Tuberculosis in South and Central Africa

Understanding epidemiology - Improving diagnosis and management

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Chapter 1

A brief introduction to tuberculosis
History and pathogen

Tuberculosis is an infectious disease predominantly caused by the pathogen *Mycobacterium tuberculosis*. Today tuberculosis continues being one of the globally most deadly infectious diseases, tuberculosis has cost more lives than any other infectious disease in the past [1].

Tuberculosis is an old disease, it has pestered humans throughout history and also human pre-history [2]. Evidence of spinal tuberculosis by means of characteristic osseous changes, such as collapse of the vertebrae, has been found in neolithic skeletons [3] and human tuberculosis in a population originating from one of the first settlements with agriculture and domestic animals could be found by molecular methods [4]. Names used for tuberculosis disease throughout history and before the discovery of the etiologic agent were amongst others consumption, phthisis, scrofula, Pott’s disease, and White Plague.

In 1882 Robert Koch, a Prussian physician, discovered the “tubercle bacillus”, i.e. *M. tuberculosis*, as infectious agent of tuberculosis by means of applying a new stain to sputa of diseased patients [5]. Today it is known that *M. tuberculosis* is an aerobic, non-motile, slow-growing, rod-shape bacterium belonging to the family of *Mycobacteriaceae* and genus *Mycobacterium*. Much of the pathogenic success of *M. tuberculosis* is due to its particular cell wall, which comprises three categories of macromolecules – peptidoglycan, arabinogalactan, mycolic acids – and a surrounding capsule [6]. Most tuberculosis cases are caused by *M. tuberculosis sensu stricto*, but other members of the *M. tuberculosis complex* (MTBC; e.g. *M. africanum* or *M. bovis*) are also causative agents of human tuberculosis [7]. Advances in molecular techniques have revealed further genetic diversity within the MTBC, which may account for varying tuberculosis transmission and clinical presentations of disease [8].

Besides hygiene improvement and rising standards of living, accounting for the largest reduction in tuberculosis prevalence in West Europe, further man-made important landmarks in the control of tuberculosis were the development and use of the BCG (*M. bovis* bacillus Calmette–Guérin) vaccine in 1921, the discovery of streptomycin and para-aminosalicylic acid as the first antiinfective drugs against tuberculosis in 1944, the value of combination regimens to delay development of drug resistance in 1952, the quadruple short course therapy in 1980, and in the early 1980ies the recognition of the effect of the HIV/AIDS epidemic on tuberculosis [9].

Clinical aspects

Tuberculosis is an airborne infectious disease that is usually transmitted from human to human via cough aerosols of *M. tuberculosis*. Following exposure to *M. tuberculosis* the host can typically react by either eliminating the bacteria, suppressing the pathogen in a latent state (LTBI, latent tuberculosis infection), developing
subclinical tuberculosis, or progressing to tuberculosis disease which can be mild to life-threatening [7].

Almost a quarter of the global population is estimated to have LTBI [10]. This large clinically unapparent reservoir which can progress to development of active tuberculosis constitutes an important challenge for control and elimination of tuberculosis. Active tuberculosis develops in 5 –10% of individuals with LTBI during their lifetime; in children and patients with immune compromising conditions this rate is higher [11].

If tuberculosis disease develops immediately after infection without a prior interval of LTBI, it is considered primary tuberculosis; if tuberculosis disease is due to activation after a period of LTBI it is considered secondary tuberculosis. With active tuberculosis disease patients suffer general symptoms, such as fever, exhaustion, and/or loss of weight [7]. Localized symptoms depend on the site of tuberculosis disease. Patients with pulmonary disease, the most common form of tuberculosis disease, can have chronic cough, haemoptysis, or breathing difficulties. Although tuberculosis is primarily a pulmonary disease, tuberculosis can affect any part of the body. Symptoms due to extra-pulmonary disease depend on the affected organ or anatomical site (e.g. tuberculous meningitis usually manifests with neurological symptoms). Tuberculosis disease can also be disseminated and affect multiple body sites; disseminated tuberculosis often presents nonspecifically and has a high mortality rate [12].

HIV infection is the most pronounced established risk factor for tuberculosis disease [7, 13]. Other known factors that increase the risk of tuberculosis are diabetes mellitus [14], heavy alcohol use and alcohol use disorders [15], and tobacco smoke [16]. Poverty markers, such as crowded living conditions, malnutrition, and exposure to indoor air pollution are also associated with an increased risk of tuberculosis [17]. Furthermore, the risk of tuberculosis disease is age-dependent [18].

Young children under the age of 2 years have a very high risk of progression to active and severe tuberculosis disease after exposure. Between the age of 2 to 10 years this risk is then much lower, but rises again during adolescence [19]. While disease patterns in the young children under the age of 2 years with immature immunity resemble those disease patterns observed in the immune compromised HIV-infected patients (higher rates of extra-pulmonary and disseminated tuberculosis) tuberculosis infection after 10 years of age frequently progresses to adult-type disease [19].

Epidemiology

Tuberculosis was an urgent health issue with epidemic sizes in Europe and North America in the 18th and 19th centuries [2]. In pre-colonial Africa tuberculosis may
have accounted for disease in some regions preceding exploration by Europeans, but was possibly not present elsewhere [20]. In northern Europe a decline in tuberculosis mortality began before the discovery of the tubercle bacilli and before the discovery of effective antituberculous drugs; it is therefore assumed that general improvements in hygiene and socioeconomic conditions accounted for much of this decline [7]. While progress in tuberculosis decline continued in several parts of the world towards the end of the 20th century; in parallel the incidence of active tuberculosis disease increased in Africa, mostly due to the impact of the HIV epidemic [7]. In 1993, the World Health Organization (WHO) declared “tuberculosis a public health emergency” and urged to make scaling-up tuberculosis control to an immediate priority [21]. During the following two decades multiple tuberculosis control strategies and programs were conceived and implemented by various international institutions.

One of the first strategies was launched by the WHO in 1995. This strategy focused on directly observed therapy short course (DOTS) to reduce periods of infectiousness and drug resistance. It comprised a bundle of five components (government commitment, case detection, standard short course chemotherapy, regular drug supply systems of essential antituberculosis drugs, and monitoring system), that established a frame for tuberculosis control [9, 22]. Further landmarks in tuberculosis control were the creation of the Global Alliance for TB (Tuberculosis) Drug Development in 2000 with the primary goal to develop a novel treatment regimen for drug sensitive pulmonary tuberculosis to shorten treatment duration [23]. In 2000 the Green Light Committee engaging in appropriate treatment for drug-resistant tuberculosis with quality-certified second-line drugs was established [24]. The Stop TB Partnership governance was formalized in 2001 as a body with the power to align actors all over the world in the fight against tuberculosis [25] and launching the first Global Plan to Stop TB 2001-2005 [26]. This plan constituted an agenda to orchestrate key partners, promote research and development, and have a timely effect on tuberculosis in regions affected most from the epidemic. Later on, the Global Plan to Stop TB 2006-2015 followed and represented an increase in yearly funding in tuberculosis control [26]; and then the Global Plan to Stop TB 2011-2015 followed in 2010 focusing on promoting existing interventions for the diagnosis and treatment of tuberculosis and promoting new technologies and diagnostic tests [26]. The Global Fund to Stop Fight AIDS, Tuberculosis and Malaria, established in 2002 constituted a partnership by governments, civil society, private sector and affected people aimed at accelerating the end of AIDS, tuberculosis and [27]. In 2004 the WHO TB-HIV policy was launched [28] and followed by the WHO’s Post-2015 Global Tuberculosis Strategy Framework promoting the vision of a world free of tuberculosis – zero deaths, disease and suffering due to tuberculosis [29].
The global tuberculosis incidence rate is estimated to have had its maximum peak in 2004 and declined by less than 1% per year since then [30]. Nevertheless, tuberculosis remains an ongoing and serious public health issue in many countries in terms of case numbers, drug resistance, and failing health systems, especially in sub-Saharan Africa [17], but also in high and middle income countries [31, 32]. At the time of primary data collection for this thesis (2012 – 2014), i.e. in 2013, a quarter of the overall estimated 9 million people who developed tuberculosis lived in the African Region, which also had the highest rates of cases and deaths relative to population [33]. Research for this thesis has mainly been performed in Gabon and South Africa, two sub-Saharan countries with high tuberculosis and HIV related morbidity and mortality. For Gabon, as for other countries in the Central African region, detailed data on local tuberculosis epidemiology were limited [34] but according to WHO Gabon ranked 10th among the top countries in terms of tuberculosis incidence rate per population [33]. At the same time South Africa ranked 5th among the top countries for numbers in absolute tuberculosis incidence and 3rd for numbers in tuberculosis incidence rate per population [33].

Historically, childhood tuberculosis has been relatively neglected by clinicians, policy makers, academics, and advocates due to the opinion that children are seldom infectious and therefore add little to the spread of tuberculosis [35]. For a long time this resulted in under-diagnosis and under-reporting of childhood tuberculosis and estimates on the tuberculosis burden in children were subject of large uncertainty intervals. The proportion of tuberculosis that is found in children depends on the population structure, risk factors for tuberculosis, and prevalence of tuberculosis in the community. The overall contribution of childhood tuberculosis to the tuberculosis burden is predicted up to 20% in low and middle income countries [36]. With increasing global attention to child health, there has been growing demand for, and interest in, estimates of tuberculosis disease burden among children. Respective estimates on childhood tuberculosis have been included in the yearly WHO tuberculosis reports since 2012 [37]. The estimate of global tuberculosis incidence among children in 2013 was 550 000 (range, 470 000–640 000), equivalent to about 6% of the total number of 9.0 million incident cases; the estimated total number of deaths from tuberculosis among HIV negative children in 2013 was 80 000 (range, 64 000–97 000), equivalent to about 7% of the total number of 1 100 000 tuberculosis deaths among HIV-negative people in 2013 [33].

**Diagnostics**

For diagnosis of active tuberculosis disease, different diagnostic tools are used and often applied in combination to establish the diagnosis.
Direct visualization of mycobacteria by light microscopy has long been the primary method for diagnosing pulmonary tuberculosis in low- and middle-income countries where most tuberculosis cases occur. Microscopy is quick, relatively easy to perform, low-cost, and highly specific in settings where tuberculosis prevalence is high [38]. LED (light-emitting diode) fluorescence microscopy was endorsed by the WHO in 2011 as sensitivity and specificity were found to be superior compared to conventional light microscopy [39]. Childhood tuberculosis is, however, usually smear negative due to the low bacillary load in children; smear microscopy has therefore a poor sensitivity in childhood tuberculosis. Culture is the gold standard microbiologic test for the diagnosis of tuberculosis disease [40]; advantages of liquid over solid mycobacterial cultures are the shorter time to detection, greater positive rates and a more convenient technology [41]. Because of better accuracy than sputum smear microscopy, the WHO now conditionally recommends a molecular test, Xpert MTB/RIF, as the first-line diagnostic test in all adults or children who are suspected of having active tuberculosis disease [42]. This cartridge-based test allows rapid detection of *M. tuberculosis* DNA and simultaneously rifampicin resistance by nucleic acid amplification; it can be considered as a “close-to point-of-care” test [43].

In the light of drug-resistant tuberculosis drug sensitivity testing (DST) is of utmost importance [44]. Knowledge on drug sensitivity pattern allows targeted treatment with antituberculous drugs to improve outcomes, avoids unnecessary toxicity, prevents further development of resistance, and saves resources. Drug sensitivity testing can be performed using traditional phenotypic or novel and more rapid genotypic tests; both approaches come with advantages and limitations, and correlation between phenotypic and genotypic DST remains challenging due to unsatisfactory understanding of mutations underlying drug resistance [45].

Indirect tuberculosis diagnostic tests measure immunologic response to tuberculosis antigens. The interferon-gamma-release-assay (IGRA), an in-vitro blood test, is specific for *M. tuberculosis*. However, it is not recommended for diagnosis of active tuberculosis disease primarily as there is insufficient knowledge on its performance in low- and middle-income countries, typically those with a high tuberculosis and/or HIV burden [46, 47]. While the tuberculin skin test (TST) is not recommended for diagnosis of active tuberculosis disease in adults, in low- and middle-income countries the TST may be used as an adjunct in diagnosing tuberculosis in children with signs and symptoms of tuberculosis and in conjunction with other diagnostic tests [47]. A major limitation of these immunologic tests, especially of the IGRA, is that they cannot distinguish between infection and disease.

Imaging has been used for many decades to diagnose pulmonary tuberculosis. Chest radiography is widely available and rapid. Pulmonary tuberculosis can present with characteristic radiologic features, i.e. cavities, in immune-competent adult
patients. However, chest radiography for the diagnosis of active pulmonary tuberculosis is limited by poor specificity and reader inconsistency, especially in children, who can present with various radiographic changes suggestive of tuberculosis [48, 49]. Besides chest radiography, other imaging modalities also play a role in diagnosis of tuberculosis; especially for cases with suspected extrapulmonary tuberculosis. Depending on the available radiologic infrastructure and indication ultrasound, computed tomography, magnetic resonance imaging, and positron emission tomography may be applied to visualize features compatible with tuberculosis disease.

Treatment

Smear-positive tuberculosis among immune-competent individuals has a reported 10-year case fatality between 53% and 86% in the absence of treatment and the time from tuberculosis onset to recovery in the absence of treatment or death is around 3 years [50]. Paediatric studies from the pre-chemotherapy era, summarized by Marais et al. [19], report progression to death within one year for the majority of children with cavitation and within 6 months for the majority of children with military tuberculosis.

With the discovery of antituberculous drugs the potential of higher cure rates was given; following initial administration of antituberculous drugs as mono-therapies emergence of drug resistance was quickly recognized as common complication with regimens comprising too few drugs. Today the standard regimen for drug-sensitive tuberculosis comprises four different drugs for the first two months and thereafter treatment is continued for another 4 months with two of these four drugs [51]. In young children, the initial two-month intensive treatment period may comprise only three different drugs [47]. For drug-resistant tuberculosis, second- and third-line drugs combined according to resistance pattern need to be used, and treatment periods are much longer than 6 months [44, 52]. Second-line drugs are usually more toxic, some need to be administered by injections, and availability as well as related costs pose common challenges that limit access to second-line drugs. Establishment of shorter and cheaper regimens for drug resistant tuberculosis are an active field of research [53].

Background and outline of this thesis

The work accomplished in the framework of this thesis addressed different aspects of tuberculosis control in two African countries which both harbour a high burden of tuberculosis but differ considerably in tuberculosis control infrastructure.

At the time of research for this thesis, Gabon and South Africa ranked among the top countries in terms of tuberculosis incidence as described above. The uncontrolled HIV/tuberculosis co-pandemic in South Africa, virtually dominating the country
and resulting in extremely high morbidity and mortality numbers, had attracted international attention and donor support which entailed establishment of a decentralized integrated HIV/tuberculosis care infrastructure and also a highly active research landscape continuously contributing to improvement of tuberculosis control. In contrast, Gabon with a much lower population number and a relatively high gross domestic product did not attract international attention and donor support despite being a largely underdeveloped country with a basically absent functioning HIV/tuberculosis care infrastructure. The lower absolute case numbers in Gabon possibly appeared less threatening to the international community fighting against tuberculosis; however on the ground the relative threats of tuberculosis in terms of multidrug resistance and unfavorable tuberculosis outcomes achieved comparable or even higher levels in Gabon compared to other countries with international support.

Children are particularly vulnerable to tuberculosis. First, children have an increased risk for developing active tuberculosis disease after infection; in children younger than 1 year up to 50% may progress to disease after infection [35]. Second, children are more likely to develop disseminated forms of tuberculosis [35] such as tuberculous meningitis or miliary tuberculosis or other forms of extrapulmonary tuberculosis such as abdominal tuberculosis. Because a diagnosis of tuberculosis is mostly difficult to establish in children, children are often under-diagnosed or diagnosis is delayed. Last but not least, children are dependent on their caregivers for treatment administration and compliance with tuberculosis care. Improving management of childhood tuberculosis therefore needs to address child-specific aspects of tuberculosis diagnosis and treatment, which cannot be extrapolated from experiences in adult tuberculosis patients.

The agenda towards global tuberculosis control - and finally tuberculosis pre-elimination - needs to include not only settings and populations most affected in terms of absolute numbers, but also settings and populations that have been historically neglected. Deficiencies on knowledge of local epidemiology as well as deficiencies in diagnostic and management infrastructure of countries such as Gabon, which may be comparatively small but carry a considerable burden of tuberculosis, have to be included into the global pursuit on tuberculosis control. Also children, who are generally less infectious than adults and therefore often considered less significantly contributing to the spread of tuberculosis, warrant increased attention within tuberculosis control as diagnosing tuberculosis in children is particularly challenging and children can constitute a reservoir for later tuberculosis re-activation if not managed appropriately. Childhood tuberculosis accounts for up to 21% of the total tuberculosis incidence in high burden countries [36] and, recently, tuberculosis has been identified as “a top ten cause of death in children worldwide” [54].
Work done in this thesis addressed three pillars of tuberculosis care which provided the structural organization for the following three sections. The aims of this thesis were (1) to improve the understanding of local tuberculosis epidemiology in Gabon, (2) to improve diagnosis of childhood tuberculosis, and (3) to identify factors impeding effective antituberculosis treatment in Gabon and in management of childhood tuberculosis.

Section I: Improving Knowledge on Local Epidemiology of Tuberculosis

Knowledge on local epidemiology of tuberculosis is a prerequisite for targeted effective tuberculosis control. Overall numbers on prevalent and incident tuberculosis cases and number of cases per treatment center as well as HIV co-infection rates and treatment outcomes need to be known in order to calculate and provide required health care infrastructure and allocate necessary resources.

In Gabon, at the time of conduct of this thesis’ fieldwork, no reliable epidemiologic data on tuberculosis care was available as neither prospective studies had been performed by national or international institutions nor was there a functioning notification system for tuberculosis patients in place. The reported personal experience of health care workers and patients suggested that tuberculosis was a serious and common health issue within the country that was inadequately managed. Few retrospective reports available from selected tuberculosis cohorts in Gabon [55-61] and WHO estimates [62] supported the apprehension that in Gabon tuberculosis was a common disease with a high rate of HIV co-infection and a high rate of adverse treatment outcomes.

Furthermore, reports from patients and health care workers on interrupted or premature termination of tuberculosis treatments, regular stock outs of tuberculosis drugs, absence of diagnostic means for drug-resistant tuberculosis beyond history taking and smear microscopy, unavailability of second-line antituberculous drugs, and an early case series on drug-resistant tuberculosis form the capital of Gabon [56] suggested a situation of threatening emergent and circulating drug-resistant tuberculosis.

In order to improve immediate patient care, to advocate for investment in the national tuberculosis program and to establish a platform for tuberculosis research and long-term improvement of tuberculosis care in a highly endemic country, research on local epidemiology of tuberculosis in Gabon was urgently needed.

Section II: Improving Diagnosis of Tuberculosis Disease in Children

Diagnosing tuberculosis disease in children is challenging because children often present with non-specific signs and symptoms and the yield of microbiological investigations is limited due to difficulties in collection of adequate sample material
with low mycobacterial loads [63]. At the same time, rapid diagnosis and delineation of disease extent is crucial to promptly initiate adequate anti-tuberculous treatment as deferral of treatment can result in complications and unfavorable treatment outcomes. Microbiological confirmation of tuberculosis should always be attempted in order to attain a definite diagnosis, have the conversion parameter as tool for assessing treatment response, and to perform drug sensitivity testing, if needed. However, confirmation of tuberculosis is only achieved in a minority of children. In most cases, childhood tuberculosis is diagnosed on clinical grounds in combination with chest radiography [64], an imaging modality that has shown low interreader agreement for childhood tuberculosis [49], exposing the child to ionizing radiation, and not always affordable for patients in resource-constrained settings [65].

At the time of the research, clinical experience and few reports suggested that a considerable proportion of children diagnosed with pulmonary tuberculosis also had concurrent extrapulmonary tuberculosis, i.e. abdominal tuberculosis which can be visualized by means of ultrasound [66]. At the same time point-of-care ultrasound for improving diagnosis of HIV-associated extrapulmonary tuberculosis in adults proved useful and found its way into routine patient care in sub-Saharan Africa [67-70]. The utility of point-of-care ultrasound within the diagnostic tuberculosis work-up in children was unknown but appeared promising as a considerable proportion of paediatric tuberculosis patients were expected to have features of intra-thoracic or abdominal extrapulmonary tuberculosis. Furthermore, ultrasound is a highly suitable imaging tool for children, as children provide very good scanning conditions, ultrasound is free of ionizing radiation, and can be performed at the patient’s bedside without the need for sedation.

The research of this thesis performed in South Africa therefore aimed at investigating the prevalence of common manifestations of extrapulmonary tuberculosis that are detectable by ultrasound in children and at evaluating the novel imaging approach of point-of-care ultrasound as a bedside imaging tool for improving childhood tuberculosis.

Section III: Improving Management of Tuberculosis
Timely and effective treatment of drug-sensitive and drug-resistant tuberculosis is important for favorable patient outcomes and is essential to avoid spread of tuberculosis and development of drug resistance. Effective antituberculous treatment always consists of a drug combination because drug resistance is developing rapidly under monotherapy or under too few active drugs. As for effective antituberculosis treatment continuous effective drug levels are vital, intake of the drug combinations over defined periods without interruption and appropriate dosing is crucial.
In Gabon, at the time of research, patients reported that the official tuberculosis treatment facilities were not continuously providing antituberculous treatment due to repeated drug stock-outs (a problem which has not been sustainably resolved by the time of writing). Consequently, tuberculosis patients were referred to pharmacies or “drug shops” in order to buy their antituberculous medication out of their private budget. Such practice and the purchasability of antituberculous drugs involves the risk of treatment interruptions or premature termination because of referral and private sourcing; entails the risk of incorrect regimens as only parts of antituberculous combination regimens would be affordable and purchased; and implies the risk of presumptive tuberculosis patients purchasing antituberculous drugs without having been diagnosed and notified with tuberculosis and prescribed with a proper treatment regimen. Research to understand the local availability and flow of anti-tuberculous drugs was urgently required to define possible risk factors for emergence of drug resistance. Furthermore, as there was no official tuberculosis treatment center providing second-line drugs for drug resistant tuberculosis, the knowledge on the availability of second-line drugs through the private sector was urgently needed in order to be able to treat patients with drug-resistant tuberculosis.

Compliance with a correct anti-tuberculous treatment regimen is especially challenging in the treatment of childhood tuberculosis. Children’s compliance is entirely dependent on their caregiver who, in the absence of paediatric drug formulations at required dosages, needs to proceed the tablets by splitting or crushing and mixing with food in order to make sure that the child is able to swallow the treatment. Common practices of caregivers on preparation of antituberculosis medication for children was largely unknown; research for unveiling potential sources for suboptimal antituberculous treatment was required to improve management and successful antituberculous drug administration in children.
REFERENCES

4. Hershkovitz, I; Donoghue, HD; Minnikin, DE; Besra, GS; Lee, OY; Gernaey, AM; Galili, E; Eshed, V; Greenblatt, CL; Lemma, E; Bar-Gal, GK; and Spigelman, M. Detection and molecular characterization of 9,000-year-old Mycobacterium tuberculosis from a Neolithic settlement in the Eastern Mediterranean. PLoS One, 2008. 3(10): p. e3426.
7. Pai, M; Behr, MA; Dowdy, D; Dheda, K; Divangahi, M; Boehme, CC; Ginsberg, A; Swaminathan, S; Spigelman, M; Getahun, H; Menzies, D; and Raviglione, M. Tuberculosis. Nat Rev Dis Primers, 2016. 2(1): p. 1047-56.
15. Rehm, J; Samokhvalov, AV; Neuman, MG; Room, R; Parry, C; Lonnroth, K; Patra, J; Poznyak, V; and Popova, S. The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. BMC Public Health, 2009. 9: p. 450.
17. Lonnroth, K; Castro, KG; Chakaya, JM; Chauhan, LS; Floyd, K; Glaziou, P; and Raviglione, MC. Tuberculosis control
31. de Vries, SG; Cremers, AL; Heuvelings, CC; Greve, PF; Visser, BJ; Belard, S; Janssen, S; Spijker, R; Shaw, B; Hill, RA; Zumla, A; van der Werf, MJ; Sandgren, A; and Grobusch, MP. Barriers and facilitators to the uptake of tuberculosis diagnostic and treatment services by hard-to-reach populations in countries of low and medium tuberculosis incidence: a systematic review of qualitative literature. Lancet Infect Dis, 2017. 17(5): e128-e143.
32. Heuvelings, CC; de Vries, SG; Greve, PF; Visser, BJ; Belard, S; Janssen, S; Cremers, AL; Spijker, R; Shaw, B; Hill, RA; Zumla, A; Sandgren, A; van der Werf, MJ; and Grobusch, MP. Effectiveness of interventions for diagnosis and treatment of tuberculosis in hard-to-reach populations in countries of low and medium tuberculosis incidence:


38. Steingart, KR; Henry, M; Ng, V; Hopewell, PC; Ramsay, A; Cunningham, J; Urbanczik, R; Perkins, M; Aziz, MA; and Pai, M. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis, 2006. 6(9): p. 570-81.


40. Lewinsohn, DM; Leonard, MK; LoBue, PA; Cohn, DL; Daley, CL; Desmond, E; Keane, J; Lewinsohn, DA; Loeffler, AM; Mazeurek, GH; O’Brien, RJ; Pai, M; Richeldi, I; Salfinger, M; Shinnick, TM; Sterling, TR; Warshauer, DM; and Woods, GL. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. Clin Infect Dis, 2017. 64(2): p. 111-115.


44. Grobusch, MP; Schaumburg, F; Alpter, E; and Belard, S. [Drug-resistant tuberculosis. Epidemiology, diagnostics and therapy]. Internist (Berl), 2016. 57(2): p. 126-35.

45. Van Deun, A; Martin, A; and Palomino, JC. Diagnosis of drug-resistant tubercu-


53. Trebucq, A; Schwoebel, V; Kashongwe, Z; Bakayoko, A; Kuaban, C; Noeske, J; Hassane, S; Souleymane, B; Piubello, A; Ciza, F; Fikouma, V; Gasana, M; Ouedraogo, M; Gnañofon, M; Van Deun, A; Cirillo, DM; Koura, KG; and Rieder, HL. Treatment outcome with a short multidrug-resistant tuberculosis regimen in nine African countries. Int J Tuberc Lung Dis, 2018. 22(1): p. 17-25.


58. Mve, MT; Bisvigou, U; Barry, NC; Ondo, CE; and Nkoghe, D. [Reasons for stopping and restarting tuberculosis treatment in Libreville (Gabon)]. Sante, 2010. 20(1): p. 31-4.


64. Frigati, L; Maskew, M; Workman, L; Munro, J; Andronikou, S; Nicol, MP; and Zar, HJ. Clinical Predictors of Culture-confirmed Pulmonary Tuberculosis in Children in a High Tuberculosis and HIV Prevalence Area. Pediatr Infect Dis J, 2015. 34(9): p. e206-10.


69. van Hoving, DJ; Lamprecht, HH; Stander, M; Vallabh, K; Fredericks, D; Louw, P; Muller, M; and Malan, JJ. Adequacy of the emergency point-of-care ultrasound core curriculum for the local burden of disease in South Africa. Emerg Med J, 2013. 30(4): p. 312-5.