-Guérin
BMI	-	Body mass index
^{14}C	-	Carbon-14 (radioactive isotope)
CD4	-	Cluster of differentiation 4
CERMEL	-	Centre de Recherches Médicales de Lambaréné
CHRGR	-	Centre Hospitalier Regional George Rawiri
CI	-	Confidence interval
CM	-	Capreomycin
CS	-	Cycloserine
CRF	-	Case record form
CTA	-	Centre de Traitement Ambulatoire
DOTS	-	Directly observed therapy, short course
DR-TB	-	Drug resistant TB
DST	-	Drug susceptibility testing
EMB	-	Ethambutol
EPTB	-	Extrapulmonary tuberculosis
ETO	-	Ethionamide
FASH	-	Focused assessment with sonography for HIV-associated TB
FCFA	-	Franc de la Communauté Financière d'Afrique
FM	-	Fluorescence microscopy
FU	-	Follow up
GPS	-	Global positioning system
HAS	-	Hôpital Albert Schweitzer
HIV	-	Human immunodeficiency virus

ID	-	Identification
IFNγ	-	Interferon Gamma
IGRA	-	Interferon-Gamma release assay
INH	-	Isoniazid
IPT	-	Isoniazid preventive therapy
IQR	-	Interquartile range
LJ agar	-	Löwenstein-Jensen agar
LTBI	-	Latent tuberculosis infection
LTFU	-	Lost to follow up
MDR-TB	-	Multi-drug resistant tuberculosis
MDG	-	Millennium development goal
MGIT	-	Mycobacterium Growth Indicator Tube
M.tb.	-	Mycobacterium tuberculosis
NA	-	Not available
NRC	-	National Reference Center
NTM	-	Non-tuberculous mycobacteria
OR	-	Odds ratio
OFX	-	Ofloxacin
PAS	-	Para-aminosalicylic acid
PCR	-	Polymerase chain reaction
PNLTB	-	Programme national de lutte contre la tuberculose
PTB	-	Pulmonary TB
PZA	-	Pyrazinamide
RIF	-	Rifampicin
SD	-	Standard deviation
STR	-	Streptomycin
TB	-	Tuberculosis
TST	-	Tuberculin Skin Test
ZN	-	Ziehl-Neelsen
WHO	-	World Health Organization
XDR-TB	-	Extensively-drug resistant tuberculosis

1 Introduction

1.1 Tuberculosis

Tuberculosis (TB) is one of the oldest diseases known to humanity [1]. While for thousands of years its etiology had been mysterious, it is today known to be caused by bacteria. TB is described as a disease of poverty and ranks among the five most common causes of death due to infectious diseases worldwide [2]. In our globalized world new challenges are arising in the fight against TB, such as its epidemiologic promotion through the human immunodeficiency virus (HIV) pandemic and the emergence of drug resistances. However, global collaboration efforts have brought up new tools towards disease control and the human potential to overcome the threat has reached an unprecedented high.

1.1.1 Epidemiology

The illustration of the current epidemiological situation of TB as described in the following section refers to the World Health Organization (WHO) global tuberculosis report 2014 [3] unless otherwise noted.

In 2013, 20 years after TB has been declared a public health emergency by the WHO, global strategies and joint efforts to fight the disease are showing effect: global incidence is falling and prevention of deaths due to TB is on track to reach the targets set by the global community for 2015. However, some regions are dropping behind in effective disease control. The central African country Gabon is particularly affected, requiring special attention now in order to achieve global TB control.

1.1.1.1 TB incidence

In 2013 there has been a total number of 9.0 million new TB cases worldwide, equivalent to 126 cases per 100,000 population. After an incidence peak in 2003 with an estimated 9.3 million new cases, incidence has been slowly declining by about 2% per year. The Millennium Development Goal of halting the rise in incidence before 2015 (section 1.2.2.) has been reached since around a decade.

In absolute numbers the Asian Region* harbors the highest incidence (56% of new cases in 2013) with India and China in lead. The numbers of incident TB cases relative to population size however are highest in southern Africa with Swaziland, Lesotho and South Africa being the leading countries with up to 1000 new cases per 100,000 population. TB hot spots outside Africa include Myanmar, Cambodia, the Philippines and Pakistan.

With an incidence rate of estimated 423 per 100.000 population in 2013, Gabon ranks among the top ten countries worldwide.

1.1.1.2 TB mortality

In 2013, 1.5 million people have died from TB with an estimated 360,000 being co-infected with the human immunodeficiency virus (HIV). Mortality rates have been falling by around 45% since 1990, but forecasts suggest that an accelerated decline would have been necessary to reach the target of the *Stop TB partnership* to reduce them by 50% until 2015. Approximately 78% of all TB deaths occurred in the African and South East Asian Region*. Mortality is declining in all six regions and the regions of the Americas and the West pacific region have already reached the target.

In Gabon, 910 and 180 deaths from TB were reported in 2013 in HIV uninfected and infected patients, respectively, showing the disease to be a considerable cause of death in the country [4].

1.1.1.3 TB prevalence

Eleven million people have suffered from TB in 2013, equivalent to 159 cases per 100,000 people. Although prevalence has fallen since 1990 by 41% forecasts suggest that the *Stop TB Partnership* target of halving TB prevalence by 2015 has not been reached. Prevalence is falling in all six WHO regions. While the target has been reached in the region of the Americas and West Pacific Region,

* Regions defined by WHO:
http://www.who.int/about/regions/en/ [last accessed: August 18, 2015]

is seems unlikely to be reached in the African and Eastern Mediterranean Regions.

With 578 per 100.000 population having suffered from TB in 2013, Gabon is among the high-prevalence countries [4].

1.1.1.4 HIV/TB co-infection

Since the 1980s, the HIV pandemic has become a major public health threat globally and in many African countries. The HIV pandemic was a driving source for the re-emergence of TB. In 2013, 1.1 million (13%) of new TB patients were co-infected with HIV, 78% of which were from the African Region. In the African Region, 34% of new TB cases were HIV co-infected.

In Gabon, while an HIV/TB co-Infection rate of 11% has been reported to the WHO in 2013 [4], some studies give evidence of much higher rates of up to 46% in the last years [5-7].

Linking-up of efforts to fight TB and HIV in an integrated approach is now accepted as mandatory to overcome the co-pandemic [8, 9]. Remarkable efforts have been made following the WHO recommendations on interventions needed to prevent, diagnose and treat TB in people living with HIV [10]: in 2013, 48% of globally notified TB cases have been tested for HIV, showing a more than 15-fold increase from 2004. 76% have been tested in the African Region, and 100% in Gabon according to WHO data [4]. However, the worldwide target of antiretroviral therapy (ART) coverage for all TB patients living with HIV is still far: 70% of global HIV co-infected TB patients have been on ART in 2013 and 69% in the African region. Equally, further effort is required in providing isoniazid preventive therapy (IPT) to people living with HIV who do not have active TB: in 2012, globally 31% received IPT, which is below the target of the 50% in eligible patients [11]. For Gabon, data on ARV and IPT coverage is urgently needed.

1.1.1.5 Drug resistant tuberculosis

The emergence of drug resistant *M. tuberculosis* strains (DR-TB) is one of the major challenges in the global fight against TB. Multi-drug resistant TB (MDR-TB) is defined as resistance to at least isoniazid (INH) and rifampicin (RIF), the two

most efficient drugs. Extensively-drug resistant TB (XDR-TB) is defined as resistance to INH and RIF as well as any fluoroquinolone and any of the second-line injectable drugs [12] (section 1.1.6).

In 2013, 3.5% of newly diagnosed TB cases and 20.5% of retreatment were MDR-TB on a global scale. While India and China harbor the highest total burden of MDR-TB, the highest proportions are found in Eastern Europe and central Asia. In Belarus, 35% of new cases and 55% of retreatment cases were due to MDR-TB. XDR-TB was found in 9.0% of global MDR-TB cases and was reported in 100 countries by the end of 2013. The proliferation of *Xpert MTB/RIF* usage (section 1.1.5.6) helped to delineate extends of MDR-TB and detection rates have increased by 42% from 2011 to 2012. While on a global scale second-line treatment was started in 71% of MDR-TB patients notified, only 44% of MDR-TB patients in the African Region received appropriate treatment and waiting lists for second-line treatments persist or grow in several countries. The absolute number of newly started second-line treatments increased by more than two times from 2009 to 2013 [11].

For Gabon only anecdotal data report on the existence of MDR-TB [13] and valid data on the local MDR-TB burden and available treatment options is urgently needed. In 2013, 2.6% of new TB cases and 13% of retreatment cases were estimated by WHO to be MDR-TB [4]. However, no systematic data is available on drug resistances.

1.1.1.6 TB in children

In 2013, the WHO estimated 550,000 children to have acquired TB, which is 6% of total new TB cases. Eighty thousand HIV negative children infected with TB died in 2013 (7% of total HIV negative TB deaths). However, the value of available data is limited due to both underreporting of TB in children and frequent misdiagnosis [14].

In Gabon, 4% of new TB cases were notified to be children younger than 15 years old [4].

1.1.2 Causative organisms

The following description of the microbiological characteristics of mycobacteria refers to the “Comprehensive Clinical Reference” by Schaaf and Zumla [15] unless otherwise noted.

Tuberculosis disease in humans and other mammals are caused by mycobacteria of the *mycobacterium tuberculosis (M.tb.) complex*. Mycobacteria are aerobic, non-motile, non-spore forming, non-encapsulated, acid-fasts rods. They are classified as acid-fast due to the fact that the lipid-rich cell wall is relatively impermeable to most dyes, requiring a special stain for microscopy (section 1.1.5.3).

In principal, mycobacteria can be divided into two slow growers and rapid growers. Both have simple nutritional requirements and are able to grow and survive in difficult conditions. Slow growers take up to 24 hours to divide, which is extremely slow compared to other bacteria. While they are divided ecologically into obligate pathogens and so-called environmental mycobacteria, the most important differentiation from a medical point of view is that into *M.tb. complex* and Non-tuberculous mycobacteria (NTM).

Mycobacteria of the *M.tb. complex* are obligate intracellular pathogens, which persist in macrophages and thereby avoid human immune responses (section 1.1.3.2). Species grouped in the *M.tb. complex* are *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti* and *M. canetti* [16].

M. tuberculosis is the major cause for TB in humans and also the first mycobacterium discovered and identified by Robert Koch in 1882 [16]. *M. africanum* is the name of a heterogeneous group of strains isolated from patients in equatorial Africa. *M. bovis* is mainly causing TB in cattle, but has the ability to cross the species barrier and caused around 6% of all human TB deaths in Europe before the introduction of milk pasteurization [16].

1.1.3 Pathophysiology

The illustration of the pathophysiological specifics of TB as described in the following section refers to “Robbins Basic Pathology” 8th edition [17] unless otherwise noted.

1.1.3.1 Transmission

Transmission occurs predominantly by inhalation of aerosols arising from expectoration of infected persons. More uncommon ways of transmission include digestion of unpasteurized milk or direct inoculation of mycobacteria.

The pathogenesis and host response differ crucially between immunocompetent and immunocompromised individuals as well as between those having and having not been previously sensitized to TB.

1.1.3.2 Latent tuberculosis infection

Of previously unexposed individuals being in contact with *M.tb.,* only a small fraction develops active disease. Over 90% of those infected are able to clear the infection or contain the pathogens in a latent stage, which is called latent tuberculosis infection (LTBI). Individuals in this stage are asymptomatic and not contagious. Worldwide, the number of people with LTBI is estimated to be two billion [16, 18].

In immunocompetent individuals, the infection initiates cell-mediated immunity, providing resistance to the organism and tissue hypersensitivity. The latter results in formation of caseating granulomas and cavitation. In most cases, the host is able to contain the primary infection focus through granuloma formation and the disease fades into a latent stage.

More specifically, mycobacteria gain entry into macrophage endosomes, but are able to inhibit microbicidal responses by endosomal manipulation, which includes change of endosomal pH and arrest of endosomal maturation. During this phase mycobacteria can proliferate freely in alveolar macrophages. After approximately three weeks, when processed mycobacterial antigens reach the draining lymph nodes, cell mediated immunity develops: antigens are presented by dendritic cells to CD4+ T cells, which thus differentiate into T_H1 cells. Through secretion of

interferon γ, they activate macrophages which, in turn, cause a variety of down-stream effects, one being the recruitment of monocytes and their differentiation into epitheloid histiocytes, which characterize granuloma formation.

1.1.3.3 Progressive primary tuberculosis

In immunocompromised persons, the host immune system cannot set up the CD4+ cell-mediated response required to contain the primary focus, allowing the pathogens to spread without granuloma formation and thus leading to progressive primary tuberculosis. This often includes atypical forms of TB (section 1.1.4). The overall risk of developing progressive disease is only around 5%, but can increase dramatically through a number of risk factors, such as coinfection with HIV or malnutrition and is also higher in children [16, 18, 19].

1.1.3.4 Secondary or postprimary TB

Postprimary TB is the disease that develops in previously exposed individuals. It may either be caused by reactivation of latent TB (endogenous reactivation, more likely in low-prevalence areas) or by reinfection with another pathogen (exogenous reinfection, more likely in high-prevalence areas). The lifetime risk in infected persons is only about 10% and the triggers for reactivation in otherwise healthy individuals are not yet completely understood. However, reactivation arises particularly when host immunity is weakened, e.g. due to HIV-Infection or malnutrition [16, 18].

The classical localization of lesions in postprimary TB is at the apex of the upper lobes of the lungs. Due to a prompt response of the host immunity, the local lymph nodes are less prominently involved than in primary TB. Instead the host response leads to cavitation and thus to dissemination along the airways and increased infectivity of the patient.

In persons with advanced immunosuppression such as AIDS, the clinical picture resembles that of progressive primary TB. This atypical presentation of TB includes lower and middle lobe lesion, hilar adenopathy and extrapulmonary forms such as lymph node TB, pleural effusions, tuberculous meningitis and

miliary TB. Due to diminished destruction of bronchial walls, bacillary loads in the sputum are often lower than in immunocompetent individuals.

1.1.4 Clinical course

The classical symptoms of pulmonary TB (PTB) consist of chronic cough, weight loss, fever, night sweats and hemoptysis [20]. In early stages of the disease patients can be asymptomatic or show only mild symptoms [21]. In most cases cough is worsening and sputum production increasing with progressive pulmonary involvement. Sputum often is initially mucoid, later purulent and blood stained. Fever is usually of low grade and remittent [17].

In HIV-infected patients clinical manifestation of TB differs in correlation with the degree of immunosuppression. Extrapulmonary TB (EPTB) manifestations are more frequently seen and the classical symptoms such as purulent cough are often missing [22, 23], making the diagnosis more difficult. In cases of EPTB, symptoms depend on the organ or body site involved. Possible complains include pleuritic pain, lymph node swelling, neurologic deficits, infertility and others [17].

1.1.5 Diagnostics

1.1.5.1 Chest x-ray

In the vast majority of TB cases, chest x-ray films show abnormalities. The hallmark of primary TB is hilar or mediastinal lymphadenopathy, which is most common in children and decreases with age. Parenchymal involvement shows an opposite trend, being more common in adults [20].

Characteristics of postprimary TB are opacities in the apical and posterior segments of the upper lobes, while hilar or mediastinal lymphadenopathy is rare. Cavities are visible in 40-45% of cases and often show air-fluid levels. Other radiographic signs include pleural effusions, atelectasis and mediastinal shift [20].

Miliary TB appears in the chest x-ray as innumerable nodules scattered throughout both lungs and is mostly associated with lymphadenopathy [20].

1.1.5.2 Tuberculin skin testing and Interferon-Gamma-release-assay

In 1980 Robert Koch discovered the immune response to a purified protein derivate (PPD) obtained from TB culture filtrates called tuberculin. Later, diagnostic test were developed using this tuberculin reaction by the physicians Felix Mendel and Charles Mantoux. Thus the test is also called Mendel-Mantoux Test [24].

Tuberculin causes a delayed type hypersensitivity reaction when injected in the skin of a person currently or previously infected with *M.tb*. The host response is detectable starting from 2-12 weeks after infection. The reaction usually begins 5-6 hours after injection and reaches its maximum after 48-72 hours [25].

The Tuberculin Skin Test (TST) cannot differentiate between active and latent TB infections. Its value is limited by its sensitivity and specifity: false-negative reactions can be caused through cutaneous anergy due to immunosuppression as seen in HIV or malnutrition, but also in overwhelming TB disease. False-positive reactions occur after contact with NTM and in persons who have been vaccinated with BCG [25].

Against the background of these limitations, another method was developed: the interferon-gamma-release-assay (IGRA). This blood test measures the immune response to *M.tb.* antigens. Secretion of IFNγ by specific effector memory t-cells of the host is stimulated *in vitro* through exposure with *M.tb*. antigens. The level of secreted IFNγ varies between exposed and unexposed individuals [26].

IGRAs have shown to be less prone to false-positive results caused by NTM or BCG-vaccine. However, they are not more sensitive than TST and they are not useful in the differentiation of active and latent TB, neither can they rule out a LTBI [27].

According to WHO, IGRAs are not preferable to TSTs in low- and middle-income countries with high TB and/or HIV burdens [28].

1.1.5.3 Microscopy

The diagnosis of mycobacteria through microscopy is the oldest available diagnostic method, and still the most commonly used method in many low-and middle income countries. Smear microscopy is done mainly from sputum samples, but can also be done from gastric aspirates or extrapulmonary samples such as lymph node aspirate or pleural fluid. Since mycobacteria are acid-fast bacilli they need a special preparation and staining.

A) Ziehl-Neelsen (ZN) stain

This is the “conventional” method with a long history of clinical use. The preparation for this stain includes coloration with carbol fuchsin, decolorization with acid alcohol and a counterstain with methylene blue. Reading is done with an ordinary microscope. While this method is quite specific for mycobacteria, sensitivity only reaches 54 – 64% [29], making different or complementary diagnostic steps necessary.

B) Fluorochrome staining

The preparation for this stain is done with the acid-fast dye auramine, decolorization with acid alcohol and counterstain with potassium permanganate solution. Reading requires a ultraviolet (UV) microscope with an intense light source. Fluorescence microscopy (FM) allows faster screening methods, is more sensitive and equally specific compared to the *ZN* method [29, 30].

While traditional fluorescence microscopes are expensive, equally sensitive and much cheaper fluorescence microscopes with light-emitting diodes (LEDs) can be used [31].

1.1.5.4 Culture and drug susceptibility testing

While culture is the gold-standard for the detection of *M.tb.*, it is difficult due to the slow growth of mycobacteria and the required infrastructure often not available in low-income countries.

A) Solid medium

The first method invented to culture mycobacteria was an egg-based solid agar called *Löwenstein-Jensen* (*LJ*) Agar. Limitations to this method are the low

sensitivity (62%) and the long detection time of around one month in average [32].

In 1958, Middlebrook and Cohn described the earlier detection of mycobacteria under growth stimulation with carbon dioxide . However, average detection times with this method are still three to four weeks [33].

B) Radiometric method

A method using ^{14}C-labeled palmitic acid for the radiometric detection of mycobacteria growth was introduced in the 1980s, called the *BACTEC 460 TB System*. It showed increased sensitivity and significant reduction in detection time compared with the conventional solid method, with an average detection time of around two weeks [32].

C) Mycobacteria Growth Indicator Tube (MGIT)

Due to the problems regarding the use of radioactive substrate, development of a non-radiometric technique was promoted, resulting in the development of the *Mycobacteria Growth Indicator Tube*.

The *MGIT* system is based on growth of mycobacteria in a liquid medium. The tube contains a fluorochrome which is initially inhibited by free oxygen but starts to fluoresce when oxygen is used by growing mycobacteria. Reading is done under UV light manually or automatically [30]. Showing similar sensitivity but clearly shorter detection time than conventional methods, and not having the disadvantages of radiometric methods, *MGIT* is the gold standard for detection of mycobacteria today [16, 34].

D) Drug susceptibility testing (DST)

Drug susceptibility testing can be done with all culturing methods mentioned above. In principal growth in two sub-cultures is compared, one with an added test drug (section 1.1.6). Both automated and manual *MGIT* have shown to be as accurate and rapid as conventional methods [35] and have therefore been recommended by the WHO to be used in low-and middle-income countries [36].

For resource-constrained settings, inexpensive alternatives such as microscopically observed drug susceptibility or nitrate reductase assay have been recommended as an interim solution [31].

1.1.5.5 Line-probe-assays

Line probe assays allow rapid molecular testing for resistances in *M.tb.* They can be performed with culture isolates or directly to smear positive sputum specimens. The *GenoType MTBDR assay* (*Hain LifeScience GmbH*, Nehren, Germany) allows detection of RIF and INH resistance genes and therefore rapid diagnosis of MDR-TB [37].

The *GenoType MTBDRsl* allows resistance testing on important second-line TB drugs, therefore being an effective measure to diagnose XDR-TB [38].

1.1.5.6 Automated nucleic acid amplification tests

One of the newest and most promising developments in TB diagnostic is the *Xpert MTB/RIF*. It uses series of molecular line probe assays and real-time polymerase chain reaction (PCR) to detect *M.tb.* and RIF resistance genes. Sensitivity in smear positive and smear negative pulmonary TB reached 98% and 73%, respectively, and specifity was 99%. Moreover, TB diagnosis and resistance testing can be achieved within hours, giving this fully automated and commercially available diagnostic tool the potential to become a major breakthrough in TB diagnostics [16].

1.1.5.7 Serologic and other assays

The use of serologic tests in the diagnosis of TB has been discouraged by the WHO [39] due to a lack of sensitivity [40]. In contrast, a urine test for the mycobacterial antigen lipoarabinomannan (*LAM*-test) has been shown to have increased sensitivity in HIV patients with advanced immunodeficiency [41]. Being still in development, it might be an attractive tool combined with microscopy in settings with high HIV burden.

1.1.5.8 TB diagnostic capacity available in Gabon

By the time of study, TB was diagnosed on clinical ground supported by conventional smear microscopy as only available microbiological tool in Gabon;

culture and DST were not available outside study settings. Equally, any rapid diagnostic tool, such as the *Xpert MTB/RIF* was not available. Therefore, data on the epidemiology of TB in Gabon, especially data on drug resistance, can only be considered as estimates.

1.1.6 Antituberculous treatment

Treatment recommendations have been last revised by the WHO in 2010 [42] and the following section refers to these recommendations unless otherwise noted. Treatment for TB is based on a combined chemotherapy with several antibiotics. Current treatment regimens last over at least 6 months, but can be much longer depending on drug susceptibility. In general, treatment can be divided in a shorter intensive phase and a longer continuation or consolidation phase.

1.1.6.1 First-line drug regimens

In TB patients with drug susceptible TB, treatment should be done with first-line drugs, which have best ratios of efficacy and adverse effects. First-line oral agents are INH, RIF, ethambutol (EMB), pyrazinamide (PZA) and streptomycin (STR). The most effective drugs, INH and RIF are indicated throughout the whole course of the treatment, while other drugs may be stopped in the continuation phase, depending on the treatment regimen. Drug intake should be daily and ideally under direct observation (section 1.2.1).

If possible, every patient should get DST at the beginning of his treatment in order to have the ability to adapt treatment to the resistance profile of the *M.tb.* strain. During treatment, regular supervision is crucial in order to detect treatment failure and adverse effects. The effect of treatment should be monitored bacteriologically in terms of sputum conversion.

Retreatment regimens can differ from those for new patients, since there is increased risk of drug resistances. They should be defined by national TB programs taking into account the local resistance data.

1.1.6.2 Second-line drug regimens

In case of drug resistances, especially MDR-TB, a second-line drug regimen should be used. Second-line drugs are generally less effective and have more adverse effects. They are parenteral, such as kanamycin, amikacin (AM) and capreomycin (CM) or oral, such as ofloxacin (OFX), ethionamide (ETO), cycloserine (CS) and para-aminosalicylic acid (PAS). Second-line drug regimens should contain at least four effective drugs and be directly observed during the whole course of the treatment. Monitoring of treatment effects requires monthly smears and cultures until culture conversion and then at least another 18 months of treatment [43].

1.2 Global strategies to fight tuberculosis

During the 44th World Health Assembly in 1991, global strategies to fight TB were recognized to be insufficient, regarding the increasing incidence in developing as well as industrialized countries. The need for worldwide political commitment was stressed, and initially two targets were set: Achieving 1) a worldwide case detection rate of 70% and 2) a worldwide cure rate of 85% until the year 2000 [44]. Later these targets were postponed to 2005.

1.2.1 DOTS

In 1993 the WHO declared TB to be a public health emergency. Subsequently increased efforts to improve TB care were made at national and international levels, resulting in the development of the *DOTS* strategy. *DOTS* means “Directly Observed Therapy, short course” and is a framework for effective TB control developed by the WHO in 1994 [45]. Its five basic components include:

- Political commitment with increased and sustained financing
- Case detection through quality-assured bacteriology
- Standardized treatment with supervision and patient support
- An effective drug supply and management system
- A standardized monitoring and evaluation system and impact measurement

1.2.2 Millennium development goals and the STOP TB Partnership

During the millennium assembly of the United Nations in 2000, eight goals were set for the year 2015, the *Millennium development goals* (*MDG*). Goal 6c specifically addresses TB: “halt and begin to reverse the incidence of tuberculosis by 2015” [46].

Although many countries have adopted the *DOTS* strategy and there was considerable progress towards the target, additional efforts seemed to be necessary for global TB control. Following two political commitments to stop TB in Amsterdam [47] and Washington [48], the global *Stop TB partnership* was founded in 2001.The *Stop TB Partnership* is an international organization closely linked to the WHO. Its six components are:

- Pursue high-quality *DOTS* expansion and enhancement.
- Address TB and HIV, multidrug resistant TB, and the needs of poor and vulnerable populations.
- Help improve health policies, human resource development, financing, supplies, service delivery, and information.
- Engage all care providers.
- Empower people with TB and communities through partnership.
- Enable and promote research

Its major first action was the publication of the “*First Global Plan to Stop TB 2000-2005*” and the setting of additional targets linked to the *MDG*: 1) By 2015, reduce the prevalence of TB and deaths due to TB by 50% compared with the baseline of 1990 and 2) By 2050, eliminate TB as a public health problem as defined by achieving a worldwide incidence of TB of less than one case per million population per year [46, 49].

Each in 2006 and 2010, the *Global Plan to Stop TB* was reevaluated and updates were published with scenarios how to reach the targets in regions with high burden of TB and more specific guidance on how to reach the targets [50, 51].

A post-2015 global TB strategy (the *End TB strategy*) has been developed in 2013 and approved by all Member States at the May 2014 World Health Assembly [52].

1.3 TB Treatment outcomes

Over the last two decades, TB treatment success has remarkably improved on a global level. From a success rate of 57% in 1995 in new smear positive TB cases, it has risen to 86% in 2012, meeting the target of 85% set in 1991 [3]. This progress can be attributed to global efforts and strategies, such as the increasing implementation and acceptance of *DOTS* [53] (section 1.2.1), strengthening of national TB programs and increased financial aid. New molecular diagnostics and the development of new drugs and vaccines offer promise for further improvement of TB control [16].

In principal, classification of treatment outcomes is done differently between patients with drug-susceptible TB and those with MDR-TB receiving second-line treatment. Different outcomes as defined by the WHO are successful treatment, treatment failure, loss to follow up, and death. Treatment success can be assessed clinically (treatment completed) or, better, microbiologically by sputum conversion (cured) [42].

1.3.1 Treatment outcomes of drug-susceptible TB

In 2012, 86% of worldwide new TB cases have been treated successfully. The American and European regions are behind, having a 76% and 75% treatment success rate in 2012, respectively [3].

According to data reported to the WHO, in the African region treatment success was 79% in 2011, 6.2% of TB patients died, 0.9% of treatments failed and 5.6% of patients defaulted [54]. For Central Africa, data of high quality and visibility is scarce. Published epidemiological studies from Gabon are mainly retrospective, available exclusively in French, and refer to particular subgroups. However, the little data available suggests that in the time period between 2004 and 2012 treatment success in Gabon has been alarmingly low (39-48%) [7, 54] and defaulting rates exceptionally high (28-61%) [5, 7, 54].

1.3.2 Treatment outcomes of drug resistant TB

In 2011 the global treatment success for MDR-TB rate was 48%, while in 25% of treatments patients were lost to follow up or no outcome was reported. Treatment success was best in the Eastern Mediterranean (64%) and lowest in the African and European Region with less than half of the patients treated successfully. The target of a 75% treatment success rate set by the *Stop TB Partnership* was reached only by 29 out of 126 countries [3].

In 2009, a meta-analysis of treatment outcomes of MDR-TB patients under strict *DOTS* conditions found a pooled success proportion of 69% [55]. A study on XDR-TB in South Africa showed culture conversion in only 20% and deaths in 42% [56].

A recent case series on drug resistant TB cases in Gabon reported that only 3 out of 16 patients (18%) were cured and 5 (31%) died [13].

1.4 Barriers towards successful TB treatment

On the way to global TB control, treatment outcome is crucial: while successful treatment does not only reduce morbidity and mortality but prevents further transmission of the disease, adverse treatment outcome increases the local disease burden, therefore also placing a higher financial strain on the country. Moreover, high defaulting and treatment failure rates bare the risk to fuel the emergence as well as the spread of drug resistances and therefore endanger disease control also on a global level.

Worldwide, the greatest barriers towards successful treatment are the emergence and spread of drug resistances and the insufficient management of HIV co-infections [11]. Furthermore, the global economic crisis endangers successful treatment by posing economic barriers in low-income countries, causing unbearable treatment costs for patients and insufficient drug supply through the governments [18, 57, 58]. Besides, other problems identified as cause of adverse treatment outcome remain unsolved [59]. These include geographical barriers [60], gender differences [61, 62], stigmatization and malnutrition [63, 64].

For Gabon, reasons for adverse TB treatment outcome are insufficiently understood. Almost no data are available about the extent and outcome of DR-TB. The few data on HIV co-infection rates in TB patients suggests a major public health challenge, but more data are required to evaluate integration of TB and HIV services and quantify treatment outcome. Evidence of dramatic defaulting rates and alarmingly low treatment success rates suggest failure of the national TB program and a lag in TB control compared to the African average and the rest of the world. Here too further investigation is necessary.

1.5 Study rationale

The rationale of the *Panepi* study (section 2.1) and this thesis is to prospectively investigate local TB epidemiology, quantify TB treatment outcome in a semi-rural area in Gabon and to identify determinants of successful and unsuccessful TB treatment.

Special emphasis is set on the extent and outcome of drug resistant TB, the outcome of TB/HIV co-infection and the investigation of high defaulting rates in Gabon. Furthermore, the correlation of potential other risk factors and treatment outcome in Gabon is investigated.

2 Methods

2.1 Study design and objectives

The *Panacea Epidemiology* (*Panepi*) study was a prospective observational epidemiologic cohort study assessing patients treated for TB disease. The full title was “Epidemiology of Tuberculosis in Lambaréné”.

The overall study objective was to assess the local TB burden, including demographic, clinical, microbiological, and treatment outcome aspects of TB. The study was conducted in close collaboration with Gabon’s national TB program *“Programme national de lutte contre la tuberculose” (PNLTB)*.

Specific objectives of the study were the evaluation of the:

- Rate of TB/HIV co-infection
- Rate and patterns of DR-TB, especially MDR-TB
- Treatment outcomes
- Risk factors for unfavorable treatment outcome and reasons for non-adherence to treatment regimens

2.2 Study setting

2.2.1 Study period

The study was conducted between June 2012 and July 2015. Recruitment of study participants took place between June 2012 and October 2013. The last follow up visit was done in June 2014. Microbiological analysis were completed in September 2014. Data analysis was completed in July 2015.

2.2.2 Study sites

All involved Gabonese study sites are located in Lambaréné, a 30,000 inhabitant town in a semi-rural area in the Moyen-Ogooué province of Gabon.

2.2.2.1 Centre de Recherches Médicales de Lambaréné (CERMEL) :

The *CERMEL* is situated next to the Albert Schweitzer Hospital. It consists of several laboratories, such as the TB laboratory, a clinical laboratory, a parasitology laboratory, a microbiology laboratory as well as an immunology

laboratory. At the *CERMEL* numerous epidemiological studies and clinical trials, have been conducted; a main focus has been on malaria. For some years *CERMEL* is expanding its research activities to other important diseases endemic in sub-Saharan Africa such as TB and nosocomial infections, as well as to immunological studies.

At the time of study the TB laboratory provided the infrastructure for study planning, coordination and handling of samples for studies on TB and HIV. Besides, parts of screening, recruitment and follow up procedures were done here as well. Data storage, data entry and first data analyzes are also performed at the TB laboratory of *CERMEL*. Laboratory procedures performed at the TB laboratory of *CERMEL* comprised sputum microscopy (*ZN* and FM), sputum conservation and preparation of samples for shipment to Germany. At the time of study, no validated mycobacterial culture was available on site. Furthermore, HIV testing as well as hematology and biochemistry analyzes were done at the clinical laboratory of *CERMEL*.

2.2.2.2 Hôpital Albert Schweitzer de Lambaréné (HAS):

HAS is the biggest hospital in Lambaréné and has a total capacity of 150 beds. It has an internal medicine ward, a pediatric ward, a maternity ward and a surgical ward as well as an emergency room. The laboratory belonging to the hospital performs sputum microscopy (*ZN* stain), HIV testing, hematology and biochemistry analyzes. The radiology department of the hospital is able to provide x-ray films in digital or printed form but no radiologist is on site. Routinely, TB patients cared for at *HAS* are admitted to the internal medicine or pediatric ward and followed up at the outpatient department. In the context of this study, patients with suggestive TB were referred to the *CERMEL*, after having been consulted in an outpatient setting.

2.2.2.3 Centre Hospitalier Regional "Georges Rawiri" de Lambaréné (CHRGR)

CHRGR is the second biggest hospital in Lambaréné next to *HAS*. The hospital laboratory performs HIV testing, hematology and biochemistry analysis, but no sputum examination. The radiology department of the hospital is able to provide

x-ray films in printed form. Routinely, TB patients cared for at *CHRGR* are admitted at the internal medicine ward and followed up at the outpatient department. In the context of this study, sputum and other samples eligible for mycobacterial search were sent to the TB laboratory of *CERMEL* for analysis.

2.2.2.4 Centre de Traitement Ambulatoire (CTA)

CTA is the HIV clinic in Lambaréné. It provides outpatient HIV testing, counseling and treatment. Patients are not admitted, but referred to *HAS* or *CHRGR* if in-hospital treatment is indicated. On the other hand, HIV patients from other health care centers, such as *CHRGR* are referred to *CTA* for HIV counseling and treatment. The laboratory performs HIV testing as well as hematology and biochemistry, but no sputum examination. A radiology department is able to provide x-ray films in printed form. TB patients co-infected with HIV receive ambulatory antituberculous treatment at *CTA*. In the context of this study, sputum and other samples eligible for mycobacterial search were sent to the TB laboratory of the *CERMEL* for analysis.

2.2.2.5 Base d'épidémiologie (BELE)

BELE is the TB clinic in Lambaréné and in charge of implementation of the national TB program *PNLTB*. It provides counseling and treatment for TB, but not sputum examination, blood analysis or x-ray. Routinely, TB patients receive ambulatory treatment at *BELE*. In the context of this study, sputum and other samples eligible for mycobacterial search were sent to the TB laboratory of the *CERMEL* for analysis.

2.2.2.6 National Reference Center (NRC) for mycobacteria in Borstel/Germany

The NRC for mycobacteria in Germany participates in the coordination of measures in the fight against and the surveillance of TB and acts as a supranational reference laboratory of the WHO.

At the time of study, the NRC in Borstel performed culture and identification of mycobacteria, as well as DST and quality control of sputum microscopy

performed in Lambaréné. All sputa examined at *CERMEL* were conserved and shipped to Borstel.

2.2.3 Study population

The study population consisted of patients being treated for active TB in the various health care institutions caring for TB patients in Lambaréné. Adults as well as children with TB were enrolled in the study. Participants lived in urban as well as semi-urban and rural areas.

2.2.4 Inclusion and exclusion Criteria

Inclusion criteria were:

- Initiation of curative TB treatment
- Provision of informed consent for participation in the TB epidemiology study; in case of minority the participant's legal representative had to give informed consent.

There were no specific exclusion criteria.

2.3 Study procedures

An overview of the study flow regarding clinical and microbiologic study procedures is presented in *Figure 1.*

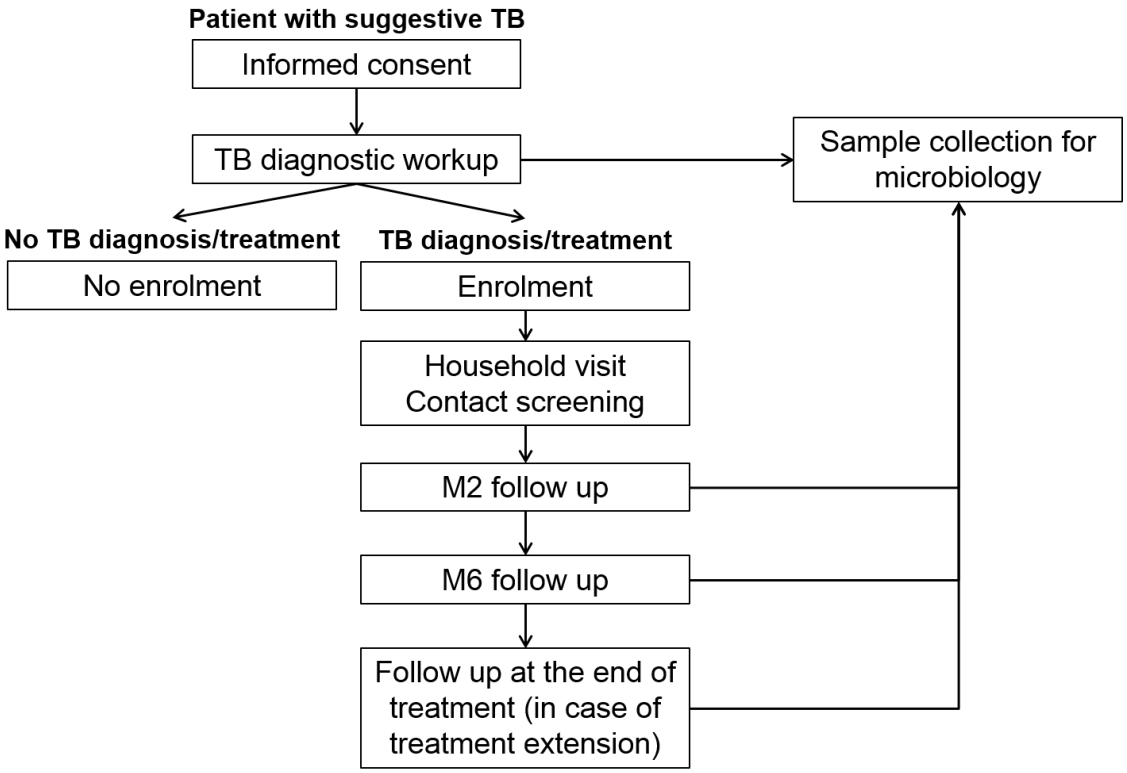

Figure 1: Study flow

Time period: June 2012 – June 2014, TB: tuberculosis, M2: month two, M6: month six

2.3.1 Clinical procedures

2.3.1.1 Consenting procedure

Patients with symptoms suggestive of TB and initiating TB workup were approached and invited for participation in the TB epidemiology study. The study was explained in detail (risks, requirements, samples to be taken and analyzed, etc.). A copy of the informed consent form in French was given to the patients to read. For illiterate patients, the investigator read the informed consent form to them. Any questions and/or concerns voiced by the patients were answered by designated study staff. If the patients were willing to participate, they were asked to sign the informed consent. For illiterate patients a thumb fingerprint was asked for instead of a signature and this was witnessed by an independent literate observer. For inclusion of minors the legal representative needed to consent the participation.

2.3.1.2 Screening procedure

Patients admitted to *HAS* or *CHRGR* with symptoms suggestive of TB were identified during ward rounds. For these patients, screening for TB was done in collaboration with the *CERMEL* (i.e. sputum microscopy and culture was done at or via *CERMEL*, while chest x-rays were taken onsite). Study relevant information was copied from the patient file after obtaining the patient's informed consent.

Outpatients presenting to *CTA* with suggestive TB were identified during consultations. Consecutively, screening was done in collaboration between *CTA* and the *CERMEL* (i.e. sputum microscopy and culture was done at or via *CERMEL*, while chest x-rays were performed onsite).

Outpatients at *HAS* and *BELE*, as well as patients seen directly at the *CERMEL*, who presented with suggestive TB were identified during consultations. Screening was done at the *CERMEL* (i.e. sputum microscopy and culture was done at or via *CERMEL*, chest x-rays were performed at *HAS*).

After ascertaining study eligibility and obtaining informed consent, a questionnaire was used to assess essential demographics and the current and past medical history. A protocol-based detailed clinical examination was performed and the findings were recorded in a standardized way on a case record form (CRF).

Diagnostic workup comprised the collection of sputum and blood samples for microbiological and laboratory analyzes, TST and chest x-ray examination. Further diagnostic investigations were performed if clinically indicated and initiated by the attending health care staff. If diagnostic measures had already been undertaken by the treating health facility before recruitment, the relevant information was copied from the patient file. All results of laboratory analyzes performed in the context of the study were reported back to the treating health facility.

2.3.1.3 Sample collection

Sputum samples were obtained during three consecutive days, ideally early in the morning. Samples from participants attended at *HAS* and *CERMEL* were

collected directly by a study clinician or a field worker and brought to the TB laboratory for further processing. Samples were collected in sterile screw topped containers without additives.

Samples from patients attended at *CHRGR*, *CTA* and *BELE* were examined at the TB laboratory outside the study setting in cooperation between the TB laboratory and the health institutions. After recruitment of study participants and obtaining informed consent, their samples were later on assigned to them.

Other samples eligible for mycobacteriology were pleural aspirates, lymph node aspirates, cerebrospinal fluid (from patients with suggestive EPTB) as well as gastric aspirates in children. Sample collection was done in the same way as the collection of sputum samples.

Blood samples were taken from the patient by the study clinician and brought to the clinical laboratory of the *CERMEL* for serology, hematology and biochemistry analysis.

2.3.1.4 Enrolment procedure

Diagnosis of active TB and decision to initiate TB treatment was taken by the attending clinician of the respective health facility and based on the national TB guidelines [65]. All patients who were diagnosed with active TB and had given informed consent, were consecutively enrolled into the study and assigned a study identification (ID) number.

2.3.1.5 Management of study participants

Patients diagnosed with active TB were initiated treatment by the treating physician/nurse according to guidelines of the national TB program [65]. Study participants who underwent diagnostic TB workup at *CERMEL* only were referred to *HAS* for TB treatment. The treating health facility coordinated treatment and treatment supervision, while the study investigators recorded duration and form of treatment.

2.3.1.6 Follow up

Follow up (FU) information was obtained two and six months (M2, M6) after initiation of TB treatment. In case of treatment extension decided upon by the attending physician, the FU period was extended until the end of the TB treatment.

FU visit dates at the *CERMEL* were assigned to all patients, where a clinical and a mycobacteriological FU was scheduled. Outside of the study, patients had additional FUs at their respective health care institutions. Patients that missed a study FU visit were called on the phone by study staff. At least three attempts were made on different days to reach the patients. In case patients could not be reached on the phone FU information was looked up in the patients' hospital file. In case no FU information could be retrieved from patient files at M6 FU, patients' treatment outcome was classified as not evaluated.

Collected FU information comprised data on medical history and current TB treatment. Treatment adherence was assessed by interviewing the participant on regular drug intake and treatment interruptions or reviewing the patient's hospital file. In case of non-adherence, reasons for non-adherence and extent of non-adherence were verbally investigated. Sputum smear and culture investigations were attempted for all patients at M2 and M6. Further diagnostic evaluations (e.g. laboratory, x-ray, etc.) during FU were done if adjudged to be clinically indicated by the attending health care staff. In case of a patient's death, FU information was sought from patient files or by a verbal autopsy with the patient's household members.

The FU information was used to

- Assess TB treatment outcome according to national and international definitions
- Asses patients' adherence to treatment and reasons for non-adherence
- Detect acquired resistance of mycobacteria

2.3.1.7 Household visits and geo-referencing

In the first weeks after study enrolment and treatment initiation, patients were visited at their homes by a field worker and/or a study clinician. The location of their residence was geo-referenced using the *Garmin Dakota ™ 20 GPS-device*. A field worker explained the risk of transmission and the importance of contact screening to the patient and his household contacts. Household contacts were invited to come to the *CERMEL* for a TB screening examination and further diagnostics, if necessary.

2.3.1.8 Acquisition on travel determinants

In order to evaluate the impact of travelling from home to the TB treatment facility on TB treatment outcome, information about travel distance, duration and costs was assessed for each study participant.

Travel distance was defined as the distance between the patient's residence and the site of recruitment. If available, distance was calculated using the GPS coordinates taken during household visits. If no GPS coordinates could be obtained, distance was estimated using the patient's reported residence.

Travel duration was defined as the time patients needed to come to the hospital using public transport, such as taxi, boat or ferry. Information on travel duration was gained in an interview with local taxi drivers, using the patient's description of his residence.

Travel costs were defined as the fees for public transport, including taxi and boat. Information on costs was taken from the list used for compensation of travel expenses at the *CERMEL*.

2.3.1.9 Interviews about TB treatment supervision and patient support

To obtain a picture of TB treatment supervision and patient support, two questionnaires were done with a subset of study participants between September 2013 and January 2014 .

One questionnaire on patient support aimed at assessing current practice of counseling, management of TB/HIV co-infection and duration of hospital admission in the view of patients.

A questionnaire on treatment supervision aimed at assessing the setting and organization of control visits and measures to ensure treatment adherence.

2.3.2 Laboratory procedures

An overview of mycobacteriological study procedures is presented in *Figure 2.*

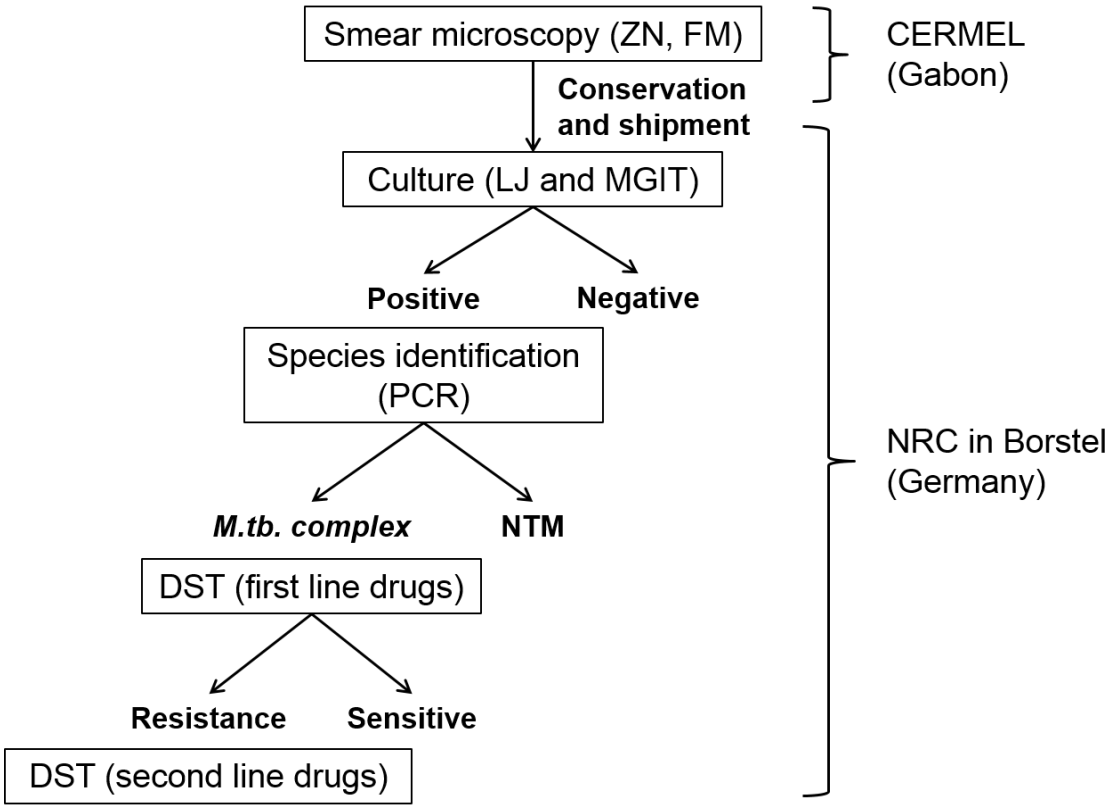

Figure 2: Overview of mycobacteriological study procedures

Time period: June 2012 – September 2014. ZN: Ziehl – Neelsen stain, FM: fluorescence microscopy, LJ: Löwenstein-Jensen agar, MGIT: Mycobacterium Growth Indicator Tube, PCR: polymerase chain reaction, *M.tb.*: *mycobacterium tuberculosis*, NTM: nontuberculous mycobacteria, DST: drug susceptibility testing, CERMEL: Centre de Recherches Médicales de Lambaréné, NRC: national reference laboratory

2.3.2.1 Sputum smear examination by microscopy

Sputum samples were analyzed by FM and by standard *ZN* staining. Precise procedures for preparation and staining of slides as well as for reading and reporting of results have been described by Siddiqi et al. [30].

2.3.2.2 Microscopy of samples other than sputum (extrapulmonary)

The procedures for microscopy of other samples were the same as for sputum smear microscopy. If the volume was over 10ml, concentration was done by centrifugation at 3000x g for 15-20 minutes before decontamination.

2.3.2.3 Sample conservation, storage and shipment

All samples for mycobacterial investigation were conserved and stored for shipment. During the first months of the study, specimens were conserved in 50% cetylpyridinium chloride; afterwards specimens were conserved without additive. Batched shipments to the NRC in Borstel, Germany, were done in intervals of four weeks. Shipment was organized through the international delivery services *DHL* and *TNT*, following packaging requirements for category B Biological and Infectious Substances (UN3373), it took around one week from sending to delivery. Samples were shipped at room temperature.

2.3.2.4 Mycobacterial culture

Sputum cultures were attempted for both smear positive and smear negative samples. Culturing of mycobacteria was done at the NRC in Borstel/Germany. Two parallel techniques, solid agar (*Lowenstein-Jensen* agar) and liquid culture tubes (*MGIT*) were used.

A small volume of the decontaminated sample was inoculated onto two Lowenstein-Jensen medium slants and grown at 37°C. Growth was monitored visually twice per week for up to eight weeks, after which absence of growth was deemed as agar culture negative.

In addition to the agar-based culture method, 500 uL of resuspended decontaminated sediment was used to inoculate a 4ml MGIT tube, and incubated in an automated BACTEC™ MGIT™ 960 Mycobacterial Detection System (BD Diagnostics, Belgium). Growth was recorded daily for five weeks [30].

2.3.2.5 Identification of M. tuberculosis complex

The differentiation of mycobacteria and identification of the species was done at the NRC in Borstel/Germany. For differentiation between *M.tb. complex* and NTM

a *Genotype MTBC assay* (*Hain Life Sciences, GmbH Germany*) was performed on mycobacteria grown by culture. [66]

2.3.2.6 Drug susceptibility testing

Drug susceptibility testing (DST) was done at the NRC in Borstel/Germany. For all positive cultures identified as *M.tb. complex*, DST for the first-line antituberculous drugs (RIF, INH, EMB, PZA, STR) was performed. In case of any resistance to the first-line drugs except for STR, DST for the following second-line drugs was performed: ETO, OFX, PAS, CS, AM and CM.

Liquid culture DST was performed using the *MGIT* culture tube manual system according to the manufacturer's instructions (*BBLTM MGIT™ SIRE* and *PZA test kits, Becton Dickinson*) [30]. Testing of mycobacteria for susceptibility to INH, STR, RIF, EMB, PZA and to the above mentioned second-line drugs was based on the detection of growth in antibiotic-containing media compared to antibiotic-free control tubes.

The NRC reported results of culture, species identification and DST back to the study investigators at *CERMEL* via email on a weekly basis; the study investigators forwarded the results to the respective physicians on designated paper forms.

2.3.2.7 HIV testing

All patients enrolled in the study were strongly recommended to undergo HIV testing. If the testing had not yet been done by the treating health institution, patients were asked for their consent to assess their HIV status. For HIV testing patient sera were isolated from whole blood by centrifugation at 3000x g at 4°C. 50µL of the patient's sera were used to perform both the *Determine HIV 1/2* (*Inverness Medical Innovations*) and the *Vikia HIV-1/2* (*Standard Diagnostics, Kyonggi-do*, South Korea) rapid diagnostic HIV tests. If both tests were in agreement with each other (both positive or both negative) the result was used as defining the patients HIV status. In case of a positive result, the patient was referred (back) to a local health institution for counseling and treatment. If the two

rapid diagnostic tests contradicted one another, a deciding third test was performed. This was done in the laboratory of *HAS* using a p24 Antigen test.

2.3.2.8 Hematology and biochemistry

Routine hematology and biochemistry analyzes were done either in the clinical laboratory of the *CERMEL* or at the laboratories of the recruitment sites. Since hematology and biochemistry results are not relevant for this thesis, they will not be further described in the following.

2.4 Data management

2.4.1 Data collection and storage

All information about the study participants was collected on paper CRFs and kept as dedicated individual case files in the TB laboratory during the duration of the study. At the end of the study all study files were stored in the archive of the *CERMEL*. Only the study coordinator and designated study physicians and field workers had access to patient-identifiable information.

2.4.2 Data entry

Data collected on paper CRFs was entered into *OpenClinica* (*OpenClinica*® version 3.0.4, Boston, USA) and stored on the server of *CERMEL*. Data entry was done by the study field worker as well as designated data entry clerks and later verified by a study physician. Data of microbiology results such as culture, species identification and DST were entered in a *Microsoft Excel spreadheet* (*Microsoft Corporation*).

2.4.3 Data cleansing

After extracting data from *OpenClinica* in a *Microsoft Excel* worksheet, data cleansing was done as follows. Completeness of participants and variables was checked. Data of individual participants was checked and if possible, gaps were filled by querying the source data. Missing data was marked as such (not available (NA)). Consistency of available data was checked and microbiology data was added to the database. Free text entries were harmonized and standardized into categories.

2.4.4 Case definitions and definition of TB treatment outcomes

Case definitions and definition of TB treatment outcomes were done according to the WHO [67] and Graham et al. [68] for adults and children younger than 18 years, respectively. They are presented in *Table 1*.

Table 1: Case definitions and definition of TB treatment outcomes

* age below 18 years, + also if no information could be obtained directly from the patient himself and the patient was documented as LTFU at the treating health institution, TB: tuberculosis, WHO: World Health Organization, INH: isoniazid, RIF: rifampicin

	Summary of Definition
Adults (WHO [67])	
Bacteriologically confirmed TB	Positive microscopy or culture
Clinically diagnosed TB	Diagnosis by clinician or other medical practitioner
Children* (Graham et al. [68])	
Confirmed TB	Microbiological confirmation
Probable TB	Chest x-ray consistent with TB and one of the following: Exposure to TB or positive TB treatment response or immunological evidence of TB
Possible TB	Chest x-ray consistent with TB or exposure to TB or positive TB treatment response or immunological evidence of TB
TB unlikely	Not fitting the above definitions
Anatomical site (WHO [67])	
Pulmonary TB	Involving lung parenchyma or tracheobronchial tree
Extra-pulmonary TB	Exclusively involving organs other than the lungs
History of previous tuberculosis treatment (WHO [67])	
New TB	No previous TB treatment
Previous TB treatment	Previous TB treatment for one month or more
Relapse	Previously declared cured or TB treatment completed
Treatment after failure	Most recent TB treatment failed
Treatment after loss to follow up	Lost to follow up at the end of most recent TB treatment
Other previously treated patients	Most recent TB treatment outcome unknown
Patients with unknown previous TB history	do not fit into any of the categories listed above

Table 1 (continued)

Drug resistance (WHO [67])	
Monoresistance	Resistance to one first-line anti-TB drug only
Polydrug resistance	Resistance to more than one first-line anti-TB drug other than both INH and RIF
Multidrug resistance	Resistance to at least both INH and RIF
TB Treatment outcome (WHO [67])	
Treatment success	Total of “cured” and “treatment completed”
Cured	Smear or culture negative in the last month of treatment and on one previous occasion
Treatment completed	No evidence of failure but not meeting the criteria for “cured”
Treatment failed	Smear or culture positive at month 5 or later
Died	Death for any reason
Lost to follow up	Treatment interruption for two consecutive months or more[+]
Not evaluated	Treatment outcome unknown

Survival was documented if any information on the patient being alive at M6 +/- one month or later was available. Unfavorable treatment outcome was defined as outcome classification other than treatment success.

2.5 Statistical analysis

For statistical analysis data were extracted to a *Microsoft Excel* worksheet and then analyzed using *R Statistical software* version 3.1.3. (R Foundation for Statistical Computing, Vienna, Austria). The likelihood ratio test was used to test the linear trend for the continuous variables. Univariate logistic regression was used to calculate crude odds ratios with 95% confidence intervals. Stepwise selection with the *Akaike Information Criterion* was used to select the factors for the multivariate logistic regression model to calculate the adjusted odds ratios with 95% confidence intervals. Using the *Mantel-Haenszel method*, confounding was investigated for risk factors with at least a 20% significant modification between crude and adjusted odds ratios. Missing data analysis was performed and the database for variables with more than 15% missing data was completed

using multiple imputations by chained equations. Survival analysis was performed and *Kaplan-Meir plots* used for visualization.

To assess the possible influences of travelling on treatment outcome the travel factor was calculated, which is a measure originally designed in the context of retention to HIV care (Janssen S, personal communication). Z scores (the number of standard deviations from the mean) were calculated from the three variables travel distance, duration and costs and later averaged to a single factor. Multivariate logistic regression was used to calculate the respective impact in treatment outcome.

2.6 Ethical aspects

The study was approved by the scientific review committee as well as the institutional ethics committee of *CERMEL* in June 2012. A notification about the study was given to the national ethics committee of Gabon. Participation in the TB epidemiology study was voluntary and written informed consent was obtained from all participant (section 2.3.1). All data collected was saved anonymously and patient files were kept in a secure room located at *CERMEL*. Samples were labeled anonymously (patient ID, date, type of specimen, study ID) preventing direct identification of the patient.

Direct benefits for the patients participating in this study were comprehensive diagnostic procedures related to TB, which are only partly available on site in the routine setting. Active FU efforts by calling and inviting patients for FU supported patients in adhering to and completing their treatment. Another benefit was the free contact screening of study participants' contacts.

Indirect benefits were the capacity for TB diagnostics set up through the study, including FM, culture and DST in Lambaréné; these diagnostic tests were not previously available in the country. By systematically studying the local TB epidemiology this study supported the understanding of the most urgent needs to improve local TB care and control. Besides, this cohort study contributed to establishing a platform and network for future TB drug, TB vaccine, and new TB diagnostics trials all urgently needed to find quicker, safer and more effective

methods for detecting, treating and curing TB in Gabon, Central Africa and globally.

2.7 Funding

The study was supported by the European and Developing Countries Clinical Trials Partnership (EDCTP) through a grant to the Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) Consortium.

3 Results

During the study recruitment period (from June 2012 to October 2013), 723 patients submitted specimens to the TB laboratory for TB microscopy and culture. Additionally, 55 patients were clinically investigated for active TB without microbiological analyzes (patients unable to produce sputum). A total of 201 patients with microbiologically or clinically diagnosed active TB and starting antituberculous treatment were enrolled; 103 (51.3%) were enrolled at *HAS*, 50 (24.9%) at *CHRGR*, 28 (13.9%) at *CERMEL*, 15 (7.5%) at *CTA*, and 5 (2.5%) at *BELE*.

3.1 Demographic, clinical and diagnostic findings

3.1.1 Baseline demographics of the study population

One hundred seventy (84.6%) patients were adults and 31 (15.4%) were children younger than 18 years. Twenty-five (12.4%) patients sought care for TB in Lambaréné despite other TB treatment centers being closer to their residency. Baseline demographic data including sex, age and HIV status are presented in *Table 2*. Travel determinants are presented in *Table 3*.

Table 2: Baseline demographics of the study population

Data collection period: June 2012 – October 2013, IQR: interquartile range, HIV: Human immunodeficiency virus, CD4: Cluster of differentiation 4, NA: not available, * Available for 200 patients, + Available for 51 (73% of HIV infected) patients, # living in villages or towns smaller than Lambaréné (around 30 000 inhabitants)

	Total cohort	**Adults**	**Children**
Total cohort (%)	201 (100)	170 (84.6)	31 (15.4)
Female sex, n (%)	92/201 (45.8)	80/170 (47.1)	12/31 (38.7)
Median Age, years (IQR)*	32.0 (22.6;42.1)	36.3 (26.9;43.3)	6.1 (2.0;15.4)
HIV infected, n (%)	70/183 (38.3)	66/158 (41.8)	4/25 (16.0)
New HIV diagnosis, n (%)	22/183 (12.0)	20/158 (12.7)	2/25 (8.0)
Median CD4 counts, cells/µl (IQR)+	130 (56;227)	130 (56;227)	NA
Rural residence, n (%)#	69/201 (34.3)	61/170 (35.9)	8/30 (25.8)

Table 3: Travel determinants

Data collection period: June 2012 – October 2013, TB: tuberculosis, IQR: interquartile range, FCFA: Franc de la Communauté Financière d'Afrique, * distance <5km represents living in Lambaréné, + available for 148 adults and 30 children, # Available for 148 adults and 29 children,

	Total cohort	**Adults**	**Children**
Median distance to TB treatment center, km (IQR)	3.6 (2.9;67.0)	3.7 (2.8;67.0)	3.6 (3.4;31.5)
Distance < 5km, n (%)*	110/184 (59.8)	90/154 (58.4)	20/30 (66.7)
Median travel costs, FCFA (IQR)+	500 (400;3200)	500 (400;3450)	500 (475;2313)
Median travel duration, minutes (IQR)#	20 (15;60)	20 (15;60)	25 (20;30)

3.1.2 Baseline characteristics related to TB

Baseline characteristics related to TB (TB contact, symptoms, clinical findings, prior non-antituberculous antibiotics, chest x-ray findings, and TST) are presented in *Table 4*.

Table 4: Baseline characteristics related to TB

Data collection period: June 2012 – October 2013, TB: tuberculosis, NA: not available, SD: standard deviation, IQR: interquartile range, BMI: body mass index, TST: Tuberculin Skin Test, * Available for 58 adults and 11 children, + Available for 143 adults and 21 children, # Available for 109 patients, ~ Percentiles provided by WHO: 3rd percentile for children <2 and 5th percentile for children ≥2 years

	Total cohort	**Adults**	**Children**
Total cohort, n (%)	201 (100)	170 (84.6)	31 (15.4)
TB contact known, n (%)	72/189 (38.1)	48/160 (30.0)	24/29 (82.8)
Main TB contact	family member	sibling	parent
Prior non-antitubercular antibiotics, n (%)	74/113 (65.5)	65/98 (66.3)	8/14 (57.1)
Most frequent antibiotics	NA	amoxicillin/ clavulanate ciprofloxacin cotrimoxazol	gentamycin

Table 4 (continued)

Reported TB symptoms			
Weight loss, n (%)	173/199 (86.9)	151/169 (89.3)	22/30 (73.3)
Mean weight loss, kg (SD)[*]	NA	8.0 (6.5)	3.7 (2.5)
Cough, n (%)	167/200 (83.5)	143/170 (84.1)	24/30 (80.0)
Fever, n (%)	162/198 (81.8)	138/169 (81.7)	24/29 (82.8)
Night sweat, n (%)	100/194 (51.5)	88/168 (52.4)	12/26 (46.2)
Chest pain, n (%)	76/200 (38.0)	69/170 (40.6)	7/30 (23.3)
Hemoptysis, n (%)	44/197 (22.3)	43/168 (25.6)	1/29 (3.4)
Dyspnea, n (%)	43/200 (21.5)	40/170 (23.5)	3/30 (10.0)
Median duration of cough**,** days (IQR)[+]	30 (21;90)	30 (21;90)	26 (14;128)
Clinical examination			
Pathologic lung auscultation, n (%)	136/188 (72.3)	120/162 (74.1)	16/26 (61.5)
Axillary temperature > 37.5°C, n (%)	77/162 (47.5)	71/142 (50.0)	6/20 (30.0)
Lymphadenopathy, n (%)	36/188 (19.1)	28/160 (17.5)	8/28 (28.6)
BMI, mean (SD)[#]	NA	18.9 (2.8)	NA
Children < 3rd/5th percentile, n (%)[~]	NA	NA	5/20 (25.0)
Diagnostic findings			
Pathologic chest x ray, n (%)	171/177 (96.6)	144/148 (97.3)	27/29 (93.1)
Infiltrates, n (%)	144/177 (81.4)	122/148 (82.4)	22/29 (75.9)
Cavitations, n (%)	56/177 (31.6)	51/148 (34.5)	5/29 (17.2)
Hilar lymphadenopathy, n (%)	55/177 (31.1)	43/148 (29.1)	12/29 (41.4)
Pleural effusion, n (%)	21/177 (11.9)	19/148 (12.8)	2/29 (6.9)
Positive TST, n (%)	36/58 (62.1)	26/41 (63.4)	10/17 (58.8)

3.1.3 Baseline mycobacteriology

Baseline mycobacteriology (smear microscopy, culture, species identification and DST) is presented in *Table 5.*

Table 5: Baseline mycobacteriology

Sample collection period: June 2012 – October 2013, PCR: Polymerase chain reaction, TB: tuberculosis, MDR: multi-drug resistance, * Ziehl-Neelsen stain or fluorescence microscopy

	Total cohort	**Adults**	**Children**
Mycobacteriology performed, n (%)	179/201 (89.1)	164/170 (96.5)	15/31 (48.4)
Microscopy* and culture			
Sputum microscopy positive	125/173 (72.3)	119/160 (74.4)	6/13 (46.2)
Sputum culture positive	105/150 (70.0)	98/138 (71.0)	7/12 (58.3)
Extrapulmonary microscopy positive	1/10 (10.0)	1/8 (12.5)	0/2
Extrapulmonary culture positive	4/8 (50.0)	4/7 (57.1)	0/1
Total positive mycobacteriology	134/179 (74.9)	126/164 (76.8)	8/15 (53.3)
Drug susceptibility testing and PCR			
M. tuberculosis, n (%)	89/108 (82.4)	83/101 (82.2)	6/7 (85.7)
M. africanum, n (%)	17/108 (15.7)	16/101 (15.8)	1/7 (14.3)
M. intracellulare, n (%)	2/108 (1.9)	2/101 (2.0)	0/7
Drug sensitive TB, n (%)	88/105 (83.8)	82/98 (83.7)	6/7 (85.7)
Mono-resistance, n (%)	7/105 (6.7)	6/98 (6.1)	1/7 (14.3)
Poly-resistance, n (%)	2/105 (1.9)	2/98 (2.0)	0/7
MDR, n (%)	8/105 (7.6)	8/98 (8.2)	0/7

Sputa were available for 160 (94.1%) adults and 13 (41.9%) children. Sputum smear microscopy and culture was done from three, two and one sputa in 67.6%, 23.1% and 9.2% patients, respectively. The positivity rate of sputum samples investigated by *ZN* microscopy was 63.2% and 36.4% for adults and children, respectively. The additional use of FM increased the positivity rate to 74.4% and 46.2%, respectively. Due to temporary lack of reagents, some samples were

stained only either with *ZN* or for FM. Of patients who submitted one sputum only, 14 out of 16 were positive. Cultures were performed for 166 (82.6%) patients, of which eight (4.8%) were contaminated. Of 125 microscopy positive patients, 17 (13.6%) cultures were negative; of 48 microscopy negative patients, nine (18.8%) cultures were positive. Mean time span between sample collection and available culture results was 2.9 (SD 0.8) months.

EPTB samples were investigated for eight adults and two children. Specimen from adults comprised four pleural fluids, two lymph node aspirates, one pericardial fluid, and one cerebro-spinal fluid. From children, one lymph node aspirate and one gastric aspirate were available. Of patients classified as EPTB, four (44.4%) were HIV-infected.

DST was performed for 105 cultures and revealed drug resistance in 17 (16.2%) cases. Resistance pattern are presented in *Table 6*. Among culture confirmed TB patients the MDR-TB rate was 4/91 (4.4%) and 4/13 (30.8%) in new and previously treated TB patients, respectively. No XDR-TB was observed.

Table 6: Drug resistance patterns of mono-, poly- and multidrug resistant TB

Sample collection period: June 2012 – October 2013, TB: tuberculosis, RIF: rifampicin, INH: isoniazid, PZA: pyrazinamide, EMB: ethambutol, STR: streptomycin, OFX: ofloxacin, ETO: ethionamide, CS: cycloserine, AM: amikacin, PAS: para-aminosalicylic acid, CM: capreomycin, R: resistant, S: sensitive, B: borderline, NA: Not available

	First-line TB drugs					**Second-line TB drugs**					
	RIF	**INH**	**PZA**	**EMB**	**STR**	**OFX**	**ETO**	**CS**	**AM**	**PAS**	**CM**
Mono-resistance to first-line drugs n = 7											
n = 2	S	R	S	S	S	S	S	S	S	S	S
n = 1	S	R	S	S	S	S	R	S	S	S	S
n = 1	S	R	S	S	S	R	S	S	S	S	S
n = 3	S	S	S	S	R	NA	NA	NA	NA	NA	NA
Poly-resistance to first-line drugs n = 2											
n = 1	S	R	S	S	R	S	S	S	S	S	S
n = 1	S	R	S	S	R	S	R	S	S	S	S
Multidrug-resistance n = 8											
n = 1	R	R	S	R	R	S	R	S	S	S	S
n = 1	R	R	S	R	R	R	S	S	S	R	S
n = 1	R	R	R	R	R	S	S	S	S	S	S
n = 1	R	R	R	R	R	S	S	S	S	S	S
n = 1	R	R	R	R	R	S	R	S	S	S	S
n = 1	R	R	S	R	R	S	B	S	S	S	S
n = 1	R	R	S	S	S	S	R	S	S	S	S
n = 1	R	R	R	R	R	S	R	S	S	S	S

3.1.4 Classification at treatment initiation

Classification of TB patients by case definitions, anatomical site and history of TB treatment is presented in *Table 7*.

Table 7: Classification of TB patients according to WHO case definitions, anatomical site, and previous TB treatment

Data/Sample collection period: June 2012 – October 2013, TB: tuberculosis, WHO: World Health Organization, NA: not available, LTFU: loss to follow up, * For two children data for classification based on anatomical site were not available

	Total n = 201	**Adults** n = 170	**Children** n = 31
WHO case definitions adults [67]			
Bacteriologically confirmed, n (%)	NA	126 (74.1)	NA
Clinically diagnosed, n (%)	NA	44 (25.9)	NA
Children according to Graham et al [68]			
Confirmed TB, n (%)	NA	NA	7 (22.6)
Probable TB, n (%)	NA	NA	17 (54.8)
Possible TB, n (%)	NA	NA	5 (16.1)
TB unlikely, n (%)	NA	NA	2 (6.5)
WHO classification based on anatomical site [67]			
Pulmonary, n (%)	190 (94.5)	161 (94.7)	29 (93.5)
Extra-pulmonary*, n (%)	9 (4.5)	9 (5.3)	0
WHO classification based on history of previous TB treatment [67]			
New TB, n (%)	165 (82.1)	140 (82.4)	25 (80.6)
Previous TB treatment, n (%)	30 (14.9)	24 (14.1)	6 (19.4)
Relapse, n (%)	13 (6.5)	11 (6.5)	2 (6.5)
Treatment after failure, n (%)	3 (1.5)	3 (1.8)	0
Treatment after LTFU, n (%)	11 (5.5)	8 (4.7)	3 (9.7)
Other previously treated patients, n (%)	3 (1.5)	2 (1.2)	1 (3.2)
Patients with unknown previous TB history, n (%)	6 (3.0)	6 (3.5)	0

3.2 Antituberculous treatment and treatment outcome

Most patients were treated by the health facility where they presented for diagnosis. Of those diagnosed at *CERMEL* most (22/28, 78.6%) were referred to *HAS* for treatment and five patients received treatment in prison coordinated by *BELE*. Follow-up data including clinical response to treatment and treatment adherence could be obtained for 120/201 (59.7%) and 80/183 (43.7%) patients at M2 and M6, respectively; follow-up continued beyond M6 for 48/181 (26.5%)

patients. Follow-up was done via phone call, face-to-face or by checking hospital files in 55.5%, 33.6%, and 11.5% of cases. Treatment outcomes by WHO definitions are presented in *Table 8*.

Table 8: TB Treatment outcome according to WHO definitions [67]

Data/Sample collection period: June 2012 – June 2014, TB: tuberculosis, WHO: World Health Organization, childr: children, HIV: human immunodeficiency virus, neg: negative, pos: positive, excl: excluding, MDR: multi-drug resistance

	Total n = 201	**Total adults** n = 170	**Total childr.** n = 31	**Total HIV neg.** n = 113	**Total HIV pos.** n = 70	**Total excl. MDR** n = 193
Treatment success, n (%)	107 (53.2)	91 (53.5)	16 (51.6)	65 (57.5)	30 (42.9)	106 (54.9)
Cured, n (%)	15 (7.5)	13 (7.6)	2 (6.5)	10 (8.8)	4 (5.7)	15 (7.8)
Treatment complete, n (%)	92 (45.8)	78 (45.9)	14 (45.2)	55 (48.7)	26 (37.1)	91 (47.2)
Treatment failed, n (%)	6 3.0)	5 (2.9)	1 (3.2)	4 (3.5)	2 (2.9)	5 (2.6)
Died, n (%)	21 (10.4)	20 (11.8)	1 (3.2)	1 (0.9)	20 (28.6)	17 (8.8)
Lost to follow up, n (%)	34 (16.9)	26 (15.3)	8 (25.8)	21 (18.6)	12 (17.1)	34 (17.6)
Not evaluated, n (%)	33 (16.4)	28 (16.5)	5 (16.1)	22 (19.5)	6 (8.6)	31 (16.1)

For 47/133 (35.3%) surviving patients for whom the precise TB drug regimen was documented, the treatment regimen deviated from the national TB guidelines in terms of treatment interruptions, inadequate drug combinations (following drug stock outs), imprecise timing of control visits, or incorrect drug dosage. At least seven patients stopped treatment directly after being discharged from the hospital. Of patients classified with treatment success 30/107 (28.0%) admitted any interruption of TB treatment with a median duration of 5 (IQR 2-14) days; treatment interruption or discontinuation occurred after a mean of 1.9 (SD 1.5) months. Most common reasons reported for treatment interruption or discontinuation were non-affordability of transportation costs (38.8%) and forgetting drug intake (28.6%); further reasons reported in decreasing frequency were subjective recovery, ignorance of the need to continue TB therapy, travel,

drug stock outs, family problems, side effects and immobility. Thirteen (9.4%) patients reported of consulting a traditional healer additionally to their standard TB treatment.

At M2 and M6, 52 (57.8%) and 11 (19.0%) adult patients and six (27.3%) and three (25.0%) children still had any TB symptom, respectively. In adults remaining symptoms were mostly cough and fatigue, while in children remaining symptoms were mostly cough and fever. At M2 and M6, 67 (78.8%) and 52 (88.1%) adult patients had gained weight, respectively, with mean of 3.2 (SD 3.8) and 6.1 (SD 6.0) kg. Among children, 18 (85.7%) and 10 (90.9%) had gained weight, respectively.

Sputum was obtained at M2 and M6 from 46 (27.1%) and 30 (17.6%) adult patients and six (19.4%) and three (9.7%) children, respectively. Follow up sputum smear microscopy was done from three sputa in 74.6% and from two and one sputa in 11.9% and 13.5%, respectively. Of initially smear or culture positive patients 47/134 (35.1%) and 26/134 (19.4%) patients provided sputum samples at M2 and M6, respectively, and nine (19.1%) and two (7.7%) were still smear or culture positive at M2 and M6, respectively. Of seven smear or culture positive patients at M6 or later, one patient had been diagnosed MDR-TB at treatment initiation; one patient had received an under-dosed TB regimen; two interrupted treatment for more than two months; and for the remainder no treatment default was reported but could not be excluded. No initially sputum or culture negative patient became sputum or culture positive later on. No acquisition of MDR-TB was detected; one patient who retrospectively was on functional mono-therapy with PZA due to MDR-TB developed additional resistance to PZA within the first month of treatment.

All patients whose cultures showed MDR-TB had been initiated on oral first–line TB drugs, three of them additionally had received STR for two months. M6 follow-up information could be obtained for six of the eight MDR-TB patients: four patients had died, one patient was still on first-line TB treatment and one patient was taken off first-line treatment by M6.

3.3 Risk factors for unfavorable treatment outcome

Risk factors for unfavorable treatment outcome are presented in *Table 9*.

Table 9: Risk factors for unfavorable treatment outcome (n=193)

Unfavorable treatment outcome defined as outcome classification other than treatment success and excluding patients with MDR-TB; linear trend for continuous variables tested by likelihood ratio test; crude odds ratios calculated by univariate logistic regression; factors for the multivariate logistic regression selected stepwise with Akaike Information Criterion; confounding investigated by Mantel-Haenszel method for risk factors with at least a 20% significant modification between crude and adjusted odds ratios; database completed using multiple imputations by chained equations for variables with more than 15% missing data. OR: odds ratio, CI: confidence interval, BMI: body mass index, TB: tuberculosis, * Very close < 3km; close 3-15km; far ≥ 15km, + Adjusted on age, distance (groups), cavitation, confirmed clinical and HIV

	Crude OR (95%CI)	**P-value**	**Adjusted OR^{+} (95%CI)**	**P-value**
Age	1 (0.98-1.02)	0.70	1 (0.96-1.05)	0.67
BMI (kg/m^2)	0.91 (0.78-1.05)	0.23	1.06 (0.85-1.31)	0.58
Cough duration	1 (0.99-1.01)	0.21	1 (0.99-1.01)	0.17
Gender				
Female	Reference	-	Reference	
Male	0.80 (0.42-1.48)	0.47	1.08 (0.41-2.92)	0.86
Distance*				
Very close	Reference	-	Reference	-
Close	1.26 (0.56-2.80)	0.58	1.22 (0.34-4.23)	0.75
Far	2.10 (1-4.44)	0.05	3.20 (1.10-9.90)	**0.04**
Anatomy				
Extrapulmonary	Reference	-	Reference	-
Pulmonary	2.45 (0.50-12.21)	0.27	5.48 (0.60-125.60)	0.18
HIV				
Not infected	Reference	-	Reference	
Infected	2.18 (1.10-4.36)	0.03	1.33 (0.47-3.67)	0.58
Cavitation				
No	Reference	-	Reference	
Yes	0.40 (0.20-0.80)	0.01	0.32 (0.10-0.98)	0.05
Confirmed /clinical TB				
Confirmed	Reference		Reference	
Clinical	2.47 (1.08-5.64)	0.04	2.81 (1.03-7.92)	**0.04**
New/retreatment TB				
Retreatment	Reference	-	Reference	
New	1.24 (0.47-3.26)	0.66	2.53 (0.50-15.30)	0.28

Risk factors for unfavorable treatment outcome were far distance to treatment center (≥15km) (aOR 3.20, 95%CI 1.10-9.90, p 0.04), and clinical (not microbiologically confirmed) diagnosis of TB (aOR 2.81, 95%CI 1.03-7.92, p 0.04). Risk factors significantly associated with death were HIV-infection (aOR 6.93, 95%CI 1.62-29.70, p 0.02) and clinical (not microbiologically confirmed) diagnosis of TB (aOR 3.26, 95%CI 1.13-9.37, p 0.03); mean survival time of deceased patients was 53 (SD 50) days after treatment initiation. The only risk factor significantly associated with treatment default was long duration of cough prior to diagnosis (aOR 3.55, 95%CI 1.13-11.12, p 0.04). Survival analysis is presented in *Figure 3*.

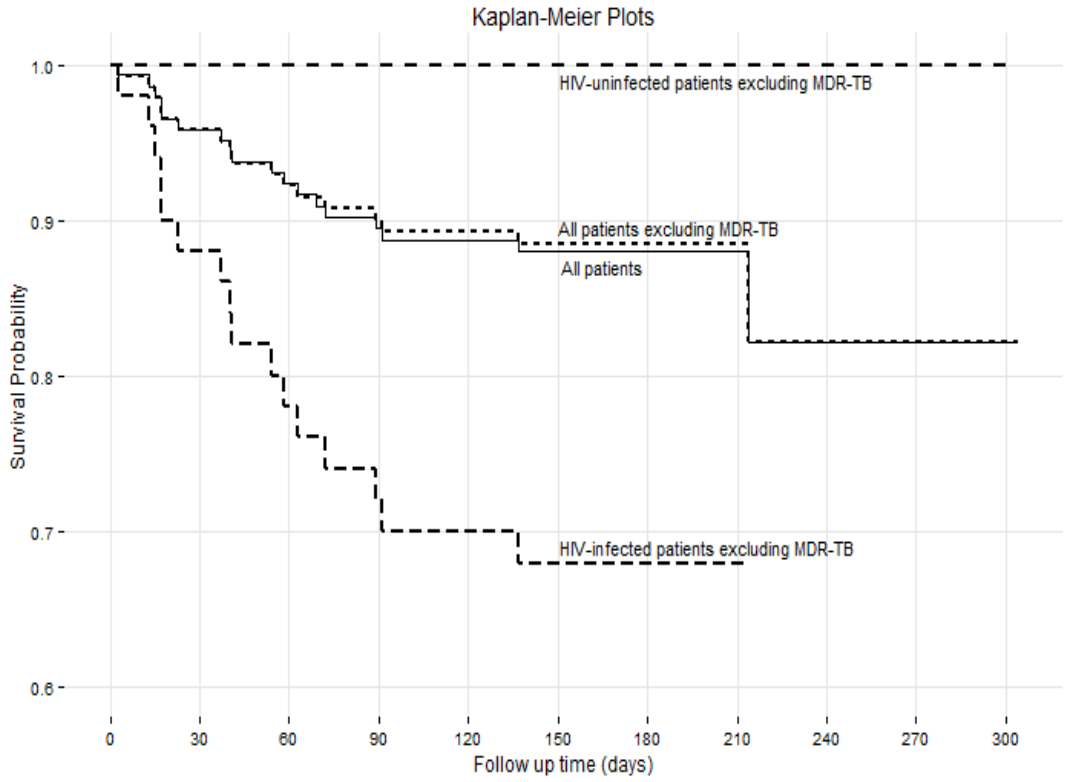

Figure 3: Survival analysis of TB patients (n=167)

Data collection period: June 2012 – June 2014, patients without the date of death, loss of follow up or study end were excluded, TB: tuberculosis, MDR-TB: multi-drug resistant TB

Risk factor analysis for unfavorable treatment outcome regarding the assessed travel determinants is presented in *Table 10*. The travel factor as proposed tool to investigate influences of travelling showed no significant correlation to treatment outcome.

Table 10: Influence of travel determinants on the risk of unfavorable TB treatment outcome (n=184)

Unfavorable treatment outcome defined as outcome classification other than treatment success and excluding patients with MDR-TB; linear trend for continuous variables tested by likelihood ratio test; crude odds ratios calculated by univariate logistic regression; factors for the multivariate logistic regression selected stepwise with Akaike Information Criterion; confounding investigated by Mantel-Haenszel method for risk factors with at least a 20% significant modification between crude and adjusted odds ratios.
TB: tuberculosis, OR: odds ratio, CI: confidence interval, * Adjusted on age, cavitation, confirmed clinical and HIV, + the number of standard deviations from the mean, # equates to the proposed travel factor

	Crude OR (95%CI)	P-value	Adjusted OR* (95%CI)	P-value
Z-score+ for distance	1.13 (0.82-1.57)	0.44	1.05 (0.71-1.56)	0.77
Z-score for time	1.01 (1-1.01)	0.17	1.16 (0.81-1.67)	0.41
Z-score for cost	1.28 (0.90-1.83)	0.17	1.47 (0.98-2.23)	0.07
Average of three Z-scores#	1.02 (0.99-1.04)	0.16	1.01 (0.98-1.05)	0.33

3.4 Counseling, patient support and treatment supervision

Twelve patients were interviewed concerning counseling and patient support. The results are presented in *Table 11*.

Nine patients were interviewed concerning treatment supervision. The results are presented in *Table 12*

.

Table 11: Interviews regarding counseling and patient support (n=12)

Data collection period: September 2013 – January 2014. Patients interviewed were treated at the internal medicine department of HAS (9 patients) and CHRGR (3 patients).
TB: tuberculosis, HIV: human immunodeficiency virus, NA: not available

Counseling		**HIV care**	
"Who told you that you suffer from TB?"		"Have you been tested for HIV at TB diagnosis?"	
a doctor	10	yes	10
a nurse	1	previously known HIV infection	2
diagnosis unknown	1		
"When have you been told to have TB?"		"Who informed you about your HIV status?"	
before TB treatment initiation	8	a doctor	8
after TB treatment initiation	2	a nurse	2
NA	2	NA	2
"Did you have the possibility to ask questions when you were told to have TB?"		"Is HIV care available at the same institution treating you for TB?"	
yes	8	yes	3
no	2	no	1
NA	2	NA	8
Hospitalization			
"Were you admitted to the hospital at the beginning of TB treatment?"		"How many days were you admitted?"	
yes	12	mean duration, days range (n)	13 10-15, 11

Table 12: Interviews regarding treatment supervision (n=9)

Data collection period: September 2013 – January 2014. Patients interviewed were treated at the internal medicine department of HAS (4 patients) and CHRGR (4 patients) and at the pediatric department of HAS (1 patient).
TB: tuberculosis, excl: excluding, NA: not available, FCFA: Franc de la Communauté Financière d'Afrique

Treatment supervision	
"How many control visits did you attend so far?"	"How many days did the control visits take?"
two 2	one 5
four 2	two 3
five 2	three 1
six 3	
"How long were the intervals between consecutive control visits?"	"Did you attend all control visits at the date scheduled?"
a) during the intensive phase:	yes 6
30 days 9	no 3
b) during the continuation phase:	"If no, why not?"
30 days 4	no transport to clinic available (1)
60 days 3	not been received in clinic (1)
NA 2	unaffordable transport costs (1)
"Have the control visits always been done by the same physician?"	"On average, how much did you pay for a control visit (excl. transport)?"
yes 4	mean costs, FCFA 9,222
no 5	range (n) 0 - 30,000 (9)
"Did you receive a log for the documentation of your TB treatment?"	"Did your treating physician call you on the phone to remind you of a control visit?"
yes 3	yes 2
no 6	no 7

4 Discussion

Panepi was the first systematic prospective cohort study on clinical and microbiological aspects of TB in Gabon, a middle-income country in Central Africa ranking among the top ten countries in terms of TB incidence per population in 2013.

4.1 Demographic, clinical and diagnostic findings

4.1.1 Age considerations

The median age of 36 years and a trend towards more male patients (53%) among adult TB patients was in line with previous reports from Gabon [5, 7, 69] and the WHO [3].

Commonly, adult and pediatric TB patients are not considered within the same cohort as many aspects around TB differ between adults and children. This study however chose a comprehensive approach to gain a cross-sectional insight into all services that care for TB patients in the region. Incidence rates of childhood TB have been reported to depend on the overall TB incidence in the population, and the proportion of TB cases among children may increase to 40% where incidence is 1,000 per 100,000 population [14]. The proportion of 15% of childhood TB in this cohort (especially given the cut-off of <18 years for childhood TB) may under-represent the true burden in the pediatric population; the uncommonly high positivity rate of sputum smear microscopy further underlines the assumption that microbiology negative pediatric TB patients are under-recognized and missed. The proportion of 4% of pediatric TB cases officially notified in Gabon [4] certainly reflects significant under-recognition and/or reporting of childhood TB.

4.1.2 High HIV co-infection rate

The HIV/TB co-infection rate of 38% in patients with known HIV status was 3.5-fold higher than reported by the WHO in the 2013 country profile (11%) [4], higher but closer in line with reports from retrospective studies evaluating TB patients hospitalized in the capital Libreville (26%-32% in 2001-2010) and Lambaréné

(34% in 2007-2012) [5-7, 69] and similar to a recent report from Cameroon [70]. The true HIV/TB co-infection rate may be even higher as for almost 10% of patients the HIV status could not be ascertained. Every tenth patient presenting with TB was newly diagnosed with HIV-infection underlining the importance of the current WHO guidelines that every TB patient should be tested for HIV. During the study period, all patients who were newly diagnosed TB at the internal medicine department of HAS and CHRGR received HIV testing at their respective health facility. Overall, CD4 cell counts of HIV-infected patients were low at presentation with median 130 (IQR 56; 227) cells/µl; 37/56 (72.5%) patients were in a state of severe immunosuppression [71] or CDC category 2 [72], making them prone to progression of TB disease.

4.1.3 Typical clinical presentation

Weight loss, cough and fever were the three most common TB symptoms reported; a quarter of patients reported hemoptysis, a rate generally in line with the varying rate ranges reported [73] but much higher than reported in a retrospective study from an urban cohort in Gabon [69]. In patients with hemoptysis but lack of microbiological confirmation of TB from sputum (5% of patients in this cohort) paragonimiasis, a parasitosis that shares similar clinical manifestations with pulmonary TB and has repeatedly been reported endemic in Gabon [74-78], should be considered as differential but was not investigated for. For other co-endemic regions it has been proposed that routine exclusion of paragonimiasis be carried out as the costs of incorrect diagnosis and treatment may be significant [79].

4.1.4 Reliable quality of diagnostics

In Gabon the only implemented TB diagnostic tool for microbiological confirmation of TB is smear microscopy by *ZN*. The gradual replacement of *ZN* technique by FM using light-emitting diode lamps, recommended by the WHO in 2009 based on improved sensitivity and reduced workload [29], has not been introduced in Gabon. This study was the first to evaluate FM in this setting and FM performed superior to the *ZN* technique by increasing the sputum positivity rate by 10%. While the overall benefits of FM outweigh *ZN* technique and FM

technique is readily picked up after short training, breakdown of electrical parts including lamps of FM is a concern [80], this needs to be considered before a general roll out of FM as in Gabon respective maintenance service and access to replacement parts is not established.

Mycobacterial culture and molecular diagnostics are not yet routinely available in Gabon. For this study mycobacterial cultures were performed overseas requiring storage and shipment of samples which may have impacted the results. The culture contamination rate of <5% was acceptable compared with other studies [81, 82], and positive cultures in some patients with negative microscopy reflect the expected higher sensitivity of culture compared to microscopy; Decreased viability following either unreported intake of TB drugs prior to sampling or sample storage and shipment may however have led to false negative culture samples.

4.1.5 High rate of microbiological confirmed TB

Two thirds of adult patients had microbiologically confirmed TB, a rate comparable to the retrospective study from Lambaréné [5] but higher than reported in retrospective studies from the capital [7, 69]. Since the population of this study profited from intensified case finding through fluorescence microscopy and culture the higher rate might be closer to the real value. On the other hand, as health care levels between Lambaréné and the capital differ, establishing diagnosis of microbiologically negative TB may be more challenging for physicians in Lambaréné and microbiologically negative TB cases may be missed.

4.1.6 Low rate of EPTB

Unexpectedly few (<5%) patients were diagnosed with exclusive EPTB. In settings with high HIV prevalence EPTB may account for a considerable part of the total TB burden and in previous retrospective report from Gabon EPTB accounted for 20%/15% and 39%/35% of TB cases in HIV-uninfected and HIV-infected hospitalized patients, respectively [5, 69]. Therefore under-diagnosis of EPTB in this cohort is highly probable. A recent report on inpatients from Cameroon with a similar TB/HIV co-infected rate found EPTB without concurrent PTB in 35% [70]. Interestingly, no difference in the rate of EPTB in HIV-infected

versus uninfected patients was reported in one previous study from Gabon and other factors than HIV accounting for higher susceptibility for TB in the Gabonese population, e.g. alcohol consumption or other infectious diseases, were postulated [69]. The eminent health concern of EPTB and the pronounced difficulties with its diagnosis are increasingly recognized [83]. Implementation of point-of-care focused assessment with sonography for HIV-associated TB (FASH) has been reported to improve diagnosis of EPTB in adult patients with HIV infection in resource-limited settings [84, 85]. Awareness and knowledge on EPTB needs to be increased in the setting of this study and implementation of FASH may contribute to improved diagnosis and care of EPTB [86].

4.1.7 High prevalence of *M. africanum*

Mycobacteria other than *M. tuberculosis* were isolated in 18% of patients. *M. africanum,* a subspecies within the *Mycobacterium tuberculosis* complex and only endemic in West African countries, had a prevalence of 17.5% in this Gabonese cohort. While *M. africanum* has never been reported from Gabon, in neighboring Cameroon its prevalence was previously reported with 56% 30 years ago (based on biochemical speciation) [87] and more recently reported with 9% using molecular methods [88]. In the Gabonese cohort *M. africanum* infection was not associated with HIV-infection nor with outcome of TB treatment. *M. africanum* generally responds to regular TB treatment [89] therefore its high prevalence in Gabon does not require local treatment adaption; however, resistance pattern must be monitored carefully as 12% (2/17) of isolates showed INH resistance plus OFX or STR and ETO resistance, respectively.

4.1.8 High rates of drug resistant TB among previously treated TB patients

Given previous anecdotal report on MDR and XDR-TB [13], the limited availability and access to DST and TB drugs [90] and low treatment completion rates [4] in Gabon, concerns about the prevalence and extent of DR-TB were high. With 4.4% and 30.8% of MDR-TB in new and previously treated patients the MDR-TB rates exceeded the overall global rates reported with 3.5% and 20.5% for 2013 by the WHO [3]. As DST was only performed for half of the cohort, the true rate

of DR-TB may be higher or lower; continuous surveillance data are needed to better understand the epidemiology of DR-TB in Gabon.

4.2 Antituberculous treatment and treatment outcome

The overall treatment success rate was concerningly low with 53% which is far from the WHO target treatment success rates of 85%. Given the research framework of this study with designated human and laboratory resources, the true treatment success rate outside of this research setting must be estimated even lower.

Only 8% of patients could be classified as cured according to the WHO guidelines. This low rate can be attributed to the increased efforts required from both the patients and the health institutions to gain enough specimen during the follow up period to declare a patient cured, exceeding the capabilities in a resourced-limited setting such as Lambaréné. The rate of patients routinely undergoing microbiological control examinations must be considered to be even lower than in the context of this study (27.1% and 17.6% at M2 and M6, respectively), taking into account the active follow up strategy applied by the study in terms of reminding patients of FU visits and supporting logistics and costs related to diagnostic FU.

Loss to follow up was the most common reason for adverse treatment outcome. The LTFU rate of 17% was lower than previously reported by studies from Lambaréné and the capital [5, 91]; the lower rate in this cohort might have been biased by the active follow up strategy applied by the study. Furthermore, the portion of patients whose treatment outcome could not be evaluated (16%) probably harbors patients actually being LTFU. Of concern, for at least more than a third of all patients, including those classified as treatment success, deviations from the recommended TB treatment regimens were documented.

The death rate of 10% was twice as high as suggested by previous studies from Gabon [5, 7]. The higher rate might as well be explained by the active follow up strategy employed in this study; probably patients are often classified as LTFU if

they do not come back to the hospital but in this study calling patients' relatives allowed for documenting death as a reason for not presenting for FU visits.

For the first time in Gabon, treatment failure was investigated for and the rate of 3% is in line with a recent study from Cameroon [92]. However, the lack of *DOTS* outside the hospital makes it impossible to reliably distinguish treatment failure from default.

Among children, the high rate of patients LTFU equally points towards deficiencies in patient support and treatment supervision, consistent with the global experience of childhood TB being neglected in the fight against TB [14]. Children are a vulnerable group of patients and depend on their caregivers for successful treatment completion; implementation of *DOTS* for children may require a person other than the direct caregiver to be responsible for treatment supervision.

Established risk factors such as male sex [61, 62, 93-95] and malnutrition [63, 64] did not show significant correlation with default and death in this study. This might be explained by the limited cohort size of the study. Further research is needed in order to rule out gender differentials and nutritional components as reason for low treatment success.

4.3 Barriers towards successful treatment

4.3.1 Drug resistant TB

Treatment outcomes of the eight MDR-TB patients in this study could not be classified according to WHO guidelines since intake of appropriate second-line treatment is a condition for such outcome classification; at the time of the study no second-line TB drugs were available in Gabon and none of the MDR-TB cases therefore received appropriate treatment. Half of the MDR-TB patients died during the study period, this would have been preventable in a setting where MDR-TB patients had access to appropriate care. As surviving MDR-TB patients were not receiving appropriate treatment and continued to live within their communities spreading of MDR-TB to further individuals is highly probable; this

situation of uncontrolled MDR-TB depicts a major public health threat and impairs successful TB control on a regional and national level.

All MDR-TB isolates had further resistance; all but one showed additional resistance to EMB and STR and half were resistant to PZA. Therefore, for most MDR-TB cases no first-line antituberculous drugs were left as partner drugs for a second-line treatment. Concerning second-line drugs almost half of the isolates were resistant to or had reduced susceptibility to ETO and two isolates showed any second-line drug resistance other than to ETO. The high rate of ETO resistance may be related to the high rate of INH resistance as both drugs share common pathways which can lead to cross-resistance [96] but further molecular analyzes are required to elucidate this.

Among mono- and polyresistant TB strains, INH and STR showed the highest rates of resistance (among isolates with any drug resistance 82% and 76%, and among all isolates with DST 13% and 12%, respectively). INH is the cornerstone of first-line antituberculous therapy, INH mono-resistance reduces the probability of treatment success if standard treatment is administered and increases the risk of acquiring additional resistance and thereby MDR-TB [97]. Importantly, in countries with high HIV prevalence INH resistance renders IPT ineffective and hampers prevention and control of HIV-associated TB. INH resistance and effectiveness of IPT in Gabon should be carefully monitored. As almost half of patients with previous TB treatment and 7/8 patients with MDR-TB had STR resistant TB, the current local guidelines recommending addition of STR to the four oral first-line antituberculous drugs for previously treated TB patients may need revision

One of the biggest challenges in the fight against MDR- and XDR-TB is the worldwide availability of affordable and quality-assured second-line drugs. During the last decade, the global framework to support expansion of MDR-TB services and care (green light committee initiative) has made considerable progress and 90 countries and 130.000 patients had received aid in 2011 [98]. However, neither guidelines for diagnosis or second-line regimens nor access to second-line drugs to treat DR-TB existed in Gabon at the time of conducting this study.

Furthermore, there is widespread usage of antibiotics (probably including potential second-line Tb drugs) without prescription among TB patients due to misinterpretations of symptoms and drug stock outs [90, 99]. Decentralized TB treatment centers with different health care staff lacking specialized TB training and initiating TB treatment promotes incorrect TB treatment prescriptions [90, 99]. All these factors fuel the development of resistance against first- and second-line drugs [100].

4.3.2 HIV co-infection

In this study HIV co-infection was the most significant risk factor for death. Treatment success rates are significantly lower in HIV-infected patients on a global level and in the African region; in 2012, the global treatment success rate was 74% compared to 88% and death rate was 11% compared to 3.4% in HIV-infected and HIV-uninfected patients respectively [11]. In Gabon, increased rates of LTFU have been described in HIV patients coinfected with TB (Janssen S, personal communication). Reasons for adverse treatment outcome and death include TB-related conditions such as disseminated TB, immune reconstitution inflammatory syndrome and treatment side effects as well as non TB-related conditions [101, 102]. However, predominant reasons for death in TB/HIV co-infected patients differ between various regions related to the level of TB and HIV care [103, 104]. Further research is needed to identify reasons for death in TB/HIV co-infection in Gabon.

As further data on HIV care has not been comprehensively collected in this study, detailed additional determinants of HIV infection, ART and IPT or cotrimoxazol prophylaxis cannot be reported. Limited data from this study and other reports do however suggest that in Gabon uptake of ART, retention to care, IPT and integrated TB/HIV care are still to be improved (Janssen S, personal communication) [4]. At the time of the study, no integrated HIV/TB care was possible at *CHRGR*, endangering outcome of both TB and HIV treatment [105-107].

4.3.3 Geographical barriers

Far distance to the TB treatment center was identified as a statistically significant risk factor for unfavorable treatment outcome. This is in line with qualitative research on malaria prevention and vaccinations in Gabon, which reported distance to the hospital as a risk factor for non-adherence; explanations include constraints due to field and house work, long queues and limited reception times at the hospital [108, 109]. Studies on TB treatment outcome from Nigeria, Nepal and India reported similar findings [60, 62, 110] but could show at the same time how implementation of *DOTS* and the use of peripheral and ambulatory treatment options were able to increase the rate of successfully treated TB [62, 110].

The travel factor as proposed tool to investigate influences of travelling showed no significant correlation to treatment outcome in this study, which is probably due to a limited cohort size. However, comparison of the three Z-scores for travel distance, duration and costs showed a trend of the latter having the greatest influence on treatment outcome, which is in line with other findings of this study (section 4.3.6).

4.3.4 Atypical presentation of TB

Clinical diagnosis of TB without microbiological confirmation (including smear negative pulmonary and EPTB) was identified as risk factor for unfavorable treatment outcome. The most likely explanation is the high rate of HIV co-infection, with HIV being a well-established cause for TB disease dissemination and smear negativity as well as higher death rates [69, 70, 111]. However, after adjusting analyses for HIV status, the correlation remained significant, suggesting other reasons to account for inferior treatment outcomes.

Possible explanations include misdiagnosed TB in patients suffering from other diseases such as paragonimiasis (section 4.1.3), delayed case finding and treatment initiation with consecutive disease progression [112] and different disease perception in unconfirmed TB with a higher risk of default and death [99] (section 4.3.8).

4.3.5 Delay in treatment initiation

Long duration of cough was a risk factor for default in this study. Patients presented late with half of the patients coughing for at least one month and a quarter for at least months. Reasons for late presentation in Gabon and Cameroon were suggested to be stigma, costs and the use of traditional medicine [113, 114]. Apart from inferior treatment outcome, late presentation and delay in diagnosis of pulmonary TB also translates into increased infectivity in the community; inacceptable time delays across high and low income countries are still a widespread problem and a major challenge for effective TB control [115].

4.3.6 Economic barriers

The most common reason for deviation from treatment named by the patients in this study was the unaffordability of transportation costs (38.8%), which is in line with results from a study performed in Libreville in 2006 [91]. Interviews on travel costs showed that a quarter of patients had to pay more than 3,500 FCFA per way, which is more than the estimated daily family income for big parts of the local population [116]. Furthermore additional costs of up to 30,000 FCFA became due for the payment of x-rays and additional examinations during control visits.

The connection between poverty and TB is established for a long time [117]. The burden of direct and indirect costs by TB disease has been shown to be a challenge worldwide, and particularly in African countries [57, 58]. While Gabon is a comparably rich country in central Africa, people in rural areas are often not sharing the country's wealth. In 2005 a third of the Gabonese population lived below the national poverty line [118]. The fact that the *PNLTB* is generally providing free TB medication is mostly not known in the population [99]. Remarkably, in Gabon a health insurance has been established since 2008, covering 80% of the costs falling due for in-hospital treatment. However, no other TB related costs are reimbursed by the insurance and it is not yet clear if this development has made a change [119].

4.3.7 Stigma

Of note, the designated TB clinic in Lambaréné saw the fewest TB patients and every tenth patient sought care for TB in Lambaréné despite other TB treatment centers being closer to his residency. Additionally, an over-average proportion of patients presents with pronounced delay. These observations may point towards TB patients preventing to be recognized and stigmatized as suffering from TB, or patients being unaware of their possible disease preventing their direct presentation to TB care services. Travelling long distances to avoid stigmatization in the environment of daily living has been reported for a subgroup of HIV patients cared for in Lambaréné (Janssen S, personal communication). In an anthropological study, 67% of patients reported to feel problematically stigmatized in Lambaréné, partly due to double stigma with TB/HIV [99].

4.3.8 Alternative treatment approaches

As recently shown by an anthropologic study performed within a proportion of this study's population by Cremers et al, pluralistic health treatment (i.e. the use of various treatment approaches, such as hospitals, pharmacies, herbal medicine, religious and traditional healers) is common in Gabon and in most cases patients seek different treatment options before coming to the hospital. Local perceptions of TB differentiate between magical disease (vampires, poison or demons and not responding to conventional drug treatment) and natural disease. Negative microbiological examination results for TB often prompt patients to believe suffering from magical instead of natural TB, probably enhancing treatment default [99].

Interestingly, while widespread consultation of traditional healers was shown using the in depth-interviews of the anthropologic study, only 9% of participant had made use of such consultations according to the questionnaires employed by this study. This discrepancy might point towards a competing nature of traditional and hospital medicine in Gabon, making patients hide their preference of alternative treatment from hospital staff and researchers.

On the other hand, Cremers et al reported of the combined use of traditional healers and hospital treatment by patients, despite no interaction between

tradition healers and hospital staff seems to exist [99]. Interestingly, some concepts of traditional medicine show parallels to hospital medicine, like the initiation rites capable of preventing diseases resembling disease prophylaxis, e.g. in form of vaccinations.

Herbal treatment is easily accessible also in remote areas. Although the widespread usage of herbal medicine in Gabon is no uncommon finding [120], little is known about its effect and side effects and further investigation is needed in order to assess its influence on TB treatment outcome.

4.3.9 Lack in counseling, patient support and supervision

Problems arising from the variety of competing official and unofficial health care providers are amplified by the lack of patient support and treatment supervision by the hospitals. The interviews on counseling and patient support point towards several deficiencies in the support of newly diagnosed TB patients, these include insufficient attending to patients' concerns and a missing concept regarding time and content for counseling. Similar deficiencies have recently been found regarding counselling in HIV care in Lambaréné (Janssen S, personal communication).

Equally, several deficiencies in treatment supervision became evident, such as the lack of a treatment log for patients and long intervals between control visits of up to 60 days during the continuation phase, which is far from directly observed treatment. Effectively, *DOTS* was only performed during hospital admission, which on average lasted 13 days.

The fact that "forgetting drug intake" and "subjective recovery" was reported among the three most common reasons for treatment interruption or discontinuation in this study, supports the assumption that patients were not given enough information and education regarding their disease and the required treatment.

4.4 Overcoming the barriers

Several deficiencies in Gabonese health care structure became evident through this study. Strategies, concepts and recommendations for addressing these have

been established and proved efficient in other settings; respective implementation in Gabon is discussed in the following sections.

4.4.1 Patient centered TB care and DOTS

Integrated, patient centered care and prevention is one fundamental pillar of the WHO post-2015 global strategy in the fight against TB [3]. While other regions have successfully adopted the *DOTS* strategy since more than a decade [121-123], *DOTS* expansion and enhancement is urgently needed in Gabon. Currently *DOTS* is not performed outside the hospital and due to high mobility, alternative treatment seeking behavior and financial considerations, patients in remote rural areas of Gabon are in increased risk of defaulting. In a recent systematic review and meta-analysis it was shown that default rates are lowest when *DOTS* is used throughout treatment. Suggested steps towards *DOTS* implementation have been decentralization of health services with limiting of cohort sizes, training of community health workers, involvement of family members and the use of patient registration forms [53, 124].

Patient health education needs to be an inherent part of counseling and treatment supervision and to be additionally offered to people living in remote areas by community health workers in order to foster compliance and reduce stigma. Traditional and religious perceptions should not be neglected but instead be specifically addressed in health education [125].

Interaction between traditional healers and hospitals is needed in order to lower competition between health services and to fall back on the network and the confidence traditional healers enjoy in the population [126-128]. Research is needed on prospects and dangers of traditional and herbal medicine.

Childhood TB must be addressed specifically by raising awareness in the population and by offering improved patient support and treatment supervision among hospital staff. Additional diagnostic methods such as gastric aspirates and induced sputum [129] need to be established in pediatric departments to not only enhance case finding but also ensure treatment success.

4.4.2 Fight against MDR-TB

As MDR-TB does not respect geographical borders, the spread of MDR-TB in Gabon needs to be stopped in time to prevent development of a breeding ground endangering TB control in the central African region and the whole world.

The five priority actions to address the MDR-TB epidemic by the WHO (high-quality treatment of drug sensitive TB to prevent DR-TB, expansion of rapid testing and detection of MDR-TB, immediate access to quality care, infection control, and increased political commitment) [3] are important for Gabon. Through the efforts of this study and related infrastructure set-up, a TB laboratory with the capability of performing mycobacterial culture and DST has been established and application to the Greenlight Committee for second-line drugs has been submitted. Country wide access to culture and DST as well as second-line drugs is urgently needed;

4.4.3 Integration of HIV and TB care

Other regions affected by high rates of TB/HIV co-infection have pioneered and demonstrated effective measures in the fight against the co-epidemic [9]; In Gabon, despite progress made within the last decade, mortality of TB/HIV co-infection is still unacceptably high and further work on the integration of HIV and TB care is necessary. Integrated services need to be implemented in all hospitals to allow for early detection of TB and provision of IPT among people living with HIV as well as early initiation of ART among patients with HIV-associated TB [3, 9]. Further steps are the improvement of home-based care including the training of volunteer caregivers and family education on infection control. [130]

4.4.4 Addressing the social determinants of health

In order to achieve a sustainable TB care in Gabon and regarding the high efforts needed to achieve the global targets set for 2030 [52], the implementation of *DOTS* is not sufficient. Upstream factors for health care deficiencies must be addressed. These include social determinants of health such as the weak and inequitable social policy, making the health system prone to corruption. Likewise, the ongoing uncontrolled urbanization leads to overcrowded slums and overburdened health care services, therefore hampering TB control [59]. National

and global efforts must be directed towards these problems in form of social protection and urban regeneration.

4.4.5 Research

The WHO global post-2015 strategy comprises intensified research and innovation as one of three fundamental pillars. This includes not only the development of novel tools in the fight against TB but also research on implementation and impact of already existing tools and strategies [3]. In the Central African region data on the extent of TB/HIV coinfection and the coverage of ART and IPT are scarce and research is needed to evaluate integration of TB and HIV care [131]. In Gabon, further research is needed to understand reasons of adverse treatment outcome. The newly established national reference laboratory will allow closer insight on the characteristics and dynamics of DR-TB in Gabon. Prospective TB studies must be performed in the big cities of Gabon in order to gain a clearer picture of the national TB situation. Moreover, studies on specific groups in the population suffering from TB are necessary, such as children and prisoners.

4.5 Limitations

This study has some limitation. Due to the observational character and the responsibility of care being with various health care staff, data collection and documentation was sometimes incomplete. On the other hand the observational design of the study is more likely to reflect the true situation in the field; however, human and laboratory resources provided through the study may also have influenced the observations in terms of completeness of TB diagnostics and higher FU rates. Explicit limitations are the incompleteness of data on ART and other HIV-related care determinants. Due to the small sample size of the interviews regarding counseling, patient support and treatment supervision the respective findings are of limited scientific value.

However, this study has several strengths. By recruiting TB patients at all different health care sites which care for TB patients coverage of the area was highly comprehensive. Several different mycobacterial diagnostics were used to increase sensitivity; by shipping samples to the German reference laboratory we

obtained DST data despite the absence of respective infrastructure on the ground. Follow up strategy was maximized by active follow-up actions such as repeated phone calls.

Although the catchment area of this study was broad, generalizability of the findings to the rest of the country or even region must be made cautiously. 40% of the Gabonese population is living in the urban capital where HIV prevalence is lower (3.9% vs. 5.8% [132]), concurrent parasitoses may be less prevalent, and where access to health care may differ.

4.6 Conclusions

Despite its ranking among the top ten high TB incidence countries, with a low population density, Gabon had so far failed to attract international attention or financial assistance to tackle this epidemic. The country relied almost exclusively on (insufficient) domestic funding for TB control activities. With a poorly resourced and underfunded TB control program, Gabon has fallen short of reaching most of the objectives of the Stop TB Strategy and is now faced with emergence and possible dissemination of MDR-TB cases, which represents a major public health threat both on the local and global scale. Concerted action is urgently needed to control the disease.

These first prospective data on basic TB epidemiology and control in Gabon, Central Africa, document that in some parts of the world TB remains a major public health burden and a deadly infection despite the overall global progress in TB control over the past years. In this specific study area the ongoing TB burden is further determined by an unacceptably low rate of treatment success and a high default rate. The expansion of *DOTS* including decentralization of health services, promotion of health education and acknowledgement of cultural specifics is of paramount importance for effective TB control. MDR-TB is a threatening public health concern, in-country diagnostic capacity and access to second-line treatment regimens are urgently needed. Death of every third TB/HIV co-infected patient calls for urgent improvement of integrated TB and HIV care with special attention to INH resistance prevalence which may impair prevention of HIV-associated TB. National and international recognition of neglected

ongoing hot spots of the TB epidemic is a prerequisite in order to achieve global TB control and approach the vision of a world free of TB.

5 Summary

BACKGROUND: Despite overall global progress in tuberculosis control over the past years, some regions are running behind. The central African country Gabon ranks number 10 in terms of TB incidence rate per 100,000 population according to the WHO, and the limited data available suggests alarmingly low treatment success rates. Disproportionately little is known about the reasons for adverse treatment outcome. The aim of the study and this thesis was to prospectively assess local TB determinants including treatment outcomes and to better understand the barriers towards successful treatment, as a prerequisite for effective TB control.

METHODS: In a prospective observational cohort study TB patients were monitored during the course of their treatment in Lambaréné, Gabon. Clinical and microbiological data was collected at treatment initiation and after two and six months. TB treatment outcome was evaluated according to WHO definitions and risk factors for unfavorable treatment outcome and death were identified. Additionally, interviews were conducted with a subset of patients focusing on counseling, patient support and treatment supervision related to TB treatment.

RESULTS: Between 2012 and 2014, 201 adult and pediatric TB patients were enrolled and followed up. The HIV co-infection rate was 42% in adults and 16% in children. Among culture confirmed TB patients the MDR-TB rate was 4/91 (4.4%) and 4/13 (30.8%) in new and previously treated TB patients, respectively. Excluding MDR-TB patients, successful TB treatment was achieved in 55% of patients, 18% were lost to follow up and 9% died. Mortality rate was 29% in TB/HIV co-infected patients and 50% in patients with MDR-TB. Risk factors for adverse treatment outcome were far distance to treatment center and clinical diagnosis of TB, risk factors for death were HIV co-infection and clinical diagnosis, the only risk factor for default was long duration of cough.

DISCUSSION: In Lambaréné, TB epidemiology is determined by a high rate of TB/HIV co-infection and a high rate of MDR-TB among re-treatment patients. Treatment success rates are low and a high percentage of patients is being lost

to follow up. Barriers towards successful treatment include geographical, financial and cultural aspects as well as lack in patient support and treatment supervision. For an effective TB control in Gabon, implementation of *DOTS* is of paramount importance. Furthermore, the access to diagnostic capacity for MDR-TB and second-line TB drugs as well as improvement of integrated TB/HIV care are urgently needed.

6 Summary in German language

Hintergrund: Trotz der Fortschritte, welche in den letzten Jahren weltweit in der Bekämpfung der Tuberkulose erzielt wurden, liegen einige Regionen in der erfolgreichen Tuberkulosebekämpfung zurück. Das zentralafrikanische Gabun belegt gemäß WHO Platz 10 bezüglich Inzidenz pro 100.000 Einwohner und die wenigen verfügbaren Daten lassen auf eine beunruhigend geringe Erfolgsquote in der Tuberkulosetherapie schließen. Über die Ursachen ausbleibenden Therapieerfolgs ist verhältnismäßig wenig bekannt. Das Ziel der Studie und dieser Dissertation ist die prospektive Erfassung von lokalen Determinanten der Tuberkulose und das Aufzeigen von Hindernissen einer erfolgreichen Tuberkulosetherapie, als Voraussetzung für deren effektive Bekämpfung.

Methoden: In einer prospektiven Beobachtungsstudie wurden Tuberkulose Patienten über den Zeitraum ihrer Behandlung in Lambaréné, Gabun nachverfolgt. Die klinische und mikrobiologische Datenerhebung erfolgte zu Therapiebeginn sowie nach zwei und sechs Monaten. Therapieerfolgsraten wurden nach WHO Definitionen ausgewertet und Risikofaktoren für ausbleibenden Therapieerfolg identifiziert. Zusätzlich erfolgte eine Befragung einer Subgruppe der Patienten zu Tuberkulose-spezifischer Beratung, persönlicher Betreuung und Therapiekontrolle.

Ergebnisse: Zwischen 2012 und 2014 wurden 201 erwachsene und pädiatrische Tuberkulosepatienten eingeschlossen und nachverfolgt. Der Anteil an HIV-Koinfektionen lag bei 42% der Erwachsenen und 16% der Kinder. Unter Patienten mit positiver Mykobakterien-Kultur betrug der Anteil an multiresistenter Tuberkulose (MDR-TB) jeweils 4/91 (4.4%) und 4/13 (30.8%) der Erst- und Rezidiv-Fälle. Die MDR-TB Patienten ausgenommen, wurden 55% der Tuberkulosepatienten erfolgreich behandelt, 18% brachen die Therapie ab und 9% starben. Die Letalität der Patienten mit HIV-Koinfektion betrug 29% und jene der Patienten mit zu Therapiebeginn diagnostizierter MDR-TB 50%. Risikofaktoren für ausbleibenden Behandlungserfolg waren große Distanz zum Behandlungszentrum und klinisch diagnostizierte Tuberkulose, Risikofaktoren für

Tod waren HIV-Koinfektion und klinisch diagnostizierte Tuberkulose, der einzige Risikofaktor für Behandlungsabbruch war lange anhaltender Husten.

Schlussfolgerungen: Die Epidemiologie der Tuberkulose in Lambaréné wird durch einen hohen Anteil an HIV-Koinfektionen und einen hohen Anteil an MDR-TB unter Rezidiv-Patienten bestimmt. Die Erfolgsquote der Tuberkulosetherapie ist niedrig und Behandlungsabbrüche sind häufig. Hindernisse einer erfolgreichen Therapie umfassen geographische, finanzielle und kulturelle Aspekte so wie ein Defizit an persönlicher Betreuung und Therapiekontrolle. Die wichtigste Voraussetzung für die effektive Tuberkulosebekämpfung in Gabun ist die Umsetzung der DOTS-Strategie (Directly Observed Therapy, short course), welche in vielen anderen Ländern bereits erfolgreich eingesetzt wird. Weitere Anforderungen sind der Zugang zu Diagnostik von MDR-TB und Zweitrang-Medikamenten sowie eine Verbesserung der Vernetzung zwischen Tuberkulose –und HIV Therapie.

7 References

1. Iwai K, Maeda S and Murase Y (2010) Archaeology of tubercle bacilli and tuberculosis. Kekkaku 85: 465-475

2. GBD 2013 Mortality and Causes of Death Collaborators (2015) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385: 117-171

3. WHO, 2014. Global Tuberculosis report 2014. ISBN 978 92 4 156480 9. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf [Last accessed July 19, 2015]

4. WHO, 2015. TB country profile, Gabon. http://www.who.int/tb/country/data/profiles/en/ [Last accessed July 19, 2015]

5. Stolp S M, Huson M A, Janssen S, Beyeme J O and Grobusch M P (2013) Tuberculosis patients hospitalized in the Albert Schweitzer Hospital, Lambarene, Gabon-a retrospective observational study. Clin.Microbiol.Infect. 19: E499-501

6. Nkoghe D, Toung Mve M, Nnegue S, Okome Nkoume M, Iba B J, Hypolite J, Leonard P and Kendjo E (2005) HIV seroprevalence among tuberculosis patients in Nkembo Hospital, Libreville, Gabon. Short note. Bull.Soc.Pathol.Exot. 98: 121-122

7. Kombila D U, Moussavou-Kombila J B, Grobusch M P and Lell B (2013) Clinical and laboratory features of tuberculosis within a hospital population in Libreville, Gabon. Infection 41: 737-739

8. Laserson K F, Wells C D (2007) Reaching the targets for tuberculosis control: the impact of HIV. Bull.World Health Organ. 85: 377-81; discussion 382-6

9. Howard A A, El-Sadr W M (2010) Integration of tuberculosis and HIV services in sub-Saharan Africa: lessons learned. Clin.Infect.Dis. 50 Suppl 3: S238-44

10. WHO, 2012. WHO policy on collaborative TB/HIV activities. WHO/HTM/TB/2012.1. ISBN 978 92 4 150300 6. http://apps.who.int/iris/bitstream/10665/44789/1/9789241503006_eng.pdf?ua=1 [Last accessed July 27, 2015]

11. WHO, Global tuberculosis report 2013. WHO/HTM/TB/2013.11. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf [Last accessed July 19, 2015]

12. WHO, 2011. Guidelines for the programmatic management of drug-resistant tuberculosis – 2011 update. WHO/HTM/TB/2011.6. ISBN 978 92 4 150158 3. http://whqlibdoc.who.int/publications/2011/9789241501583_eng.pdf [Last accessed July 27, 2015]

13. Mounguengui D, Ondounda M, Mandji Lawson J M, Fabre M, Gaudong L, Mangouka L, Magne C, Nzenze J R and L'her P (2012) Multi-resistant tuberculosis at the hopital d'instruction des armees de Libreville (Gabon) about 16 cases. Bull.Soc.Pathol.Exot. 105: 1-4

14. Seddon J A, Shingadia D (2014) Epidemiology and disease burden of tuberculosis in children: a global perspective. Infect.Drug Resist 7: 153-165

15. Schaaf S Z A: (2009) The genus Mycobacterium and the Mycobacterium tuberculosis complex. P. 44-59. In: Saunders Elsevier (Hrsg) Tuberculosis: a comprehensive clinical reference, Elsevier Health Sciences, Philadelphia,

16. Lawn S D, Zumla A I (2011) Tuberculosis. Lancet 378: 57-72

17. Kumar V, Abbas A K, Fausto N, Mitchell R: (2007) Pulmonary infections. P. 508-528. In: Saunders Elsevier (Hrsg) Robbins Basic Pathology, 8th edition, Elsevier Health Sciences, Philadelphia,

18. Zumla A, Raviglione M, Hafner R and von Reyn C F (2013) Tuberculosis. N.Engl.J.Med. 368: 745-755

19. Delacourt C (2011) Specific features of tuberculosis in childhood. Rev.Mal.Respir. 28: 529-541

20. van't Hoog A H, Meme H K, Laserson K F, Agaya J A, Muchiri B G, Githui W A, Odeny L O, Marston B J and Borgdorff M W (2012) Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. PLoS One 7: e38691

21. Hoa N B, Sy D N, Nhung N V, Tiemersma E W, Borgdorff M W and Cobelens F G (2010) National survey of tuberculosis prevalence in Viet Nam. Bull.World Health Organ. 88: 273-280

22. Jaryal A, Raina R, Sarkar M and Sharma A (2011) Manifestations of tuberculosis in HIV/AIDS patients and its relationship with CD4 count. Lung India. 28: 263-266

23. Kouassi B, N'Gom A, Horo K, Gode C, Ahui B, Emvoudou N M, Koffi N, Anon J C, Konate K F, Itchi M, Koffi M O, Ano A, Manewa S F, Gro Bi A, Aka-Danguy E, Gnaze A and Toure K (2013) Correlation of the manifestations of tuberculosis and the degree of immunosuppression in patients with HIV. Rev.Mal.Respir. 30: 549-554

24. Nayak S, Acharjya B (2012) Mantoux test and its interpretation. Indian.Dermatol.Online J. 3: 2-6

25. Centers for Disease Control and Prevention (2000) Targeted Tuberculin testing and treatment of latent tuberculosis infection, Center for disease control. MMWR 49, No. RR-6: 10-11

26. European Centre for Disease Prevention and Control, 2011. Use of interferon-gamma release assays in support of TB diagnosis. doi:10.2900/3858. http://ecdc.europa.eu/en/publications/Publications/1103_GUI_IGRA.pdf [Last accessed August 03, 2015]

27. McNerney R, Maeurer M, Abubakar I, Marais B, McHugh T D, Ford N, Weyer K, Lawn S, Grobusch M P, Memish Z, Squire S B, Pantaleo G, Chakaya J, Casenghi M, Migliori G B, Mwaba P, Zijenah L, Hoelscher M, Cox H, Swaminathan S, Kim P S, Schito M, Harari A, Bates M, Schwank S, O'Grady J, Pletschette M, Ditui L, Atun R and Zumla A (2012) Tuberculosis diagnostics and biomarkers: needs, challenges, recent advances, and opportunities. J.Infect.Dis. 205 Suppl 2: S147-58

28. WHO, 2010. Use of interferon-γ release assays (IGRAs) in TB control in low and middle-income settings. WHO/HTM/TB/2011.17. http://www.who.int/tb/features_archive/igra_egm_report_oct2011.pdf [Last accessed August 01, 2015]

29. Steingart K R, Henry M, Ng V, Hopewell P C, Ramsay A, Cunningham J, Urbanczik R, Perkins M, Aziz M A and Pai M (2006) Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect.Dis. 6: 570-581

30. Siddiqi S.H. R S, 2006. MGIT Procedure Manual. http://www.finddiagnostics.org/resource-centre/reports_brochures/071130_mgit_manual.html [Last accessed July 27, 2015]

31. WHO, 2009. Report of the ninth meeting of the strategic and technical advisory group for tuberculosis. http://www.who.int/tb/advisory_bodies/stag_tb_report_2009.pdf [Last accessed July 19, 2015]

32. Morgan M A, Horstmeier C D, DeYoung D R and Roberts G D (1983) Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. J.Clin.Microbiol. 18: 384-388

33. MIDDLEBROOK G, COHN M L (1958) Bacteriology of tuberculosis: laboratory methods. Am.J.Public Health Nations Health 48: 844-853

34. Palaci M, Ueki S Y, Sato D N, Da Silva Telles M A, Curcio M and Silva E A (1996) Evaluation of mycobacteria growth indicator tube for recovery and drug susceptibility testing of Mycobacterium tuberculosis isolates from respiratory specimens. J.Clin.Microbiol. 34: 762-764

35. Adjers-Koskela K, Katila M L (2003) Susceptibility testing with the manual mycobacteria growth indicator tube (MGIT) and the MGIT 960 system provides rapid and reliable verification of multidrug-resistant tuberculosis. J.Clin.Microbiol. 41: 1235-1239

36. WHO, 2007. Use of Liquid TB Culture and Drug Susceptibility Testing (DST) in Low and Medium Income Settings. Summary report of the Expert Group Meeting on the use of liquid culture media. http://www.who.int/tb/laboratory/use_of_liquid_tb_culture_summary_report.pdf [Last accessed August 03, 2015]

37. Ling D I, Zwerling A A and Pai M (2008) GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur.Respir.J. 32: 1165-1174

38. Hillemann D, Rusch-Gerdes S and Richter E (2009) Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J.Clin.Microbiol. 47: 1767-1772

39. WHO, 2011. Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement. WHO/HTM/TB/2011.5. ISBN 978 92 4 150205 4. http://whqlibdoc.who.int/publications/2011/9789241502054_eng.pdf [Last accessed August 01, 2015]

40. Steingart K R, Henry M, Laal S, Hopewell P C, Ramsay A, Menzies D, Cunningham J, Weldingh K and Pai M (2007) A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis. Thorax 62: 911-918

41. Shah M, Variava E, Holmes C B, Coppin A, Golub J E, McCallum J, Wong M, Luke B, Martin D J, Chaisson R E, Dorman S E and Martinson N A (2009) Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a High HIV prevalence setting. J.Acquir.Immune Defic.Syndr. 52: 145-151

42. WHO, 2009. Treatment of tuberculosis: guidelines – 4th ed. WHO/HTM/TB/2009.420. ISBN 978 92 4 154783 3. http://www.who.int/tb/publications/tb_treatmentguidelines/en/ [Last accessed July 19, 2015]

43. Janssen S, Padanilam X, Louw R, Mahanyele R, Coetzee G, Hanscheid T, Leenstra T and Grobusch M P (2013) How many sputum culture results do we

need to monitor multidrug-resistant-tuberculosis (MDR-TB) patients during treatment? J.Clin.Microbiol. 51: 644-646

44. WHO, 1991. Tuberculosis control programme. Resolution WHA44.8. http://www.who.int/tb/publications/tbresolution_wha44_8_1991.pdf [Last accessed July 19, 2015]

45. WHO, 1994. Framework for effective tuberculosis control. WHO/TB/94.179. http://whqlibdoc.who.int/hq/1994/WHO_TB_94.179.pdf [Last accessed July 19, 2015]

46. WHO, 2014. MDG 6: combat HIV/AIDS, Malaria and Other Diseases. http://www.who.int/topics/millennium_development_goals/diseases/en/ [Last accessed July 19, 2015]

47. Ministerial Conference on TB and Sustainable Development, 2000. Amsterdam Declaration to Stop TB. http://www.stoptb.org/assets/documents/events/meetings/amsterdam_conference/decla.pdf [Last accessed July 19, 2015]

48. Stop TB partnership, 2001. Washington Commitment to Stop TB. http://www.stoptb.org/assets/documents/events/meetings/partners_forum/2001/WashCommitm_Eng12Dec.pdf [Last accessed July 19, 2015]

49. Stop TB partnership, 2001. The Global Plan to Stop TB 2001 - 2005. WHO/CDS/STB/2001.16. http://www.stoptb.org/assets/documents/global/plan/GLOBAL_PLAN_TO_STOP_TB_2001_2005.pdf [Last accessed July 19, 2015]

50. Stop TB Partnership and World Health Organization, 2006. The Global Plan to Stop TB 2006–2015. WHO/HTM/STB/2006.35. ISBN 92 4 159399 7. http://www.stoptb.org/assets/documents/global/plan/globalplanfinal.pdf [Last accessed July 19, 2015]

51. Stop TB partnership, 2010. The Global Plan to Stop TB 2011-2015. http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf [Last accessed July 19, 2015]

52. WHO, 2013. Global strategy and targets for tuberculosis prevention, care and control after 2015. EB134/12. http://apps.who.int/gb/ebwha/pdf_files/EB134/B134_12-en.pdf [Last accessed July 19, 2015]

53. Toczek A, Cox H, du Cros P, Cooke G and Ford N (2013) Strategies for reducing treatment default in drug-resistant tuberculosis: systematic review and meta-analysis. Int.J.Tuberc.Lung Dis. 17: 299-307

54. WHO, 2013. Global database, TB treatment outcomes. https://extranet.who.int/tme/generateCSV.asp?ds=outcomes [Last accessed November 20, 2013]

55. Orenstein E W, Basu S, Shah N S, Andrews J R, Friedland G H, Moll A P, Gandhi N R and Galvani A P (2009) Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect.Dis. 9: 153-161

56. O'Donnell M R, Padayatchi N, Master I, Osburn G and Horsburgh C R (2009) Improved early results for patients with extensively drug-resistant tuberculosis and HIV in South Africa. Int.J.Tuberc.Lung Dis. 13: 855-861

57. Mauch V, Bonsu F, Gyapong M, Awini E, Suarez P, Marcelino B, Melgen R E, Lonnroth K, Nhung N V, Hoa N B and Klinkenberg E (2013) Free tuberculosis diagnosis and treatment are not enough: patient cost evidence from three continents. Int.J.Tuberc.Lung Dis. 17: 381-387

58. Leive A, Xu K (2008) Coping with out-of-pocket health payments: empirical evidence from 15 African countries. Bull.World Health Organ. 86: 849-856

59. Hargreaves J R, Boccia D, Evans C A, Adato M, Petticrew M and Porter J D (2011) The social determinants of tuberculosis: from evidence to action. Am.J.Public Health 101: 654-662

60. Ahmed J, Chadha V K, Singh S, Venkatachalappa B and Kumar P (2009) Utilization of RNTCP services in rural areas of Bellary District, Karnataka, by gender, age and distance from health centre. Indian.J.Tuberc. 56: 62-68

61. Mukherjee A, Saha I, Sarkar A and Chowdhury R (2012) Gender differences in notification rates, clinical forms and treatment outcome of tuberculosis patients under the RNTCP. Lung India. 29: 120-122

62. Erhabor G E, Adewole O, Adisa A O and Olajolo O A (2003) Directly observed short course therapy for tuberculosis--a preliminary report of a three-year experience in a teaching hospital. J.Natl.Med.Assoc. 95: 1082-1088

63. Kim H J, Lee C H, Shin S, Lee J H, Kim Y W, Chung H S, Han S K, Shim Y S and Kim D K (2010) The impact of nutritional deficit on mortality of in-patients with pulmonary tuberculosis. Int.J.Tuberc.Lung Dis. 14: 79-85

64. Zachariah R, Spielmann M P, Harries A D and Salaniponi F M (2002) Moderate to severe malnutrition in patients with tuberculosis is a risk factor associated with early death. Trans.R.Soc.Trop.Med.Hyg. 96: 291-294

65. Republique Gabonaise, Ministère de la Santé, Programme National de Lutte contre la Tuberculose (2013) Guide technique de prise en charge de la Tuberculose, 5ème Edition. 19-36

66. HAIN Lifesciences, 2011. GenoType Mycobacterium CM. Ver 1.0. IFU-299-20. http://www.hain-lifescience.de/en/instructions-for-use.html [Last accessed July 27, 2015]

67. WHO, 2013. Definitions and reporting framework for tuberculosis – 2013 revision. WHO/HTM/TB/2013.2. ISBN 978 92 4 150. http://www.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf [Last accessed July 19, 2015]

68. Graham S M, Ahmed T, Amanullah F, Browning R, Cardenas V, Casenghi M, Cuevas L E, Gale M, Gie R P, Grzemska M, Handelsman E, Hatherill M, Hesseling A C, Jean-Philippe P, Kampmann B, Kabra S K, Lienhardt C, Lighter-Fisher J, Madhi S, Makhene M, Marais B J, McNeeley D F, Menzies H, Mitchell C, Modi S, Mofenson L, Musoke P, Nachman S, Powell C, Rigaud M, Rouzier V, Starke J R, Swaminathan S and Wingfield C (2012) Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J.Infect.Dis. 205 Suppl 2: S199-208

69. Ondounda M, Ilozue C, Mounguengui D, Magne C and Nzenze J R (2011) Clinical and radiological features of tuberculosis during HIV infection in Libreville, Gabon. Med.Trop.(Mars) 71: 253-256

70. Agbor A A, Bigna J J, Plottel C S, Billong S C, Tejiokem M C, Ekali G L, Noubiap J J, Toby R, Abessolo H and Koulla-Shiro S (2015) Characteristics of patients co-infected with HIV at the time of inpatient tuberculosis treatment initiation in Yaounde, Cameroon: a tertiary care hospital-based cross-sectional study. Arch.Public.Health. 73: 24-015-0075-y. eCollection 2015

71. WHO, 2007. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. ISBN 978 92 4 159562 9. http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf [Last accessed July 29, 2015]

72. Centers for Disease Control and Prevention (1992) 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep 41 (RR-17): 1-19

73. Achkar J M, Sherpa T, Cohen H W and Holzman R S (2008) Differences in clinical presentation among persons with pulmonary tuberculosis: a comparison of documented and undocumented foreign-born versus US-born persons. Clin.Infect.Dis. 47: 1277-1283

74. Malvy D, Ezzedine K H, Receveur M C, Pistone T, Mercie P and Longy-Boursier M (2006) Extra-pulmonary paragonimiasis with unusual arthritis and

cutaneous features among a tourist returning from Gabon. Travel Med.Infect.Dis. 4: 340-342

75. Voelker J, Sachs R (1985) Morphology of the lung fluke Paragonimus uterobilateralis occurring in Gabon, West Africa. Trop.Med.Parasitol. 36: 210-212

76. Sachs R, Kern P and Voelker J (1983) Paragonimus uterobilateralis as the cause of 3 cases of human paragonimiasis in Gabon. Tropenmed.Parasitol. 34: 105-108

77. Petavy A F, Cambon M, Demeocq F and Dechelotte P (1981) A Gabonese case of paragonimiasis in a child. Bull.Soc.Pathol.Exot.Filiales 74: 193-197

78. Vuong P N, Bayssade-Dufour C, Mabika B, Ogoula-Gerbeix S and Kombila M (1996) Paragonimus westermani pulmonary distomatosis in Gabon. First case. Presse Med. 25: 1084-1085

79. Belizario V,Jr, Totanes F I, Asuncion C A, De Leon W, Jorge M, Ang C and Naig J R (2014) Integrated surveillance of pulmonary tuberculosis and paragonimiasis in Zamboanga del Norte, the Philippines. Pathog.Glob.Health. 108: 95-102

80. Van Deun A, Aung K J, Khan M H, de Jong B C, Gumusboga M and Hossain M A (2014) An operational study comparing microscopes and staining variations for tuberculosis LED FM. Int.J.Tuberc.Lung Dis. 18: 964-971

81. Musau S, McCarthy K, Okumu A, Shinnick T, Wandiga S, Williamson J and Cain K (2015) Experience in implementing a quality management system in a tuberculosis laboratory, Kisumu, Kenya. Int.J.Tuberc.Lung Dis. 19: 693-695

82. Mathewos B, Kebede N, Kassa T, Mihret A and Getahun M (2015) Characterization of mycobacterium isolates from pulmomary tuberculosis suspected cases visiting Tuberculosis Reference Laboratory at Ethiopian Health and Nutrition Research Institute, Addis Ababa Ethiopia: a cross sectional study. Asian Pac.J.Trop.Med. 8: 35-40

83. Sotgiu G, Gegia M and Migliori G B (2014) Extra-pulmonary tuberculosis: the comorbidity of the near future? Int.J.Tuberc.Lung Dis. 18: 1389

84. Heller T, Wallrauch C, Goblirsch S and Brunetti E (2012) Focused assessment with sonography for HIV-associated tuberculosis (FASH): a short protocol and a pictorial review. Crit.Ultrasound J. 4: 21-7902-4-21

85. Heller T, Goblirsch S, Bahlas S, Ahmed M, Giordani M T, Wallrauch C and Brunetti E (2013) Diagnostic value of FASH ultrasound and chest X-ray in HIV-co-infected patients with abdominal tuberculosis. Int.J.Tuberc.Lung Dis. 17: 342-344

86. Janssen S, Grobusch M P and Heller T (2013) 'Remote FASH' tele-sonography - a novel tool to assist diagnosing HIV-associated extrapulmonary tuberculosis in remote areas. Acta Trop. 127: 53-55

87. Huet M, Rist N, Boube G and Potier D (1971) Bacteriological study of tuberculosis in Cameroon. Rev.Tuberc.Pneumol.(Paris) 35: 413-426

88. Niobe-Eyangoh S N, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, Rastogi N, Vincent V and Gutierrez M C (2003) Genetic biodiversity of Mycobacterium tuberculosis complex strains from patients with pulmonary tuberculosis in Cameroon. J.Clin.Microbiol. 41: 2547-2553

89. de Jong B C, Antonio M and Gagneux S (2010) Mycobacterium africanum--review of an important cause of human tuberculosis in West Africa. PLoS Negl Trop.Dis. 4: e744

90. Belard S, Janssen S, Osbak K K, Adegnika A A, Ondounda M and Grobusch M P (2014) Limited access to drugs for resistant tuberculosis: a call to action. J.Public.Health.(Oxf)

91. Mve M T, Bisvigou U, Barry N C, Ondo C E and Nkoghe D (2010) Reasons for stopping and restarting tuberculosis treatment in Libreville (Gabon). Sante 20: 31-34

92. Djouma F N, Noubom M, Ateudjieu J and Donfack H (2015) Delay in sputum smear conversion and outcomes of smear-positive tuberculosis patients: a retrospective cohort study in Bafoussam, Cameroon. BMC Infect.Dis. 15: 139-015-0876-1

93. Samman Y, Krayem A, Haidar M, Mimesh S, Osoba A, Al-Mowaallad A, Abdelaziz M and Wali S (2003) Treatment outcome of tuberculosis among Saudi nationals: role of drug resistance and compliance. Clin.Microbiol.Infect. 9: 289-294

94. Jimenez-Corona M E, Garcia-Garcia L, DeRiemer K, Ferreyra-Reyes L, Bobadilla-del-Valle M, Cano-Arellano B, Canizales-Quintero S, Martinez-Gamboa A, Small P M, Sifuentes-Osornio J and Ponce-de-Leon A (2006) Gender differentials of pulmonary tuberculosis transmission and reactivation in an endemic area. Thorax 61: 348-353

95. Chan-Yeung M, Noertjojo K, Leung C C, Chan S L and Tam C M (2003) Prevalence and predictors of default from tuberculosis treatment in Hong Kong. Hong Kong Med.J. 9: 263-268

96. Machado D, Perdigao J, Ramos J, Couto I, Portugal I, Ritter C, Boettger E C and Viveiros M (2013) High-level resistance to isoniazid and ethionamide in multidrug-resistant Mycobacterium tuberculosis of the Lisboa family is associated with inhA double mutations. J.Antimicrob.Chemother. 68: 1728-1732

97. Menzies D, Benedetti A, Paydar A, Royce S, Madhukar P, Burman W, Vernon A and Lienhardt C (2009) Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. PLoS Med. 6: e1000150

98. Green Light Committee Initiative, 2013. Green light commitee programmes applications.
http://www.who.int/tb/challenges/mdr/greenlightcommittee/en/index.html [Last accessed November 21, 2013]

99. Cremers A L, Janssen S, Huson M A M, Bikene G, Gerrets R P M, Grobusch M P (2013)
Perceptions, health care seeking behaviour and the implementation of a TB control program in Lambaréné, Gabon. Public Health Action Journal 3(4): 328-332

100. Migliori G B, Dheda K, Centis R, Mwaba P, Bates M, O'Grady J, Hoelscher M and Zumla A (2010) Review of multidrug-resistant and extensively drug-resistant TB: global perspectives with a focus on sub-Saharan Africa. Trop.Med.Int.Health 15: 1052-1066

101. Havlir D V, Kendall M A, Ive P, Kumwenda J, Swindells S, Qasba S S, Luetkemeyer A F, Hogg E, Rooney J F, Wu X, Hosseinipour M C, Lalloo U, Veloso V G, Some F F, Kumarasamy N, Padayatchi N, Santos B R, Reid S, Hakim J, Mohapi L, Mugyenyi P, Sanchez J, Lama J R, Pape J W, Sanchez A, Asmelash A, Moko E, Sawe F, Andersen J, Sanne I and AIDS Clinical Trials Group Study A5221 (2011) Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. N.Engl.J.Med. 365: 1482-1491

102. Abdool Karim S S, Naidoo K, Grobler A, Padayatchi N, Baxter C, Gray A L, Gengiah T, Gengiah S, Naidoo A, Jithoo N, Nair G, El-Sadr W M, Friedland G and Abdool Karim Q (2011) Integration of antiretroviral therapy with tuberculosis treatment. N.Engl.J.Med. 365: 1492-1501

103. Podlekareva D N, Panteleev A M, Grint D, Post F A, Miro J M, Bruyand M, Furrer H, Obel N, Girardi E, Vasilenko A, Losso M H, Arenas-Pinto A, Cayla J, Rakhmanova A, Zeltina I, Werlinrud A M, Lundgren J D, Mocroft A, Kirk O and HIV/TB study group (2014) Short- and long-term mortality and causes of death in HIV/tuberculosis patients in Europe. Eur.Respir.J. 43: 166-177

104. Worodria W, Massinga-Loembe M, Mazakpwe D, Luzinda K, Menten J, Van Leth F, Mayanja-Kizza H, Kestens L, Mugerwa R D, Reiss P, Colebunders R and TB-IRIS Study Group (2011) Incidence and predictors of mortality and the effect of tuberculosis immune reconstitution inflammatory syndrome in a cohort of TB/HIV patients commencing antiretroviral therapy. J.Acquir.Immune Defic.Syndr. 58: 32-37

105. Schulz S A, Draper H R and Naidoo P (2013) A comparative study of tuberculosis patients initiated on ART and receiving different models of TB-HIV care. Int.J.Tuberc.Lung Dis. 17: 1558-1563

106. Schwartz A B, Tamuhla N, Steenhoff A P, Nkakana K, Letlhogile R, Chadborn T R, Kestler M, Zetola N M, Ravimohan S and Bisson G P (2013) Outcomes in HIV-infected adults with tuberculosis at clinics with and without co-located HIV clinics in Botswana. Int.J.Tuberc.Lung Dis. 17: 1298-1303

107. Hermans S M, Castelnuovo B, Katabira C, Mbidde P, Lange J M, Hoepelman A I, Coutinho A and Manabe Y C (2012) Integration of HIV and TB services results in improved TB treatment outcomes and earlier prioritized ART initiation in a large urban HIV clinic in Uganda. J.Acquir.Immune Defic.Syndr. 60: e29-35

108. Gysels M, Pell C, Mathanga D P, Adongo P, Odhiambo F, Gosling R, Akweongo P, Mwangi R, Okello G, Mangesho P, Slutsker L, Kremsner P G, Grobusch M P, Hamel M J, Newman R D and Pool R (2009) Community response to intermittent preventive treatment of malaria in infants (IPTi) delivered through the expanded programme of immunization in five African settings. Malar J. 8: 191-2875-8-191

109. Schwarz N G, Gysels M, Pell C, Gabor J, Schlie M, Issifou S, Lell B, Kremsner P G, Grobusch M P and Pool R (2009) Reasons for non-adherence to vaccination at mother and child care clinics (MCCs) in Lambarene, Gabon. Vaccine 27: 5371-5375

110. Wares D F, Akhtar M and Singh S (2001) DOT for patients with limited access to health care facilities in a hill district of eastern Nepal. Int.J.Tuberc.Lung Dis. 5: 732-740

111. Hargreaves N J, Kadzakumanja O, Phiri S, Nyangulu D S, Salaniponi F M, Harries A D and Squire S B (2001) What causes smear-negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence? Int.J.Tuberc.Lung Dis. 5: 113-122

112. Ade S, Harries A D, Trebucq A, Hinderaker S G, Ade G, Agodokpessi G, Affolabi D and Anagonou S (2013) National profile and treatment outcomes of adult smear-negative pulmonary TB patients in Benin. Trans.R.Soc.Trop.Med.Hyg. 107: 783-788

113. Ondounda M, Gaudong Mbethe L, Mounguengui D, Magne C and Nzenze J R (2013) Profile of adenopathies in a tropical setting: 78 observations in Gabon. Med.Sante.Trop. 23: 304-307

114. Cambanis A, Ramsay A, Yassin M A and Cuevas L E (2007) Duration and associated factors of patient delay during tuberculosis screening in rural Cameroon. Trop.Med.Int.Health 12: 1309-1314

115. Sreeramareddy C T, Qin Z Z, Satyanarayana S, Subbaraman R and Pai M (2014) Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. Int.J.Tuberc.Lung Dis. 18: 255-266

116. Goesch J N, Schwarz N G, Decker M L, Oyakhirome S, Borchert L B, Kombila U D, Poetschke M, Lell B, Issifou S, Kremsner P G and Grobusch M P (2008) Socio-economic status is inversely related to bed net use in Gabon. Malar J. 7: 60-2875-7-60

117. WHO, 2005. Adressing poverty for TB control. WHO/HTM/TB/2005.352. http://whqlibdoc.who.int/hq/2005/WHO_HTM_TB_2005.352.pdf [Last accessed November 11, 2013]

118. Direction Générale de la Statistique et des Etudes Economiques (DGSEE) - Ministère de la Planification, de la Programmation du Développement et de l'Aménagement du Territoire, 2013. Enquête Gabonaise pour l'Evaluation et le Suivi de la Pauvreté 2005. http://catalog.ihsn.org/index.php/catalog/41 [Last accessed August 01, 2015]

119. Gabon gets everyone under one social health insurance roof. Bull World Health Organ 2013;91:318–319.

120. Towns A M, Mengue Eyi S and van Andel T (2014) Traditional medicine and childcare in Western Africa: mothers' knowledge, folk illnesses, and patterns of healthcare-seeking behavior. PLoS One 9: e105972

121. Xianyi C, Fengzeng Z, Hongjin D, Liya W, Lixia W, Xin D and Chin D P (2002) The DOTS strategy in China: results and lessons after 10 years. Bull.World Health Organ. 80: 430-436

122. Khatri G R, Frieden T R (2002) Rapid DOTS expansion in India. Bull.World Health Organ. 80: 457-463

123. Zalesky R, Abdullajev F, Khechinashvili G, Safarian M, Madaras T, Grzemska M, Englund E, Dittmann S and Raviglione M (1999) Tuberculosis control in the Caucasus: successes and constraints in DOTS implementation. Int.J.Tuberc.Lung Dis. 3: 394-401

124. WHO, 2006. Revised TB recording and reporting forms and registers - version 2006. WHO/HTM/TB/2006.373. http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.373_eng.pdf [Last accessed July 30, 2015]

125. Vecchiato N L (1997) Sociocultural aspects of tuberculosis control in Ethiopia. Med.Anthropol.Q. 11: 183-201

126. Azevedo M J, Prater G S, Hayes S C (2010) Human immunodeficiency virus and tuberculosis coinfections in Kenya: Environment, resources and culture. Int J Sociol Anthropol 2(4): 55-65

127. Pronyk R M, Makhubele M B, Hargreaves J R, Tollman S M and Hausler H P (2001) Assessing health seeking behaviour among tuberculosis patients in rural South Africa. Int.J.Tuberc.Lung Dis. 5: 619-627

128. Wilkinson D, Gcabashe L and Lurie M (1999) Traditional healers as tuberculosis treatment supervisors: precedent and potential. Int.J.Tuberc.Lung Dis. 3: 838-842

129. Getahun H, Sculier D, Sismanidis C, Grzemska M and Raviglione M (2012) Prevention, diagnosis, and treatment of tuberculosis in children and mothers: evidence for action for maternal, neonatal, and child health services. J.Infect.Dis. 205 Suppl 2: S216-27

130. Akintola O, Hangulu L (2014) Infection control in home-based care for people living with HIV/AIDS/TB in South Africa: an exploratory study. Glob.Public.Health. 9: 382-393

131. Janssen S, Huson M A, Belard S, Stolp S, Kapata N, Bates M, van Vugt M and Grobusch M P (2014) TB and HIV in the Central African region: current knowledge and knowledge gaps. Infection 42: 281-294

132. Direction Générale de la Statistique (DGS) du Gabon et ICF International, 2012. Enquête Démographique et de Santé du Gabon 2012: Prévalence du VIH. http://dhsprogram.com/pubs/pdf/HF44/HF44.pdf [Last accessed August 01, 2015]

8 Declaration of own contribution

The research for this thesis was conducted at the institute for tropical medicine, travel medicine and human parasitology at the university department of medicine of the Eberhard Karls Universität in Tübingen under supervision of P.G. Kremsner.

The study was designed by Sabine Bélard (Principal Investigator) in collaboration with Afsatou Ndama Traoré, Davy Ulrich Kombila, Matthias Frank, Betrand Lell, Abraham Alabi, Sabine Rüsch-Gerdes, Marguerite Massinga Loembé, Akim Ayola Adegnika, Martin Grobusch and P.G. Kremsner (Co-Investigators).

The data presented in table 2-4 were collected by me assisted by Sanne Bootsma, Davy Ulrich Kombila, Saskia Janssen, Jonas Ehrhardt, Kara Osbak (recruitment and provision of clinical data for part of the study participants) and Grace Bikene (field work), after being trained by Sabine Bélard. Data analysis was done by me in collaboration with Sabine Bélard.

The data presented in table 5 and 6 were collected by me assisted by Sanne Bootsma, Davy Ulrich Kombila, Jonas Ehrhardt, Grace Bikene (sample acquisition for part of the patients), Makaya Nina, Ermine Linda Nsafoe, Harry Mabala Kaba, Francis Foguim Tsombeng, Arnault Rogue Mfoumbi Ibinda (microscopy) and in collaboration with Abraham Alabi (head of TB lab, shipment) and Sabine Rüsch-Gerdes (culture, DST and PCR) after being trained by Sabine Bélard. Data analysis was done by me in collaboration with Sabine Bélard.

The classification presented in table 7 was done by me in collaboration with Sabine Bélard using the data presented in table 4 and 5.

The data presented in table 8 was collected by me assisted by Sanne Bootsma, Davy Ulrich Kombila, Saskia Janssen, Jonas Ehrhardt, Kara Osbak, Grace Bikene (provision of clinical data and sample acquisition for part of the patients), Makaya Nina, Ermine Linda Nsafoe, Harry Mabala Kaba, Francis Foguim Tsombeng, Arnault Rogue Mfoumbi Ibinda (microscopy) and in collaboration

with Abraham Alabi (head of TB lab, shipment) and Sabine Rüsch-Gerdes (culture, DST and PCR) after being trained by Sabine Bélard. Data analysis was done by me in collaboration with Sabine Bélard.

The statistical analysis presented in table 9 and 10 and figure 3 were done by me in collaboration with Sabine Bélard and Régis Maurin Obiang Mba.

The data presented in table 11 and 12 were collected by me assisted by Sanne Bootsma (conduction of part of the interviews).

Other investigators involved in the study are Justin O. Beyeme, Elie G. Rossatanga, Cosme Kokou (provision of the infrastructure of local TB treatment centers), Emmanuel Bache (internal study monitoring) and Stefan Niemann (senior supervision of laboratory procedures at the NRC in Borstel)

The manuscript of this thesis was written by myself under supervision of P.G. Kremnser, Martin Grobusch and Sabine Bélard, no other than the noted references were used.