Clinical Evaluation of New Diagnostic Tests and Development of Testing Strategies for Tuberculosis Diagnosis in Africa

Aus dem

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Table of content

Summary

Tuberculosis (TB) continues to kill more than 1.5 Mio people every year and causes a significant morbidity burden in the about 9 Mio patients who survived this infectious disease. Rapid and accurate TB diagnosis is considered one cornerstone of the global fight against TB. The “End TB Strategy” of the World Health Organization (WHO) is enforcing the need to develop new TB diagnostic tests, which are addressing the shortcomings of standard diagnostic tests that are currently used in TB epidemic and resource constrained settings like sub-Saharan Africa. In addition, to improved diagnostic tests, innovate testing strategies are needed to detect TB and control TB transmissions within specific risk groups such as prisoners, children and also TB contacts.

In the frame of the presented habilitation project, three new diagnostic tests, namely the new Xpert MTB/RIF assay, which was endorsed by WHO in 2010, and two urine- based LAM tests, were evaluated in various clinical diagnostic studies in Tanzania. Further, a cross-sectional TB prevalence study was conducted in 13 Ethiopian prisons to study risk factors for TB in inmates and how successful currently implemented diagnostics algorithms are to detect TB in detention facilities. Finally, the isolated TB strains from these research studies were further analyzed using genotyping techniques in order to analyse mechanisms of TB transmission, spread of drug resistance and pathogenicity of TB strains in different high TB risk populations in Africa.

For the Xpert MTB/RIF evaluation, the studies included adult and pediatric cohorts of patients with suspicion of TB. Further, the Xpert MTB/RIF assay was evaluated in a study with household contacts of smear-positive TB patients. Finally, the assay´s capacity to monitor TB treatment was assessed in TB patients who were enrolled in a therapeutic drug trial. The results of these studies contributed relevant information on the diagnostic accuracy of the assay and are also reflected by the current Xpert MTB/RIF policy recommendations from WHO. The most relevant findings were that Xpert MTB/RIF had a significant higher sensitivity compared to smear microscopy and detected up to 60% of smear negative, culture positive adult and paediatric TB cases. Xpert MTB/RIF performs equally well in HIV-positive and HIV-negative TB suspects and only one test in adults is sufficient to reach almost maximal sensitivity. Due to easy handling, rapid availability of test results and good performance in field conditions, the Xpert MTB/RIF test should be considered as the preferred test in contact tracing scenarios in Tanzania. However, due to sustained positive Xpert MTB/RIF results until the end of antimicrobial therapy in up to 27% of smear-positive TB patients, this assay is not useful to monitor microbiological response to TB treatment.

The diagnostic capacity of the two LAM-assays was evaluated in a cohort of children with presumed TB. This study showed that LAM-sensitivity was highest, with maximal 70%, in HIV positive TB cases but had poor sensitivity, 28%, in HIV-negative TB suspects. Importantly, those groups of children suffering most from (co-) morbidities and high mortality were more likely to be LAM-positive. Therefore, specifically HIV-positive children with presumed active TB infection and advanced morbidity might benefit most from urine LAM-testing. This is in line with current WHO recommendations on the use of urine-based LAM tests. However, further scientific evidence is needed for a final evaluation of the use of LAM tests for the diagnosis of TB in clinical routine in Africa.

The prison studies revealed that TB prevalence with about 450 TB cases among 100.000 convicts was twice as large as in the standard Ethiopian population. About 30% of existing TB cases were not detected by the prison health staff, whereby half on these were smear negative. Risk factors for TB in prisons were related to subject characteristics and behavior (e.g. alcohol drinking and TB contact at home) as well as to prison capacities (e.g. windows in prison cells). Genotypic analyses revealed that TB strains from prisoners were forming joint clusters with TB strains isolated from the Ethiopian standard population. These findings support the concept of interrelated TB epidemics among populations inside and outside prisons. Both sides need to be addressed in TB control programmes, and e.g. systematic and comprehensive TB screenings among new prisoners at entry as well as long-term prisoners might be an important strategy in order to end TB in high risk populations in Africa.

1. Introduction

1.1. Global burden of TB disease

TB is one of the most common infectious disease worldwide (1). Estimated one-third of the global population is latently infected with TB mycobacteria. In 2015, about 10.4 Mio people developed active TB disease, out of whom about one Mio were children below the age of 19 years. Up to 11% of the newly diagnosed TB cases were co-infected with HIV. An estimate of 1.8 Mio people died as a consequence of TB activation and TB disease, among them 0.2 Mio children (1). Consequently, TB belongs to the top ten killers worldwide. In 2015 more human beings died from TB than HIV or malaria. The 2015 TB mortality rate in low and middle income countries was between 5% in few countries of Northern Africa and Asia and more than 20% in countries of sub-Saharan Africa (2). This difference in percentages is also reflecting the diversity in the availability and quality of TB diagnosis and treatment in the different countries (3). Further, there are differences in the vulnerability of the various populations for TB infection and death from TB which are mainly associated with the distribution of socio-economic determinates (3).

In addition to the considerable mortality risk, active TB disease is associated with a high disease burden (4), which can last until far beyond the period of active infection (5, 6). Even years after the finalization of anti-mycobacterial therapy, a large proportion of TB patients suffers from the long-term consequences of TB disease, leading to a sustained reduction in the quality of life and shortened life expectancy (7-10). Mostly, chronic pulmonary impairment and consecutive heart disease are the medical reasons for this (11), but diabetes mellitus and cancer are also more frequently diagnosed in former TB patients (12, 13). In addition to the long term TB sequelae, active TB often represents a high risk of impoverishment for the entire family of the affected persons (14, 15), as well as social stigma and ostracism (16), especially in those countries most affected by TB. This underlines the importance of TB prevention measures in the population, including the availability of reliable diagnostic tests. An early diagnosis of TB prevents the transmission of the disease to other persons and allows the limitation of potential health (17, 18) and financial (19) damage to TB patients.

1.2. The importance of diagnostic tests for TB control in Africa

The achievement of successful TB control in the developed countries of the Western world up to the middle of the 20th century (20, 21) resulted in a diminishing interest of science in the development of new diagnostic tests and therapies. In addition to the introduction of antibiotic 4-fold therapy in the 1950s and 1960s, progress in the public health sector and raising social and economic standards have made TB a rare disease in many Western countries Regions (21).

It was only in 1991 that TB was again declared a serious global health problem by the World Health Assembly (22), and the DOTS strategy was developed in the 1990s with the aim to control TB on a global level. An important pillar of the DOTS strategy (of a total of five) was to ensure TB diagnosis by means of assured microbiological detection methods: "case detection through quality-assisted bacteriology" (23). The major aim of the WHO and the national TB programs was to stop TB transmission in the population. This was to be achieved mainly by a rapid diagnosis and effective antibiotic therapy. A decade after DOTS the WHO's Stop TB strategy was introduced, addressing additional challenges (such as the HIV co-epidemic in most sub-Saharan Africa), and setting out strategies for solutions. Again, an early and correct diagnosis of active TB as well as the promotion of research in this area were of particular importance: "Pursue high-quality DOTS expansion and

enhancement (Component 1)" & "Enable and promote research (component 6)" (24). Through the implementation of DOTS and the Stop TB strategy, some sub-Saharan African countries, including Tanzania and Ethiopia, have succeeded in achieving individual aims of the United Nations Millennium Development Goals (MDGs) and the Stop TB strategy (1). However, both countries, Tanzania and Ethiopia, still belong to the 20 "high TB burden" countries in 2015. Among other reasons, this classification as high burden country was mainly based on the ongoing high TB incidence in both countries. Although TB incidence and mortality rates in Tanzania and Ethiopia have declined significantly compared to 1990, there are still around 300/100.000 new cases per year in Tanzania and 200/100.000 in Ethiopia. In addition to economic and social development aspects (3), the limited, comprehensive availability of TB diagnostics as well as the difficult diagnosis or accessibility of risk groups (HIV-positive, children, prisoners) are decisive factors for the lack of TB control in these countries. For example, the proportion of treated TB patients with bacteriologically confirmed TB in 2015 was still only 54% in Ethiopia and 53% in Tanzania (1).

The WHO and the United Nations have formulated the goal of ending the global TB epidemic by 2030. The WHO's "End TB Strategy", developed for this purpose, describes the most important measures and changes needed to achieve this ambitious goal worldwide. As part of the first component (A), the first pillar of this strategy emphasizes once again the importance of early and correct TB diagnosis, especially in specific risk groups: "Early diagnosis of TB, including universal drug-susceptibility testing, risk groups" (1).

1.3. Standard diagnostic tests for detection of TB in Africa

The gold standard of TB diagnosis represents the microbiological detection of mycobacteria in culture, either on solid or in liquid media, with a sensitivity of 80-85% and a specificity of about 98% (25, 26). In addition, the cultivation of mycobacteria is a prerequisite for the phenotypic determination of mycobacteria and the conduction of phenotypic resistance tests (27). The detection of active TB by means of cultural procedures is, however, also associated with disadvantages and specific requirements which can create additional financial and infrastructural hurdles, especially for developing countries in sub-Saharan Africa. On the one hand, the test requires that an adequate and sufficiently alive mycobacteria-containing clinical sample, usually sputum, can be obtained from the patients. This is often not possible, particularly in children and HIV patients, who either show too low bacterial load in the sputum or develop extra-pulmonary TB. Further, culturing methods last for an average of 1-3 weeks in liquid media and 3-8 weeks on solid media (26). Thus, they are very limited tools for the therapeutic monitoring of TB patients, since in the case of a negative culture growth up to eight weeks must be waited to obtain the final result. In addition, the predictive power of culture results for the occurrence of therapy failure or relapse is only unsatisfactory with 40% (28). Furthermore, the contamination with environmental organisms, e.g. NTM, particularly in Africa, is an additional differential-diagnostic challenge in the cultural detection of mycobacteria (29, 30). Finally, in particular, automated culture methods are expensive (cost more than ten euros per test) and also dependent on a functioning infrastructure (cold chain, electricity) and, not to forget, above-average trained staff.

The by far most widely and most frequently used method for the detection of mycobacteria in sub-Saharan Africa is the microscopy of sputum samples after staining, e.g. Ziehl-Neelsen staining or auramine staining (27). The advantages of this method are: (theoretical) independence from electricity, low costs and high specificity in symptomatic patients. Important disadvantages are, in

particular, the dependence on the investigator, the relatively high turnaround time required per sample, and the lack of sensitivity in specific subgroups (detection limit of 10^5 mycobacteria/ml of sputum) (31). The sensitivity of smear microscopy lays between 50-80% in adults with pulmonary TB (27). In children and HIV positive TB patients, it is significantly lower with 10% and 50%, respectively (32). Until recently, sputum microscopy was also used during follow-up of sputum-positive TB patients, to monitor anti-mycotic therapy in Africa. Because of the low predictive value of 24% for treatment failure (28), a positive sputum result at week eight of antibiotic therapy is currently no longer recommended as a basis for the extension of antibiotic therapy (intensive phase) by the WHO (33).

The molecular detection of TB, in particular by PCR, played only a subordinate role for the diagnosis in African countries by the end of 2010, as the lack of infrastructure in most TB laboratories did not allow the development and implementation of an in-house PCR method. The commercialized line probe assays from Hain Lifescience GmbH (Hain test), which were based on the detection of mycobacterial DNA by hybridization of molecular probes after amplification of mycobacterial DNA by PCR, were used in only a few African laboratories, especially for species determination as well as rapid resistance testing in positive cultures (27, 34). The sensitivity and specificity of the different line probe assays as well as other PCR-based methods can reach up to 100%, if at least 10^5 organisms are present in the diagnostic sample (27, 34). Accordingly, sensitivity was significantly reduced (77% to 88%) when the MTBDRplus test from Hain was applied directly to sputum samples from adults with pulmonary TB (35, 36). A sterile PCR lab is a requirement for the execution of the Hain tests, as well as appropriately trained staff. In addition, the tests´ costs are about 15-25 euros, depending on the test version, which is comparatively cost-intensive.

With the endorsement of the Xpert MTB/RIF test, developed by the Cepheid Inc and the Foundation for Innovative New Diagnostics (FIND), by the WHO in December 2010, the situation changed suddenly. It seemed, this new assay had the potential to change the accuracy and strategy of TB diagnosis on the African continent fundamentally and sustainably. The Xpert MTB/RIF test, which is run on the GeneXpert testing machine, is a completely automated PCR method (37), which can also be carried out reliably by clinical personnel (38). The test duration for one sample is two hours and includes, in addition to the detection of mycobacteria of the tuberculosis complex, the detection of resistance against rifampicin. The sensitivity in a first worldwide study reached 90% with a specificity of up to 99% (39). However, this was the only available data when the test was endorsed in 2010. One test costs about 10 US dollars in Africa. With a detection limit of approximately 130 DNA copies per ml of sputum, the Xpert MTB / RIF test should be significantly more sensitive than sputum microscopy. The clinical evaluation of the Xpert MTB/RIF test in various patient populations was a major scientific focus of this habilitation project.

A further diagnostic possibility that is specifically relevant in the countries of sub-Saharan Africa is the detection of mycobacterial antigen, especially lipoarabinomannan (LAM). LAM is a component of the cell wall of mycobacteria and is accordingly present in the sputum, blood and urine of tuberculosis patients (40). While the detection of LAM for diagnostic purposes in the sputum has not yet been successful (41), the TB diagnosis by LAM detection in the urine, especially in HIV-positive TB patients and possibly also in children, is considered an attractive alternative (40). In different studies, the reported sensitivity of LAM-detection in urine of adults was between 13% (42) and 67% (43), with best performance shown in HIV-positive TB patients with advanced immune-suppression (44). At the time point of the conduction of LAM-studies within this habilitation, no LAM- data were published yet for

children. If the first test generations were still relatively elaborate ELISA tests, Alere's urine-based LAM test is now available as a point-of-care rapid test. The evaluation of various LAM tests was also a scientific goal of this habilitation project.

Immunological test methods are mentioned here only for the sake of completeness, since they were (almost) not used as TB diagnostics in the various studies within this habilitation work. So-called IGRA test methods, which are based on the detection of interferon-gamma-secreting T-lymphocytes in the peripheral blood of TB patients, play, mainly due to their complexity in the execution and interpretation of test results as well as due to costs, outside the research no role in Africa. On the other hand, positive skin tests, so-called Mendel Mantoux skin tests, are used in children with TB suspicion in order to estimate the probability of presence of active TB. Like the IGRA tests from blood, the skin tests are also based on the detection of immunological cells, especially T-memory cells, which after stimulation with a TB-specific antigen migrate into the skin and trigger a localized delayed-type immune reaction (45).

1.4. Strategies for TB detection in specific populations in Africa

Since some studies within the framework of this habilitation project deal specifically with the detection of TB, or the evaluation of new test strategies, in children and prisoners, the following are brief descriptions of the specificities and challenges of TB diagnosis in these two risk groups.

In principle, the diagnosis of TB disease in children is comparably difficult, since children often present with atypical symptoms due to extra-pulmonary manifestations of the disease. In Africa, the diagnosis is further hampered by the presence of comorbidities such as malnutrition or HIV. In African countries, diagnostic scoring systems are frequently used in clinical practice (46-49), which, in addition to the clinical examination, also include the clinical history, the Mendel Mantoux skin test and radiological imaging. Unfortunately, almost all of these scores showed unsatisfactory diagnostic accuracy in various clinical trials (49, 50). The bacteriological detection of infection with mycobacteria, however, can only be achieved in a minority, often not more than 10%, of symptomatic children (27, 32). On the one hand, this is explained by the typically low bacterial load in children. On the other hand, sample collection is naturally associated with challenges in children and often not possible. Two different, semi-invasive procedures are available in Africa for sample collection in children with TB suspicion. The first one is the so-called sputum induction, where tracheal secretion is obtained by atomization of hypertonic saline solution via respiratory mask and subsequent nasal catheterization. Alternatively, the removal of sober-gastric juice by means of oesophageal catheterization is performed through the nose. Both methods show similar (low) success rates for the recovery of mycobacteria (51, 52). New diagnostic methods, based either on the use of alternative sample material, e.g. the urine for LAM testing, or with significantly higher sensitive than microscopy in sputum or gastric juice samples, e.g. the Xpert MTB/RIF test, are therefore extremely attractive candidates for clinical evaluation in children.

TB is an important health problem in prisons worldwide, and especially in Africa. Various studies have shown that TB prevalence is constantly higher in prisons than in the normal population (53-55). This has various causes in Africa. On the one hand, TB risk factors such as low quality or even shortage of food, "overcrowding", lack of ventilation and lighting and limited medical care are closely linked to the usual conditions of detention facilities in Africa (54, 56). On the other hand, (potential) prisoners per se are constituting a TB risk populations because of their often low socioeconomic status (57) and pre-

existing diseases such as HIV (58). Usually TB is diagnosed late in prisoners, e.g. if significant disease symptoms are present or the detection by sputum microscopy succeeds. More expensive and sensitive diagnostic methods, such as PCR or culture procedures, are often not available for prisoners in Africa. In a study conducted with Tanzanian prisoners, it was shown that 41% of the detainees who were presented in a hospital due to a relevant illness were actually affected by active TB (59). In addition to the understanding of specific risk factors and typical symptoms of active TB disease in prisoners, the development of cost-effective but effective screening tests or algorithms is necessary to quickly diagnose and treat TB in African prisons and by that prevent further spread of the bacilli to other prison inmates and to community members.

2. Scientific objectives

The first, and main, focus of this habilitation was the clinical evaluation of different new diagnostic tests (Xpert MTB/RIF tests plus two different urine LAM-tests), as well as testing strategies for the detection of TB in different study populations in Tanzania. The main objective was to investigate the extent to which the new diagnostics are capable of supplementing or replacing the standard procedures (microscopy and culture).

A second focus of this work was the investigation of various aspects related to TB prevalence among prisoners in Ethiopian detention facilities and the associated risk factors and transmission routes, which could guide future TB detection and TB control strategies in African prisons.

In a third approach, the isolated TB strains from Tanzanian and Ethiopian studies were analyzed using genotyping methods with the aim to further characterize MTB strains circulating in East Africa, to explain possible TB transmission routes in the study populations and to assess the virulence of various MTB strains in HIV-positive TB patients.

2.1. Evaluation of the molecular Xpert MTB/RIF assay in different Tanzanian study populations

For the clinical evaluation of the new, and automated, molecular Xpert MTB/RIF assay, the following research objectives were in the main focus:

- Description of diagnostic accuracy of Xpert MTB/RIF assay compared to culture as a reference standard in adults with suspected pulmonary TB and with and without HIV co-infection (60).
- Description of diagnostic accuracy of Xpert MTB/RIF assay compared to culture and/or a clinical classification as reference standard in children with suspected pulmonary TB and with and without HIV co-infection (61).
- Evaluation of Xpert MTB/RIF assay as TB screening method for household contacts of patients with smear positive pulmonary TB (62).
- Evaluation of the Xpert MTB/RIF test compared to smear microscopy and culture methods as a marker for therapy monitoring in smear positive TB patients (63).

2.2. Evaluation of various urine-based LAM assays for their diagnostic capacity in Tanzanian children

For the evaluation of different urine-based LAM tests in children, the following scientific aims were the in main focus:

- Description of diagnostic accuracy of urine-based LAM assays compared to culture as a reference standard in children with suspected pulmonary TB and with and without HIV co-infection (64).
- Description of factors that are associated with TB detection by urine LAM to define the preferred target (sub-) group for LAM-based TB diagnosis in children (64).

2.3. TB prevalence and risk factors in Ethiopian prisons

A second focus of this habilitation project was the investigation of TB prevalence and various risk factors related to TB among prisoners in Ethiopian prisons. The following questions were addressed:

- What is the TB prevalence in Ethiopian prisons, and
- What are the key risk factors for the diagnosis of active TB in Ethiopian prisons (65)?

2.4. Genotypic characterization of isolated TB strains from research studies from Ethiopia and Tanzania

Finally, the isolated TB strains from the different Tanzania and Ethiopian studies were further characterized using genotyping methods. The following objectives were addressed in two sub-studies:

- What are the characteristics of TB strains isolated from Ethiopian prison inmates compared to those from the normal population? Which conclusions can be drawn concerning the TB transmission routes in the Ethiopian population and prisons (66)?
- Is there an association between specific MTB genotypes and the degree of immune-suppression in ART-naïve individuals diagnosed with active pulmonary TB and HIV coinfection in Tanzania (67)?

3. Scientific projects

3.1. Research studies performed at NIMR-Mbeya Medical Research Center (NIMR-MMRC)

The data for the evaluation of the TB diagnostic tests, namely the Xpert MTB / RIF assay and the urine-based LAM tests, were originating from several clinical research studies conducted at the NIMR-MMRC (National Institute of Medical Research, Mbeya Medical Research Center) in collaboration with the Department for Infectious Diseases and Tropical Medicine of the LMU. Most of these studies have been carried out and/or supervised by me as Head of the Department of Clinical Tuberculosis Studies at NIMR-MMRC, from 2009 to 2012. In addition to the coordination and supervision of data collection in the clinic and in the tuberculosis laboratory, I contributed to the development of the various student designs. The processing of the data as well as the data analysis I performed either independently or in close cooperation with coauthors, and as supervisor of the master thesis of Dr. Nyanda Elias Ntinginya.

3.1.1. "Evaluation of transrenal-DNA detection to diagnose tuberculosis (TB Tr-DNA)"

The TB Tr-DNA diagnostic study was performed at the NIMR-MMRC in Tanzania to develop a new PCR-based test that is suitable for detecting pulmonary tuberculosis in the urine of symptomatic patients. The main clinical study was developed by scientists from LMU, the NIMR-MMRC and the University College, London, and completed by early 2009. In short, approximately 300 patients with TB suspicion were recruited from July 2007 to September 2007 in order to determine the diagnostic performance of the urine-based PCR tests. In addition to urine samples, sputum was also collected and used as a reference standard for the diagnosis of pulmonary tuberculosis by means of microscopy and culture. The collected sputum samples were frozen at -80°C for future studies. The isolated TB strains were sent to the German reference laboratory in Borstel for further analysis.

For the evaluation of the Xpert MTB/RIF test, the frozen sputum samples of the TB Tr-DNA study were systematically used to investigate the diagnostic capacity of the new Xpert test, between January 2010 and March 2010. The planning of the study, the performance of the test in the laboratory, as well as the data entry and data processing on the NIMR-MMRC took place under my supervision. I carried out the data analysis together with the statistician of our working group (Dr. Elmar Saathoff) and my then working group leader (Prof. Michael Hoelscher) at LMU. The study results were subsequently published in PLosOne in 2011 (60).

In a further sub-study, the isolated TB strains were used to investigate the correlation between the detection of different genotypes and the degree of immunosuppression in HIV-positive TB patients. The TB Tr-DNA cohort (as well as the REMox TB cohort, see below) was particularly well suited for this analysis because of the high proportion of HIV-positive subjects with TB. I was involved in the development of the study design. I carried out the data analysis together with a colleague (Dr. Ioana Olaru) and a statistician from Research Center Borstel. The data were published in the International Journal of TB and Lung Diseases in 2014 (67).

3.1.2. "Active Detection of Active TB (ADAT) in Children"

Within the frame of the multi-site ADAT project, a prospective cohort study was carried out at NIMR-MMRC, between May 2008 and November 2010, in approximately 200 children with clinical suspicion of TB. The aim was to evaluate various new diagnostic TB tests in children against culture or a clinical reference standard for TB diagnosis, among those the Xpert MTB/RIF assay and two different urine-based LAM assays as part of this habilitation project. In order to observe the clinical course with or

without TB therapy (as a possibility of indirect TB diagnosis in children with negative TB culture) or to be able to make alternative clinical diagnoses in the further course (thus exclusion from TB), the children were examined up to one year after inclusion in the study. As of May 2009, I was involved in the supervision of data collection (clinical data and laboratory data). I was also involved in the clinical care of the children. As the basis for the data analysis and evaluation of all diagnostic tests, I defined the final (TB) diagnosis and/or alternative diagnoses of the study participants with my medical colleague at NIMR MMRC (Dr. Petra Clowes), taking into account clinical, radiological and microbiological data. I continued to supervise data management and was involved in the statistical analysis of the study data. The results of this research were published in Clinical Infectious Diseases in 2012 (61) and in the European Respiratory Journal in 2015 (64).

3.1.3. “TB screening in household contacts of pulmonary TB patients using Xpert MTB/RIF assay”

As the local supervisor of the MSc thesis of Dr. Nyanda Elias Ntinginya (MD), I was decisively involved in the conceptual design, as well as in the data collection and analysis of this Xpert MTB/RIF evaluation study. Two hundred and nineteen household members of 80 sputum smear-positive TB patients were recruited and examined for the presence of TB bacteria in their sputum, using smear microscopy, MTB culture and Xpert MTB/RIF test between April 2011 and June 2011. The results were published in the International Journal of Tuberculosis and Lung Diseases in 2012 (62).

3.1.4. “Controlled comparison of two moxifloxacin containing treatment shortening regimens with the standard regimen in pulmonary tuberculosis: REMox TB”

This study was a placebo-controlled, double-blinded therapeutic study in which moxifloxacin was evaluated as a component of shortened antibiotic TB combination therapy compared to standard therapy (2 months rifampicin, isoniazid, ethambutol and pyrazinamide plus 4 months rifampicin and isoniazid) (68). It was a multicenter study funded by the EDCTP and the TB Alliance, which was carried out among other sites at the NIMR-MMRC from March 2009 until March 2014. In my role as head of the Department of Clinical Tuberculosis Studies, I was responsible for the coordination and supervision of the study in the field of clinical, laboratory and data collection as well as for the medical care of the study patients on site.

As a sub-study, together with my working group leader at LMU (Prof. Michael Hoelscher) and our colleagues in Cape Town (Prof. Andreas Diacon, Dr. Sven Friedrich), we developed the idea of an evaluation study for the Xpert MTB/RIF as a therapeutic marker for pulmonary TB patients. For this purpose, the frozen sputum samples of approximately 260 REMox TB patients from Mbeya (Tanzania) and Cape Town (South Africa) were tested systematically by the Xpert MTB/RIF assay for various study time points up to 1.5 years after TB diagnosis in order to analyze the capacity of Xpert MTB/RIF assay to monitor TB treatment response compared to standard diagnostics. In addition to the concept of the study, I was involved in data processing and analysis (together with Dr. Elmar Saathoff). The study was published in Lancet Respiratory Medicine in 2013 (63).

3.2. Prison studies in Ethiopia

From December 2012 to May 2015, the Department for Infectious Diseases and Tropical Medicine of the LMU, conducted an EDCTP-sponsored multicenter study at various African study centers. The project was called "Epidemiology of PZA resistance in TB Clinical trials in Africa - an essential prerequisite for evaluating novel TB drug combinations" and had the main goal of investigating the

occurrence of drug resistance, with major focus on pyrazinamide resistance, in different study populations in Africa. I supervised this multi-center study as co-investigator and scientific co-ordinator at the Department for Infectious Diseases and Tropical Medicine of the LMU. Within the scope of this project, new TB studies or TB study sites were setup in order to expand the study population to as many African countries as possible. One of these newly initiated research projects was a tuberculosis jail study, which was conducted in 13 Ethiopian zonal prisons and covering more than 15.000 incarcerated persons from January 2013 until December 2013. Mr. Ali Mohammed, who initiated and supervised the study at the ground in Jimma, Ethiopia, was enrolled as a PhD student at the Center for International Health (CIH) at the LMU since October 2012 and I supervised his PhD work as a direct supervisor. In this function, I was involved in the conception of the prison study as well as in the development of the methodology. Later, I supervised the statistical evaluation and the publication of the data in 2015 and 2016 (65, 66).

4. Results and interpretation

4.1. Evaluation of the diagnostic performance of Xpert MTB/RIF assay in Tanzanian adults with clinical suspicion of TB and high HIV-coinfection rate

A retrospective, cross-sectional diagnostic evaluation study was performed, using bio-banked sputum samples, which were stored from all participants of the Tr-DNA study (see paragraph 3.1.1 above). In this study, 292 adults were consecutively enrolled, who presented themselves with clinical suspicion of active TB to the TB study clinic at NIMR-MMRC or to another collaborating health center. From all 292 subjects, three sputum samples (two spot sputum samples and one morning sputum sample) were collected for the diagnosis of TB, using smear microscopy and culture methods on liquid and solid media, and consecutive storage. Later, the same, thawed sputum samples were used for Xpert MTB/RIF evaluation. For the calculation of the diagnostic performance of Xpert MTB/RIF test, culture confirmed TB was used as reference standard.

Among the 69 (23.6% of 292) participants with microbiologically confirmed TB, the sensitivity of Xpert MTB/RIF reached 88.4% (95%CI: 78.4%-94.9%). In those 103 (35.3% of 292) participants in whom active pulmonary TB disease could be definitely excluded, the specificity was 99.0% (95%CI: 94.7%-100%). Of note, there was a significant difference in the sensitivity of the Xpert MTB/RIF test in sputum smear positives versus sputum smear negatives, with 98.0% (95%CI: 89.6%-100.0%) versus 61.1% (95%CI: 35.7%-82.7%), respectively ($p< 0.001$). There was no difference in the performance of the Xpert MTB/RIF assay among HIV-positive and HIV-negative study participants (HIV-prevalence in study: 58.9%, 95%CI: 45.0%-89.9%). The Xpert MTB/RIF correctly identified seven (9.1%) additional TB patients in the group of 77 (26.4% of 292) participants who had a clinical TB diagnosis (all of them were treated for TB and showed clinical improvement within eight weeks follow up) but were negative for TB in sputum culture. All of these seven patients were HIV-coinfected and were Xpert MTB/RIF positive in multiple sputum samples. None of them had a history of TB in the past. Interestingly, there was no significant difference in Xpert MTB/RIF sensitivity when only one morning sputum or three sputa (1 morning sputum plus 2 spot sputa) were tested. Finally, we could show a good correlation of quantitative Xpert MTB/RIF readouts (CT-value) with smear microscopy grade and time to positivity (TTP) in liquid culture (MGIT).

This study provided first information on specific diagnostic performance characteristics of Xpert MTB/RIF assay, which were also relevant for the designs and objectives of following evaluation studies and were later confirmed by other studies from developing countries. First, Xpert MTB/RIF assay could be run under field conditions without any technical problems. Second, Xpert performed equally well in HIV-positive and HIV-negative TB suspects. Third, only one sample needed to be tested to reach maximal sensitivity of the assay in adults. And finally, Xpert MTB/RIF detected additional TB cases who were M.tb culture negative. However, this study also indicated that the sensitivity of the Xpert MTB/RIF test is significantly reduced in the group of smear negative TB patients.

4.2. Evaluation of the diagnostic performance of Xpert MTB/RIF assay in a Tanzanian children cohort with clinical suspicion of TB and high proportion of HIV-coinfection

To evaluate the diagnostic accuracy of Xpert MTB/RIF in children and estimate the impact of the test on time to diagnosis and anti TB treatment initiation we used the sputum samples from 164 children with presumed TB who were enrolled in a prospective cohort and were followed for a minimum time

of 12 months. Sixty nine (42.07% of 164) children were hospitalized at recruitment, the remaining children were recruited from the ambulant sector. Up to three sputum samples (induced and not induced sputum samples) from each participant were collected at recruitment for TB diagnosis using smear microscopy, solid and liquid culture and Xpert MTB/RIF test.

All children, in whom active TB disease could be reliably excluded, were also detected as negative by Xpert MTB/RIF, resulting in a specificity of 100%. Xpert´s sensitivity was 75% (95%CI: 55.1%-89.3%) with 21 detected children out of 28 children with culture confirmed TB. Among the seven smear positive children sensitivity was even 100% (95%CI: 59.0%-100.0%), while Xpert MTB/RIF detected only 66.6% (95%CI: 43.0%-85.4%) of smear negative children (14 out of 21). In the per sample analysis of 77 sputum samples which were collected from the 28 children with culture confirmed TB, the sensitivity of smear microscopy, solid culture, liquid culture and Xpert MTB/RIF was 19.5%, 55.3%, 54.2% and 54.2%, respectively. Notably, Xpert MTB/RIF test detected four additional TB cases (sensitivity of 8.5%) among 47 children with highly probable clinical TB diagnosis. Testing a second and third sputum sample increased the overall sensitivity of Xpert MTB/RIF by 20% and 16%, respectively. Importantly, the chance of a Xpert positive sputum result was increased in children of older age and more advanced disease, while the HIV-status (cohort prevalence: 51.2%) played no significant role. Median time from enrolment to TB diagnosis for smear, Xpert (retrospectively calculated), liquid culture and solid culture was 1 day (range 1-3), 2 days (range 1-12), 21 days (range 11-59) and 30 days (range 14-79), respectively. Initiation of anti TB treatment was delayed by up to 59 days in 9 out of 28 children with culture confirmed TB and two children with confirmed TB were lost while the culture result was pending. As Xpert MTB/RIF results did not inform treatment decision it could only be speculated that Xpert-based TB diagnosis could not only shorten time to diagnosis but also reduce time to treatment start and number of children untreated for TB.

The diagnostic performance of Xpert MTB/RIF in children with culture confirmed TB was comparable to that found in adults, namely with a significant difference in sensitivity among smear-positive and smear-negative TB cases. Like in adults, HIV-status played no role for Xpert MTB/RIF performance in children. Most important in this study was to show that Xpert tripled the number of children (25 children with positive Xpert MTB/RIF results) with a potentially rapid TB result compared to when only smear microscopy (7 smear positive children) would be available. Opposed to results from adult studies, we showed, however, that up to three sputum samples need to be tested to reach the maximal sensitivity of the test.

4.3. Evaluation of the diagnostic performance of Xpert MTB/RIF in an active case finding strategy

The aim of this cross-sectional study was to access the feasibility and accuracy of Xpert MTB/RIF assay in the context of TB contact tracing among household contacts of smear-positive TB cases.

Two hundred and nineteen (219) household contacts, who were living in the same house or plot and shared meals together with 80 smear-positive index cases, were enrolled in this study. Out of these, 33 contacts could provide up to two spot sputum samples (56 sputum samples in total). All samples were tested for TB with culture methods (MGIT and LJ), smear microscopy and Xpert MTB/RIF assay. In total, five household contacts were M.tb culture positive, resulting in a TB prevalence of 2.3% (95%CI: 0.7-5.2%), which is equivalent to 2.300 TB cases per 100.000 house hold contacts and a number of 43.8 (95%CI: 19.1-134.2) needed to be screened to detect one TB case. Among those, who could provide at least one sputum sample, the proportion of TB-positives was 15.2% (5/33, 95%CI: 5.1%-

31.9%), the number to be screened to detect one case was 6.6 (95%CI: 3.1-19.6) and the point prevalence was 15.200 TB cases among 100.000 households contacts with sputum production. Xpert MTB/RIF detected all culture-confirmed cases, 5 out of 5, resulting in a sensitivity of 100% (one-sided 97.5%CI: 47.8%-100%). The sensitivity of smear microscopy was 60% (3/5, 95%CI: 14.7%-94.7%). In all TB cases already the first sputum sample collected was tested positive by Xpert MTB/RIF assay. No additional cases nor rifampicin-resistance were detected by Xpert.

Despite the small study size, the data indicated that Xpert MTB/RIF testing is a feasible, fast and accurate method for TB tracing among household contacts. Further, the high sensitivity compared to sputum culture and the challenge of low volume (~ 1ml) of many sputum samples support the use of the Xpert MTB/RIF as a single diagnostic test. Especially in this cohort, with a high proportion of asymptomatic and paucibacillary TB cases the Xpert MTB/RIF was shown to be superior to smear microscopy. The cost effectiveness and the impact of TB contact tracing using Xpert MTB/RIF has to be proven in greater studies and also for further settings.

4.4. Assessment of the Xpert MTB/RIF assay as a sputum biomarker of response to TB treatment

In the absence of a useful biological biomarker for the monitoring of response to anti TB treatment, we evaluated the diagnostic accuracy of Xpert MTB/RIF assay against sputum culture and smear microscopy in a clinical cohort of smear-positive TB patients who were enrolled in the REMox TB trial, a randomized placebo controlled phase III therapeutic drug trial (ClinicalTrials.gov, NCT00864383).

In this substudy, 2741 sputum samples were analysed which were collected from 221 patients over a treatment period of 26 weeks. While only 29% (62 out of 212), 26% (46 out of 176) and 42% (77 out of 183) of samples were positive for smear, LJ and MGIT, respectively, at week eight after TB treatment initiation, 84% (174 out of 207) of samples were still positive for Xpert. Further, 5% (10 out of 199), 3% (4 out of 157) and 4% (7 out of 169) of samples were positive for smear microscopy, LJ and MGIT, respectively, at the end of TB treatment, while 27% (22 out of 83) were still positive with Xpert MTB/RIF assay. Compared to smear microscopy and culture methods as combined reference standard, Xpert MTB/RIF had a high overall sensitivity of 97.0% (95%CI: 95.8%-97.9%) but poor specificity of 48.6% (95%CI: 45.0%-52.2%). The quantitative readouts of Xpert MTB/RIF (CT-value), smear microscopy (positivity grade), LJ (positivity grade) and MGIT (TTP) correlated very well until week 8 of treatment, with poorer correlation thereafter, resulting in an overall correlation coefficient of -0.74, -0.73 and 0.73 for smear microscopy, LJ and MGIT, respectively.

During the time point of data analysis for this substudy, the main REMox TB Trial was still ongoing. To avoid interference with the endpoints and main analysis of the main treatment trial, the sample analysis with Xpert MTB/RIF assay was restricted until week 26 and did not include TB treatment outcome data at week 26. Further, all investigators were masked to the treatment allocations of the patients included when this substudy was published. The poor specificity of Xpert MTB/RIF assay precludes the use of the test as biomarker for treatment response in its current format. It is open to show whether modifications of the sample processing and testing protocol could avoid the detection of DNA related with destroyed or non-viable mycobacteria. Further data analysis and additional studies are awaited to assess whether a positive Xpert MTB/RIF result at the end of TB treatment or a high bacterial burden measured at treatment beginning can predict treatment failure or relapse.

4.5. Analysis of performance of urine-based LAM-assays in a Tanzanian paediatric cohort with presumed TB and high proportion of HIV-coinfection

To evaluate the diagnostic accuracy of two different urine based LAM-assays (MTB-LAM ELISA assay from Chemogen and the Determine TB-LAM strip test from Alere) in children with TB symptoms, and to assess patient characteristics which might be associated with a positive LAM-result, we used urine samples from the same paediatric cohort, with an HIV-prevalence of 51%, which was enrolled to evaluate the performance of Xpert MTB/RIF assay in sputum samples (see paragraph 3.1.2 and 4.2 above).

Out of 132 children who were included in the final analysis, 18 (13.64% out of 132) were diagnosed with culture confirmed TB and 36 (27.27% out of 132) children were classified as highly probable TB cases. In those with culture confirmed TB, the sensitivity of the ELISA assay was 44% (95%CI: 22%-69%) and that of the strip test was 28% (95%CI: 10%-54%). In the subgroup analysis of HIV-positive confirmed TB cases, the sensitivity was 70% (95%CI: 35%-93%) and 50% (95%CI: 19%-81%) for the ELISA and strip test, respectively, while it was 13% (95%CI: 0%-53%) for the ELISA test and 0% (95%CI: 0%-37%) for the strip test in the subgroup of HIV-negative culture confirmed TB cases. Both test found additional TB cases, 6 (17%) out of 36 detected by ELISA assay and 2 (6%) detected by the strip test, in the group of children with highly probable TB. Both tests provided negative result for all children in whom active TB could reliably excluded, resulting in a specificity of 100% (95%CI: 84%-100%). A positive LAM ELISA result was highly and independently associated with HIV (p=0.04), proteinuria (p=0.019), low BMI (p=0.02) and one year mortality (p=0.032). In all but one (positive until month 7) children with a positive LAM result at the beginning of anti-TB therapy the test turned negative after three months of TB treatment.

Opposed to sputum samples in children, urine samples are a relatively easy to collect specimen and are therefore an attractive target for new diagnostic assays specifically designed for diagnosis of childhood TB. This study indicated that specifically HIV-positive children with presumed active TB infection might benefit from urine LAM-testing, especially in resource limited settings where culture methods and even Xpert MTB/RIF assay might not be available. This assumption is further supported by the fact that those cases with advanced disease (proteinuria, low BMI, high mortality) seem to be more likely to be detected by LAM-tests and thus, could benefit from an early TB diagnosis and treatment initiation. The observation of LAM-conversion during TB treatment among those with a positive test at baseline was shown for the first time in children and calls for further studies to explore the capacity of LAM-assays to monitor TB treatment in children.

4.6. TB prevalence and associated risk factors in Ethiopian zonal prisons

A cross-sectional research study was conducted in 13 zonal prisons in South Ethiopia, Southwest and Southeast Ethiopia, from January 2013 until December 2013. The WHO TB symptom screening algorithm was applied (69) to identify prisoners with high probability levels of TB. Those with a positive screening result, were asked to provide two sputum samples for smear and microscopy and MTB culture on solid media (LJ). A standardized questionnaire was used to collect information on risk factors from all prisoners with collected sputum samples.

In total, 15.495 inmates were included in the TB symptom screening, out of whom 765 prisoners (4.9%, 95%CI: 4.6%-5.2%) had TB symptoms and were screened for TB by microscopy and culture. Among the 765 TB suspects, 51 (6.67%) were already on TB treatment and 20 (2.8%) new TB cases could be found. This results in a TB prevalence of 9.2% (71/765; 95%CI: 7.2%–11.4%) among TB suspects and of 0.46%

(71/15.495; 95%CI: 0.35–0.57) among all prisoners. There was a great range in TB prevalence among the different prisons and Ethiopian states: the highest TB prevalence was observed in Dilla prison (SNNPRS) with 1.528 cases per 100.000 inmates (1.53%). Among the different regional states the SNNPRS had the highest tuberculosis burden with an overall prevalence of 618.8 (95%CI: 420–820) per 100.000 inmates (0.62%). We found a linear trend in prevalence of tuberculosis with advancing distance of the prisons from the centre of Ethiopia (Addis Ababa). Prisons within a radius of below 200km distance from Addis Ababa had the lowest TB prevalence of 97.98 (95%CI: 10–210) per 100.000 inmates while the highest TB prevalence of 804 (95%CI: 580–1020) per 100.000 inmates was observed in prisons located more than 400km away from Addis Ababa, (OR = 3.60, 95%CI: 2.24–5.70, p<0.0001). Apart from that, additional risk factors which were significantly associated with TB were presence of window in prison cell (AOR: 0.26, 95%CI: 0.16–0.45, p-value: <0.001), alcohol consumption in the past (AOR: 2.04, 95%CI: 1.20–3.46, p-value: 0.008) and contact with a TB case before imprisonment (AOR: 1.49, 95%CI: 1.08–2.06, p-value: 0.02).

Our research results indicated, that the average TB prevalence in Ethiopian prison inmates is twice higher than the prevalence in the general population, although a great variability of prevalence among different prisons existed. This variability, the fact that only 28% of TB cases were newly diagnosed (20 out of 71), and the TB risk factors found in this study suggest that the presence of TB in Ethiopian prisons is associated to both, factors inherent to the prisons (implementation of TB prevention methods, TB diagnosis and treatment) and also inherent to TB patients (contact to TB case, alcohol consumption). The results of this study support the notion that TB epidemics in communities and prisons are closely interrelated. Risk factors of both groups, prisoners and communities, needs to be addressed in order to control TB sufficiently at the population level.

4.7. Further characterization of MTB strains isolated from Ethiopian and Tanzanian populations

The MTB strains, which were isolated within the different research studies in Ethiopia and Tanzania were further analyzed in the frame of various research collaborations of the Department for Infectious Diseases and Tropical Medicine of the LMU, Germany, with the Research Center in Borstel, Germany.

In the first study, Ethiopian MTB strains from prisoners and hospitalized community members were analysed and compared. In total, for 109 MTB strains (21 strains from prisoners and 88 strains from hospitalized TB patients) the phenotypic drug resistance pattern and genotyping results (24 loci MIRU-VNTR and spoligotyping) were available. The majority (27.52%) of the strains belonged to Ethiopia_H37RV like, followed by Ethiopia_3 (16.51%) and Delhi/CAS (16.51%) lineages and there was no statistical difference between prisons and communities with regards to the diversity and pattern of MTB genotypes. However, the genotype patterns from different Ethiopian regions were differing significantly. In the cluster analysis, 12 clusters of two to eight strains of size were identified with a total clustering rate of 31.19%. Interestingly, clustering rates were not different between prisons and communities but, again, between different regions and different linages. Of note, two clusters included MTB strains from prisoners and community members. Clustering was not associated with drug resistance. Further, MDR prevalence in community isolates was 2.27% (2 out of 88) whereas it was 9.52% in prison isolates (2 out of 21, with one isolate from an inmate with previous history of TB), however, this difference was not statistically significant, (p = 0.112). In conclusion, this study suggested that the appearance of TB in prisons and communities are closely interrelated: MTB strains from both settings share the same genotypic pattern and clustering rates and even build the same clusters, thus, prisons as an integral part of civil societies need to be included in NTP strategies aiming for TB control.

In the second study, we investigated the association of TB infection with specific MTB linages, characterized by spoligotyping and MIRU-VNTR, and the degree of immune- suppression measured by circulating CD4 lymphocytes. The hypothesis was, that ART-naïve HIV-infected individuals with mild immune suppression develop active TB disease, which is caused by more virulent MTB strains compared to those HIV-infected TB patients with severe immune suppression. One hundred and twenty nine MTB strains, which were collected in different research studies, performed at the NIMR-MMRC, were included in this project. Sixty (46.5%) participants had advanced immune-suppression (CD4 count </= 200/mm^3). The majority of TB strains (n=55; 42.6%) belonged the Latin American Mediterranean (LAM) lineage, followed by Delhi/Central-Asian (CAS) (n=37; 28.7%) lineages. There was no significant association between the type of lineages identified (in total 8 different lineages) and the level of immune-suppression in HIV-positive TB patients. The most likely explanation for this finding was that all identified lineages have comparable in vivo virulence in HIV-infected individuals.

5. Conclusions

New diagnostics are needed to control TB globally and, specifically, in Africa. Especially, for particular sub-Saharan African risk populations, such as children, HIV-positives or prisoners, where the current standard diagnostics are either lacking diagnostic accuracy or are not feasible and affordable, innovative testing strategies are required to increase the number of microbiologically confirmed and correctly treated TB cases.

The endorsement of the automated molecular Xpert MTB/RIF assay by WHO in December 2010 hold promise to fundamentally change TB diagnosis (and diagnosis of MDR-TB) in African and other resource-limited settings. This habilitation project contributed to the clinical evaluation of Xpert MTB/RIF in African populations, mainly by the assessment of the assay´s diagnostic accuracy in various study populations and context. The main test characteristics summarized by the research results of this habilitation project were confirmed by different authors and are also reflected by the current global testing strategy for Xpert MTB/RIF recommended by WHO (70). Precisely, Xpert MTB/RIF should preferably applied in adults and children with HIV-associated TB. Further, the WHO recommendations support the use of a single sputum sample for TB diagnosis with Xpert MTB/RIF (70). As the Xpert MTB/RIF evaluation studies demonstrated in this project were mostly performed retrospectively the derived conclusion were limited to diagnostic accuracy parameters. Therefore, the calculated estimates related to impact on patient level (time to diagnose and treatment initiation, TB-related morbidity and mortality) need to be interpreted with caution and may vary between different settings. Further, while generalizability of diagnostic performance results was confirmed by several studies from other African and resource limited settings, the final judgement on Xpert MTB/RIF assay as marker for TB treatment response and, even more important, for treatment outcome is still outstanding. Additionally, although cost-effectiveness compared to previous diagnostic algorithms could be shown for Xpert MTB/RIF assay in different studies (70), the assay´s impact on patient-relevant outcome parameters (morbidity and mortality) was not yet sufficiently investigated (38). Further, the epidemiological impact (e.g. TB incidence and prevalence) of the implementation of this assay is still to be proved.

Opposed to Xpert MTB/RIF assay, the role of urine-based LAM-assays in the diagnosis of TB is still not defined. This is mainly due to the limited scientific evidence on the diagnostic accuracy and impact of the application of LAM-assays in different populations (71). E.g. apart from the publication resulting from this habilitation project there was only a maximum of four further studies published on the evaluation of LAM assays in children in 2015 (71). Further, the majority of available studies were either not comparable or of poor quality (71). Current WHO guidelines (for adults and children) do not suggest to use the LAM test as a single test for TB diagnosis. However, the assay should be considered to support TB diagnosis in HIV-positive TB subjects who are severely ill. The data of this habilitation project support this strategy, showing the highest sensitivity in HIV-positive TB cases and those with advanced morbidity and high mortality. Although, a reduction in time to treatment initiation and, by that, also, in mortality, could be shown for LAM strip test in one prospective study with adult, hospitalized and HIV-positive TB suspects (72), further research is needed to better define the testing strategy, clinical setting(s) and the target populations for this test.

The presented data from Ethiopia confirmed that TB in African prisons is a relevant health problem and poses a relevant threat to TB control in communities. Even with using a relative insensitive TB

screening method (strict symptom screening (69), followed by smear microscopy plus sputum culture on LJ in symptomatic subjects) TB prevalence in Ethiopian prisons was twice higher than in the normal population. In addition to well known risk factors for TB in prisons (e.g. alcohol consumption, circulation of air) our data provide further evidence for the interrelation of the two TB epidemics, inside and outside prison facilities. Precisely, active TB cases in prisons were more likely to have had contact with a TB case before imprisonment compared to healthy prison inmates, and the genotypic analysis revealed that TB strains from inside prison facilities build clusters together with TB strains from the normal population indicating that they were sharing the same source of TB infection. Prisoners are usually originating from vulnerable groups of society, e.g. drug and alcohol dependent, poor, homeless or mentally ill people, etc., who have per se a high risk for TB. In detention facilities, they meet an environment which amplifies that risk by malnutrition, overcrowding and extremely poor housing conditions. Therefore, active TB screening among prisoners, including both those who newly enter detention facilities and those who stay in prisons for a longer time period, should be scaled up and empowered by better staff and improved diagnostics, as it has a high potential to detect many new TB cases among those community members who carry the greatest TB risk but are considered difficult to reach by common screening programmes and by that improve TB control in sub-Saharan Africa.

Abbreviations and acronyms

ADAT	Active Detection of Active TB
AOR	Adjusted Odds Ratio
CI	Confidence Interval
CIH	Center for Internal Health
CT-value	Cycle Threshold-value (in PCR)
DNA	Deoxyribonucleic Acid
DOTS	Direct Observed Treatment, Short Course
EDCTP	European & Developing Countries Clinical Trials Partnership
e.g.	exempli gratia
FIND	Foundation for Innovative New Diagnostics
HIV	Human Immunodeficiency Virus
IGRA	Interferon Gamma Release Assay
LMU	Ludwig-Maximilians-Universität
LAM	Lipoarabinomannan
MD	Medical Doctor
MDG	Millenium Development Goals
MGIT	Mycobacteria Growth Indicator Tube
Mio	Million
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeats
MMRC	Mbeya Medical Research Center
MTB	Mycobacterium Tuberculosis
NIMR	National Institute of Medical Research
NTM	Nontuberculous Mycobacteria
NTP	National TB Program
OR	Odds Ratio (not adjusted)
PCR	Polymerase Chain Reaction
RIF	Rifampicin
SDG	Sustainable Development Goals
TB	Tuberculosis
TR DNA	Transrenal Deoxyribonucleic Acid
TTP	Time To Positivity
US	United States (of America)
WHO	World Health Organisation

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Original articles

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Hiermit versichere ich an Eides Statt, dass ich die schriftliche Habilitationsleistung selbständig verfasst und die Herkunft des verwendeten oder zitierten Materials ordnungsgemäß kenntlich gemacht habe.

Des Weiteren erkläre ich, dass ich noch kein Habilitationsverfahren im gleichen Fach ohne Erfolg beendet habe, mir kein akademischer Grad entzogen worden ist und auch kein Verfahren gegen mich anhängig ist, dass die Entziehung eines akademischen Grades zur Folge haben könnte.

München, 24. März 2018

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