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Chapter 18

# Peadiatric Tuberculosis: Is the World Doing Enough?

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## 1. Introduction

Until recently, tuberculosis (TB) had become a neglected disease, particularly in children. It was after the emergence of multi drug resistant TB, and the complications brought about by the HIV/AIDS coinfection, that TB took centre stage again. The common perception has been that children rarely develop severe forms of TB and that they do not contribute much to the spread of the epidemic. Although this could be the case in non-endemic areas, where diligent contact tracing is enforced, in children from endemic areas it is a different story as revealed by an autopsy study conducted in Zambia which demonstrated that tuberculosis was a major cause of death from respiratory disease (Chintu et al., 2002).

Children are particularly vulnerable to more rapid development of severe disease and death after infection, and those with latent infection become the reservoir of disease reactivation in adulthood, fueling the future epidemic (Nicol et al., 2011). Since focus has been on reducing transmission, previous TB control strategies have not prioritized childhood TB (Zar and Pai, 2011). Due to the difficulty in establishing an accurate diagnosis of childhood tuberculosis, the true extent of the tuberculosis-related morbidity and mortality suffered by children in endemic areas is rarely appreciated (Chintu et al., 2002). For example, Regional Data from the WHO in 2007 showed that smear-positive TB in children aged under 14 years accounted for 0.6–3.6% of reported cases but since about 95% of cases in children under 12 years of age are smear negative, these data underestimate the true burden of TB. Furthermore, in countries with a high prevalence of HIV infection, there has been a marked increase in the incidence and a decrease in the peak age prevalence of infectious TB; thus, most cases now occur in young adults, who are often parents of young children (WHO Report, 2009). This finding suggests that children in developing countries will emerge as a group at high risk. In industrialized countries, most childhood TB cases are detected through contact tracing and have good outcomes. This is in contrast to the situation in low- and middle-income countries, where

childhood TB is closely associated with poverty, overcrowding, and malnutrition, with consequently higher death and lower treatment success rates (Nelson and Wells, 2010).

Studies have revealed that children contribute a significant proportion to the disease burden and suffer severe tuberculosis-related morbidity and mortality, particularly in endemic areas. TB is now among the 10 major causes of mortality among children, with a global estimate of 130,000 deaths per year (WHO Report, 2009). Mortality has a strong correlation with socioeconomic status, underlying nutritional status and immunocompetence (Palme, 2002). TB has also been reported to be the third most common cause of death in HIV-infected children with a clinical diagnosis of acute severe pneumonia (Palme, 2002). With roughly a million cases estimated globally each year (Walls and, Shingadia, 2004) and a much higher risk of severe disease and death among young children than adults, paediatric TB remains a public health emergency and this is particularly evident in developing countries with poor public health infrastructure.

As in adults, the majority of cases occur in the 22 high burden countries, where a combination of high transmission rates and a large proportion of the population under the age of 15 years mean children account for up to 25-40% of cases, with incidence rates for paediatric TB ranging from 60-600 per 100,000 per year (Nelson and Wells, 2004). Increasing rates of childhood TB have also been reported in Eastern Europe in the wake of the explosive TB epidemic which followed the break up of the Soviet Union (Walls and Shingadia, 2007). Even traditionally low-burden countries have seen a rise in cases, mainly due to immigration from TB endemic areas. In most countries of Western Europe and North America, where children account for 4-7% cases, paediatric incidence rates vary from about 1 to 15 per 100,000 per year, although much higher rates are observed in some cities, such as London (Newton et al., 2008).

Despite this huge disease burden, children's access to anti-tuberculosis treatment in most endemic areas remains poor, as tuberculosis control programs focus predominantly on the treatment of sputum smear–positive adults (Starke, 2002). Recent technological advancements in diagnosis of TB in adults have not been validated in children and, similarly, trials of new drugs and development of pediatric formulations of standard first- and second-line drugs are lagging behind. As a result both research and surveillance data in the field of childhood TB have been greatly limited. Further research into the epidemiology, immune mechanisms, diagnosis, treatment and prevention of childhood TB is urgently needed to enhance our understanding of TB in children which may provide wider insights and opportunities to facilitate efforts to control TB in the population.

Another problem is that most programs for TB control are limited because they target and treat only active cases (Graham, et al., 2004) when most TB cases in children present as latent tuberculosis infection (LTBI) with active disease occurring mainly in developing countries (Dogra, et al., 2007). Without treatment, the majority of infants aged under 1 year die due to TB. Even with effective antimicrobial therapy, severe TB continues to occur in young children (Ávalos and Montes de Oca, 2012). Priorities for future research should, therefore, enhance collaborations between developing and developed nations.

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This chapter addresses some of the unique features of TB in children; presents existing and novel diagnostic, therapeutic and preventative measures; and outlines important areas of future research. The main challenges for future research are highlighted and in conclusion it is emphasized that well-targeted interventions, improved resources, and improved political commitment, may lead to a dramatic reduction in tuberculosis-related morbidity and mortality among children.

## 2. Epidemiology of paediatric TB

### 2.1. Global disease burden of paediatric TB

Poor case ascertainment, lack of resources for active case finding in most settings, and limited paediatric surveillance data from TB control programs all hamper efforts to define accurately the global burden of childhood TB (Nelson and Wells, 2004). Until recently, under the WHO Directly Observed Treatment Short Course (DOTS) strategy, only smear positive cases were being reported for children, yet smears are seldom performed in many high burden settings and most disease in children is smear negative.

Although limited surveillance data prevent reliable estimates of the contribution of TB to childhood mortality, available data indicate that pneumonia is the commonest cause of childhood death globally (Nelson et al., 2004) an implication that TB, being an important cause of pneumonia in many settings (Scott et al., 2008), may contribute significantly to these global childhood deaths. A necropsy study in Zambia found evidence of TB in 18% of HIV-positive and 26% of HIV-negative children dying of pneumonia (Chintu et al., 2002) although more robust regional data on the epidemiology of childhood TB are urgently needed to define the true burden of disease, and to characterize current transmission rates and circulating strains.

### 2.2. Pathophysiology of TB in children

#### *2.2.1. Natural history of TB in children*

The natural history of TB in children and pediatric patients follows a series of steps in which phase 1 occurs after an incubation period of 3–8 weeks after primary infection. This is followed by appearance of well-defined signs that include fever, erythema nodosum, a positive tuberculin skin test response, and formation of the primary complex visible on chest radiography. Phase 2 occurs 1–3 months after the phase 1 in which period, the bacillus can migrate to other parts of the body via the blood and this represents the period of the highest risk for the development of tuberculous meningitis and miliary tuberculosis in young children. This is the phase where dissemination of the bacillus most frequently occurs. Phase 3 occurs 3–7 months after primary infection and is the period of pleural effusions in children greater than 5 years old and bronchial disease in children less than 5 years. Phase 4 presents after 1–3 years after phase 1 and is during which the osteoarticular tuberculosis in children 5 years and below, appears. Phase 5 occurs up to 3 years after phase 1 and it is presented after calcification has

been completed. It is after this stage that manifestations of classical adult tuberculosis appear (Marais et al., 2004).

Extrapulmonary tuberculosis or miliary TB is a complication of primary TB in young children. It includes peripheral lymphadenopathy, TB meningitis, skeletal TB, and other organ involvement. Other unusual sites for TB include the middle ear, gastrointestinal (GI) tract, skin, kidneys, and ocular structures (Marais et al., 2006). Lymph node involvement typically occurs 6-9 months following initial infection by the tubercle bacilli. More superficial lymph nodes commonly are involved. Frequent sites of involvement include the anterior cervical, submandibular, and supraclavicular nodes. TB of the skeletal system may lead to involvement of the inguinal, epitrochlear, or axillary lymph nodes. Typically, infected lymph nodes are firm and non- tender with non erythematous overlying skin. The nodes are initially non-fluctuant. Suppuration and spontaneous drainage of the lymph nodes may occur with caseation and the development of necrosis (Marais et al., 2006).

Bone or joint TB or skeletal TB may present acutely or sub-acutely. Vertebral disease may go unrecognized for months to years because of its indolent nature. Common sites involved include the large weight-bearing bones or joints, including the vertebrae (50%), hip (15%), and knee (15%). Destruction of the bones with deformity is a late sign of TB. Manifestations for skeletal TB may include angulation of the spine (gibbus deformity) and/or Pott disease (severe kyphosis with destruction of the vertebral bodies). Cervical spine involvement may result in allantoaxial subluxation, which may lead to paraplegia or quadriplegia.

### *2.2.2. TB risk factors*

Following infection children have a higher risk not only of progression to disease, but also of extrapulmonary dissemination and death. Infants have a particularly high morbidity and mortality from TB (WHO, 2007). While many factors including host genetics, microbial virulence and underlying conditions that impair immune competence (as is the case with malnutrition and HIV infection) determine the outcome of infection, it is likely that the high rate of progressive TB seen in young children is largely a reflection of the immaturity of the immune response. Risk factors for the acquisition of tuberculosis (TB) are usually exogenous to the patient. Thus, the likelihood of being infected depends on the environment and the features of the index case. However, the development of TB disease depends on inherent immunologic status of the host. For example, tuberculosis has been reported in patients treated for arthritis, inflammatory bowel disease, and other conditions with tumor necrosis factor (TNF)-alpha blockers/antagonists.

### *2.2.3. Factors for acquiring paediatric TB disease*

Neonatal CD4 cells appear intrinsically deficient in their capacity to express Th1 effector function, partially attributed to hypermethylation of the proximal promoter of the IFN-$\gamma$ gene, (White et al., 2002) and this results in a highly restricted pattern of IFN-$\gamma$ response to a variety of stimuli (Kampmann et al., 2006). CD154 (CD40 ligand) expression is also significantly reduced compared with adult cells. These findings of generally impaired cell-mediated

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immune responses in the neonate and young children raise the question of whether antigen-specific immune responses to mycobacteria are equally affected. Delayed type hypersensitivity (DTH) to purified protein derivative (PPD) may be absent in up to 40% of HIV negative children presenting with extrapulmonary TB, (van der Weert et al., 2006) compounding the difficulties of diagnosis in young children. However, studies measuring responses to neonatal vaccination with M. bovis BCG demonstrate potent Th1 responses, possibly related to the activating properties of BCG vaccine on the potent antigen-presenting cells (APC). Indeed while the long term efficacy of BCG vaccination may be limited, it does offer protection against disseminated disease in infants and young children. The risk of serious and potentially devastating disease is nevertheless still high in the first two years of life, underscoring the need for a better understanding of the determinants of host protection particularly in this vulnerable age group.

In the natural history of childhood intrathoracic TB, primary infection before 2 years of age frequently progresses to disease within the first 12 months (Marais, et al., 2004). Young age and HIV infection are the most important risk factors for severe or disseminated disease; the risk of disease progression decreases during childhood, least at 5–10 years of age, and increases again during adolescence. Pulmonary parenchymal disease and intrathoracic adenopathy are the most common clinical manifestations of pediatric TB, accounting for 60%–80% of all cases (Jensen, 2002). Among extra-pulmonary manifestations, lymphadenopathy is the most common (67%), followed by central nervous system involvement (13%) and pleural (6%), disseminated (5%), and skeletal (4%) TB (Marais, 2006). Disseminated disease and TB meningitis are usually found in very young children (age, under 3 years) and/or HIV-infected children (Starke, 2003). More research is required to identify better strategies for case detection and contact tracing, especially in high-burden settings, and to study the role of genetic and nutritional factors that protect children from TB infection and disease.

HIV-infected children are at risk of both atypical pulmonary presentation and extra-pulmonary disease, which comprises up to 60% of TB in this population (Starke, 2003). Symptom-based diagnostic approaches perform poorly, because other HIV-related conditions, such as lymphocytic interstitial pneumonitis, broncho-ectasis, and respiratory infections (including viral pneumonitis), mimic the clinical and radiographic features of TB (Marais, 2007). Lymphocytic interstitial pneumonitis tends to occur in children aged less than two years, presents with recurrent respiratory symptoms, and is associated with clubbing and generalized lymphadenopathy and a miliary TB-like picture on chest radiograph. Although these patients improve temporarily with antibiotic therapy, antiretroviral treatment is required for sustained benefit and to avoid development of chronic lung disease. In the short term, there is little prospect of achieving a widely available gold standard diagnosis of TB in children either by means of culture, microscopy, PCR, or serological testing. Consequently, clinicians must rely on clinical criteria, chest radiography, and tuberculin testing, and attempts must be made to improve the predictive power of available tools (Swaminathan and Rekha, 2010).

*2.2.4. Host genetic susceptibility to paediatric TB*

The immunological responses to MTB are due to the interaction between the immature immune system in children, the host, bacterial and environmental factors (Meya and McAdam, 2007). Genetic as well as acquired defects in host immune response pathways greatly increase the risk of progressive disease (Kampmann et al., 2005). Results from genome wide linkage studies suggest that TB disease susceptibility is highly likely to be polygenic, with contributions from many minor loci (Hill, 2006) and a large number of TB susceptibility markers have been identified from candidate gene studies as 'disease-causing' genes which include TIRAP, HLA DQB1, VDR, IL-12β, IL12Rβ1, IFN-γ, SLC11A1 and MCP-1. However, to date the greatest evidence to support an underlying genetic basis for TB has come from the discovery of single gene defects predisposing to disseminated and often lethal mycobacterial disease. Most observations were initially made in children with reduced ability to activate macrophage antimycobacterial mechanisms through defects in the IFN-γ (Kampmann et al., 2005) /IL-12 pathway resulting in severe mycobacterial infection. However, subsequent studies have led to description of mutations in five susceptibility genes (Ottenhoff et al., 2005) confirming that up-regulation of the macrophage through the IL-12/23-IFN-γ pathway is a fundamental step in the containment of infection with mycobacteria. However, despite a growing adult literature on the role of candidate genes from this pathway, data from children is scarce. This is surprising given the marked differences in TB pathophysiology in children, which may also reflect differences in genetic factors. Further studies of TB genetics in well-defined paediatric populations are therefore needed.

**2.3. Impact of HIV epidemic paeditric TB**

Studies demonstrating a higher risk of TB among HIV- children (Jeena et al., 2002) highlight the essential role of cell mediated immunity (CMI) in preventing mycobacterial dissemination (Tena et al., 2003). Poor CMI in HIV co-infection often results in disseminated disease, especially in advanced stages of HIV-infection, resulting in poorer survival compared to HIV-negative children (Palme et al., 2002). Risk of active TB in HIV co-infected children is related to both CD4 count and more indirectly also to viral load (Elenga et al., 2005).Conversely, restoration of cellular immunity with anti-retroviral therapy partially reverses TB susceptibility (Kampmann et al., 2006).

The impact of the Human Immunodeficiency Virus (HIV) epidemic on the burden of childhood TB has been less well characterized than for adults (Corbett et al., 2003). However, the observed shift in disease burden to younger adults it has caused, suggests that children are at particularly high risk of exposure as well as disease (Graham et al, 2001). Reported prevalence of HIV co-infection among children with TB range from below 5%, in industrialized settings, to over 50% in some high burden African settings (Nelson and Wells, 2004). However, it is often difficult to draw reliable inferences about the effect of HIV on TB incidence or risk from these observational data due to ascertainment bias or diagnostic bias; incomplete ascertainment of HIV status and because denominator population data on the proportion of all children infected with HIV are usually lacking. (For example, children with HIV are more likely to be investigated for TB and diagnosis is unreliable because it is affected by HIV status). Nevertheless an

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increased TB incidence and poorer outcome have been observed among HIV infected children in a variety of settings (Palme et al., 2002) including an estimated 20-fold increased TB incidence associated with HIV infection in a study from South Africa. Methodological constraints in some studies may explain why this has not been a universal finding (Marais et al., 2007).

### 2.4. Nutrition and paediatric TB

Several observational studies from adults and children show an association between malnutrition and TB, (Cegielski and McMurray, 2004) although proving the direction of a causal link is challenging, as TB in itself causes wasting. Diagnosis is further complicated by frequently false negative TST in malnutrition, reverting to positivity only once nutrition has improved. Nevertheless these observational data, coupled with experimental animal data and impaired CMI observed in malnutrition, support its role as a risk factor for childhood TB (Cegielski and McMurray, 2004). However the effect of differing types and degrees of malnutrition, and the population at risk due to malnutrition in communities where both are endemic, are yet to be defined.

Among micronutrients, vitamin D deficiency has been most extensively studied, and shown to be associated with TB in UK immigrants. Its active metabolite 1-alpha, 25-dihydroxy-vitamin D modulates the host response to TB infection in numerous ways, including the induction of antimicrobial peptides such as Cathelicidin LL-37 (Martineau et al., 2007).

### 2.5. Host-pathogen interactions in paediatric TB

The relationship between MTB strain genotype and clinical manifestation of disease is poorly documented in children. A study in the Western Cape Province of South Africa demonstrated that the Beijing and Haarlem genotype families are significantly associated with drug resistant TB in children (Marais et al., 2006). The high prevalence of Beijing and Latin American Mediterranean (LAM) strains in children reflects considerable transmission of these genotype families in this setting (Marais et al., 2006).

Genetic markers of virulence and transmissibility, (Lopez et al., 2003) and the ability to modulate host cellular immunity have been described for the Beijing strain, HN878 (Reed, et al., 2004). Similarly the East African-Indian lineage is characterized by an LSP (Large Sequence Polymorphism) conferring an immune subverting phenotype that contributes to its persistence and outbreak potential of this lineage (Newton et al., 2006). Strain differences in immunogenicity may result in reduced detection by TST (Anderson et al., 2006) as documented in a London school contact tracing investigation - an extremely worrying phenomenon which may lead to underestimates of the true global burden of TB and underscores the need for new diagnostics. Most studies of strain-specific responses are derived from adult TB cases, and it remains to be established whether results are equally applicable to children. Further research to characterize strain differences in pathogenicity and induction of immune responses should include children as well as adults.

### 2.6. Clinical spectrum of paediatric TB disease

The clinical spectrum of childhood TB also reflects differences in the balance between the pathogen and the host immune response, with more severe disease resulting from either poor or 'over-exuberant' attempts to contain the disease. Many cases of primary TB infection in children are asymptomatic, self-healing and remain completely unnoticed or accidentally discovered at a later stage (Marais et al., 2004). In previously healthy children, what determines the differences in the host/pathogen interactions that lead to successful containment as opposed to progressive disease remains largely unknown. However, age and immunodeficiency are important factors. Thus, while an exuberant immune response, in immunocompetent adolescents, tends to result in adult-type, cavitating disease, (Marais et al., 2005) in young children and/or HIV co-infection, poor CMI is thought to allow unrestrained proliferation of bacilli with progressive parenchymal lung damage (with or without cavity formation) dissemination (Marais et al., 2005)

While dissemination can occur to almost any site, TB meningitis (TBM) is one of the commonest consequences of extra-pulmonary TB and develops three to six months after primary infection (Donald and Schoeman, 2004). It is also the most severe and potentially devastating form of childhood TB with mortality or significant long term neurological sequelae occurring in almost 50% of cases (Thwaites and Tran, 2005). Anatomical differences in children, compared with adults, also modify the presentation of TB. Complications arising from enlarging lymph nodes and small airways are common in children less than five years of age (Marais, et al., 2004; Marais et al., 2005). Post-primary TB can result in upper-lobe pulmonary consolidation and cavitation with highly infectious patients, more likely to be seen in older children.

HIV infection often mimics TB associated signs and symptoms, such as weight loss, failure to thrive and chronic pulmonary symptoms, corroborating the diagnostic difficulties (reviewed in references (Marais et al., 2007). In turn, the treatment of HIV with ART can result in unmasking signs and symptoms of underlying LTBI or active TB in the form of immune reconstitution disease (IRD) (Walters et al., 2006) in young children is largely a reflection on the immaturity of the immune response.

### 2.7. Differences in childhood immune responses to TB

The alveolar macrophage is the first line of defense in the innate immune response to TB and plays a critical role in amplifying the response to infection. Studies in the animal and human host have consistently demonstrated reduced microbial killing and diminished monocyte recruitment to the site of infection in infants compared to adults. Thus impairment of innate pulmonary defenses in the neonate and infant may allow mycobacteria to overwhelm the effects of the innate immune system prior to the initiation of an antigen-specific immune response.

Antigen presentation by dendritic cells (DC), the major antigen-presenting cell (APC) in the lung, and the efficiency with which naïve T cells respond to antigen, also appears less effective in infants and may contribute to the delay in initiating an appropriate antigen-specific response, resulting in development of active disease. Blood derived DCs are functionally

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immature at birth relative to adult DCs and continue to express a less differentiated phenotype throughout early childhood (Upham et al., 2006). Some studies also suggest that neonatal APCs lack the capacity to deliver important Th1 polarising signals to T-cells. Their capacity to synthesise interleukin (IL)-12, a key APC-derived cytokine, matures slowly during childhood (Upham et al., 2002) and neonatal, monocyte-derived DCs have a specific defect in IL-12p35 expression (Goriely et al., 2001). IL-12 is critical for the initial phases of Th1 polarisation and also for maintaining the efficiency of the interferon (IFN)-γ transcription machinery in Th1 effector cells (Goriely et al., 2001).

## 2.8. Latent tuberculosis in children

### *2.8.1. Detection of the infection*

Transmission within a community is measured by the Annual Risk of Infection (ARI) (Rieder, 2005). Infection rates rise with increased exposure in toddlers, around the ages of school entry and with increased social mobility in late teens and early adulthood (Marais et al., 2004). ARI is traditionally estimated using childhood tuberculin surveys, although this has limitations due to the poor specificity of the tuberculin skin test (TST), particularly where Bacille Calmette Guerin (BCG) vaccine is given at birth and non-tuberculous mycobacteria (NTM) are endemic. T-cell based interferon gamma release assays (IGRAs) may offer a more specific alternative (Dinnes et al., 2007), but have not yet found a use in this context due to their cost, ethical concerns about venepuncture in healthy children (Rieder, 2005), and uncertainty about the significance of a positive result for later development of active disease. Threfore, differences in the pathophysiology and clinical presentation of TB in children make diagnosis more challenging than in adults (Shingadia and Novelli, 2003) and the definitions of latent infection and disease, are less clear cut (Marais et al., 2004).

### *2.8.2. Activation from infection to disease*

Following infection, several factors appear to influence the balance of risk between latent TB infection (LTBI) or progression to active disease, including age (Marais et al., 2004) and nutritional (Cegielski and McMurray, 2004), vaccination and immune status (Chen, 2004). Children are at much higher risk of progression to active disease than adults. This risk is greatest for infants and children under 2 years of age (Marais et al., 2004). Active surveillance data from the pre-chemotherapy era suggest that the majority of children develop radiological abnormalities following infection, including 60-80% of children less than two years. However, less than 10% of those are notified, suggesting the disease is controlled by the host immune response in most cases (Marais et al., 2004). This has implications for case definitions based on radiological findings. Overall the risk of disease is highest among infants and in late teens, with the lowest risk between 5 and 10 years - the so-called "safe school years" (Marais et al., 2004). Most disease occurred in the first year following infection (Marais et al., 2004). Thus because disease in young children reflects recent infection, rather than secondary reactivation, the paediatric disease burden potentially provides a useful measure of current transmission

within a community, Marais et al., 2005) including multi-drug resistant (MDR) (Schaaf et al., 2006) and extensively drug resistant (XDR) strains.

## 3. Challenges presented by paediatric TB in the field of diagnosis and treatment

### 3.1. Concepts from the natural history of disease

The pre-chemotherapy literature documented the natural history of tuberculosis in children. Unfortunately, clinicians and researchers have limited access to these important studies as they were conducted before 1950 and are not included in modern electronic databanks. Since the discovery of safe and effective antituberculosis treatment, conducting studies on the natural history of disease became unethical and therefore these historic disease descriptions remain invaluable today. The pre-chemotherapy literature provides a strong body of evidence; multiple studies monitored large cohorts of children for prolonged periods of time and carefully documented the development of disease after primary infection with Mycobacterium tuberculosis. A critical review of the natural history of disease identified three central concepts that are important to consider when addressing current and/or future challenges in the field of childhood tuberculosis: (1) the need for accurate case definitions, (2) the importance of risk stratification, and (3) the diverse spectrum of disease pathology, which necessitates accurate disease classification (Marais et al., 2004).

### 3.2. Challenge of case definition

Accurate case definition revolves mainly around the ability to differentiate primary infection from active disease. Primary infection is believed to occur when a previously uninfected child inhales a single infectious aerosol droplet, which may contain fewer than five bacilli that penetrate into a terminal airway. A localized pneumonic process, referred to as the primary parenchymal (Ghon) focus, results at the site of organism deposition. For the first 4–6 weeks, unrestrained multiplication occurs within the Ghon focus and bacilli drain via local lymphatics to the regional lymph nodes and beyond. The upper lobes drain to ipsilateral–paratracheal nodes, whereas the rest of the lung drains to perihilar and subcarinal nodes, with dominant lymph flow from left to right (Marais et al., 2006). The Ghon complex is represented by both the Ghon focus, with or without some overlying pleural reaction, and the affected regional lymph nodes (Marais et al., 2006).

Occult dissemination frequently occurs during this early proliferative phase before cell-mediated immunity is fully activated. Bacteriologic cultures collected at this time may be positive; Wallgren demonstrated in the 1930s that M. tuberculosis is sometimes recovered from recently infected children who are not diseased. Therefore, with active contact tracing and aggressive screening that includes the collection of mycobacterial cultures in asymptomatic children it is not unexpected to find some positive cultures in recently infected children who are not diseased. This illustrates the overlap that exists between recent primary infection and

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case definitions of disease that rely exclusively on bacteriology. It is important to consider this overlap when case definitions are formulated for research purposes, particularly within the contact setting, although it is less relevant in everyday practice where there is no reason to obtain cultures from completely asymptomatic children.

Uncomplicated hilar adenopathy remains the most common disease manifestation in children and is usually regarded as the hallmark of primary tuberculosis. However, the prechemotherapy literature documented transient hilar adenopathy in the majority (50–60%) of children after recent primary pulmonary infection, of whom only a few progressed to disease (Marais et al., 2004). The natural history of disease illustrates that progression to disease is indicated by the onset of persistent, nonremitting symptoms, referred to as the breakpoint of clinical significance whereas the complete absence of symptoms usually indicates good organism containment (Marais et al., 2004). By convention, asymptomatic hilar adenopathy is currently treated as active disease, although early experience with isoniazid alone demonstrated that one-drug therapy was sufficient in these cases. In terms of pathophysiology, microbiology, and natural history, asymptomatic hilar adenopathy is more indicative of recent primary infection than active disease (Marais et al., 2004).

This indicates that radiologic signs should be interpreted with caution in the absence of clinical data. The entity of so-called asymptomatic tuberculosis, where the case definition rests exclusively on radiographic criteria, is a case in point. High-resolution computed tomography is the most sensitive tool available to detect hilar adenopathy (Andronikou et al., 2004), as demonstrated by the fact that in children with recent M. tuberculosis infection and a normal chest radiograph, prominent intrathoracic nodes are frequently demonstrated by high-resolution computed tomography. Particular caution is required when interpreting the relevance of these radiologic signs in the absence of clinical data. It is important to point out that there is no role for high-resolution computed tomography in the evaluation of asymptomatic, immune-competent children exposed to M. tuberculosis.

In reality, differences in patient selection may result in the use of different functional case definitions even though the definitions appear similar on paper. In non-endemic areas where active contact tracing is diligently enforced, more children with transient radiologic signs indicative of recent primary infection will be identified, and those with active disease will be diagnosed at an earlier, less advanced stage. Active contact tracing is rarely enforced in endemic areas and children usually present to health care facilities with suspicious symptoms and more advanced disease (Marais et al., 2006). Unlike asymptomatic contacts in which visible radiologic signs probably indicate recent primary infection only, radiologic signs in symptomatic children indicate active disease. From a research perspective it is important to be aware of these differences, as inconsistent case definitions may confound the scientific interpretation of results. In everyday practice, distinguishing between the signs and symptoms of recent primary infection and active disease is less relevant in high-risk children (less than 3 years of age and/or immune compromised) in whom infection frequently progresses to disease, sometimes with rapid disease progression.

### 3.3. Problems of risk stratification

The natural history of disease demonstrates that age is the most important variable that determines the risk to progress to disease after primary M. tuberculosis infection in immune-competent children (Marais et al., 2004). Infants are at the highest risk (Marais et al., 2004) and the risk drops but stays appreciable in the second year of life, to reach its lowest level in children infected between 5 and 10 years of age (Marais et al., 2004). Children with human immunodeficiency virus (HIV) infection and/or other forms of immune compromise, such as severe malnutrition, seem to experience a similar high risk as the very young (less than 2 years of age), immune-immature children (Marais et al., 2004). The vast majority (more than 95%) of children who progress to disease do so within 12 months of primary infection and, therefore, it seems prudent to categorize all children less than 3 years of age and/or immune-compromised children as high-risk. Because of the frequency and rapidity with which disease progression may occur, exposure to and/or infection with M. tuberculosis warrants treatment intervention in this high-risk group (Marais et al., 2004).

Immune-competent children of at least 3 years of age are at low risk of progression to disease after primary infection. However, as the vast majority of children in endemic areas become infected after 2 to 3 years of age, these low-risk children still contribute a significant percentage to the total disease burden. In addition, although these children are at low risk to progress to disease, latent infection with M. tuberculosis does pose the risk of future reactivation of the disease. In non-endemic areas, where transmission rates are low and eradicating the pool of latent infection is an achievable aim, the provision of preventive therapy to these low-risk children is warranted. In endemic areas, where the majority of disease in immune-competent adults results from ongoing transmission and not from reactivation (Saiman et al., 2001), the provision of preventive therapy after exposure and/or infection becomes less relevant. The major diagnostic challenge in this low-risk group is the differentiation between latent infection and active disease (Marais et al., 2004). Fortunately, active disease is accompanied by persistent, non-remitting symptoms and disease progression is slow, which provides a window of opportunity for symptom-based diagnosis (Marais et al., 2004).

### 3.4. Difficulties in classifying disease diversity

Childhood tuberculosis is often reported as a single disease entity, although it represents a diverse spectrum of pathology (Marais et al., 2004), and one of the obstacles has been the lack of standard descriptive terminology. Accurate disease classification is important, because of its prognostic significance and to facilitate scientific communication and optimal case management. Within the Ghon focus, containment is usually successful, but disease progression may result from either poor or "excessive" containment. Poor containment and unrestrained organism proliferation may cause progressive parenchymal damage, with ultimate breakdown of the Ghon focus. Infants (Dinnes et al., 2007) and HIV-positive children (Pai et al., 2004), who have poor cell-mediated immune responses, are most vulnerable to this type of cavitation. In contrast, immune-competent adolescents seem to mount an "excessive" (damaging) immune response in an attempt to contain the organism. The exact immune mechanisms underlying adult-type disease remain uncertain, but it is a striking observation that it emerges

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only as children enter into puberty (Marais et al., 2004). It is important to remember that children with adult-type disease are frequently sputum smear–positive and that they do contribute to disease transmission (Cegielski and McMurray, 2004), particularly in congregate settings such as schools.

Complications that arise from affected lymph nodes are most common in children less than 5 years old, because of exuberant lymph node enlargement and small airway size (Marais et al., 2004). Extraluminal compression results when the airway is encircled by enlarged lymph nodes and associated inflammatory edema (Marais et al., 2004). Intraluminal obstruction results from polyps or granulomatous tissue that develops secondary to inflammatory changes in the bronchial wall, or when caseous material is deposited into an airway after lymph node eruption (Marais et al., 2004). Radiologic signs vary from segmental or lobar hyperinflation with partial obstruction and a check-valve effect (Marais et al., 2004), to segmental or lobar collapse with total obstruction and resorption of distal air (Marais et al., 2004). The pathology that results from the aspiration of caseous material is influenced by the dose and virulence of the bacilli aspirated. The pathology may range from transient parenchymal consolidation, resulting from a pure hypersensitivity response to dead bacilli and/or toxic products, to an expansile pneumonic process with progressive caseating pneumonia in the affected segment or lobe (Marais et al., 2004). Expansible caseating pneumonia frequently leads to parenchymal destruction and cavity formation.

Thus, cavitary disease in children may result from three distinct pathologic processes: (1) poor containment at the site of organism deposition (very young and/or immune-compromised children); (2) aspiration of live bacilli when a diseased lymph node erupts into an airway, with destructive caseating pneumonia in the distal segment or lobe (children less than 5 year of age); and (3) adult-type disease (mainly children greater than 10 year of age). The fact that immune-competent children 5 to 10 yr of age experience the lowest risk to progress to disease after primary infection with M. tuberculosis is an interesting immunologic phenomenon that is poorly understood. A better understanding of age-related differences in the immune response to M. tuberculosis may provide important insight into immune correlates of disease and protection.

Disseminated disease occurs predominantly in very young (immune-immature) and/or immune-compromised children, such as the HIV-infected or severely malnourished (Pai et al., 2004; Shingadia and Chen, 2004). These children have suboptimal cellular immune responses and demonstrate poor containment of the organism, both within the regional lymph nodes and at the multiple sites of occult dissemination. TB meningitis (TBM) is the most dangerous complication of disseminated disease, occurring in 20 to 30% of cases (Chen, 2004).

### 3.5. Challenges in diagnosis

#### *3.5.1. Overview of diagnostic challenges*

Diagnostic difficulties pose the greatest challenge to childhood TB management (Marais and Pai, 2007). There are diagnostic complications because: (i) TB can mimic many common childhood diseases, including pneumonia, generalized bacterial and viral infections, malnutrition,

and HIV (Marais and Pai, 2007); (ii) the absence of a practical reference test or gold standard (Marais et al., 2006); (iii) of the inability of child patients to expectorate sputum (Nelson and Wells, 2004) (iv) of the nonspecific clinical presentation (Nicol. et al., 2009); (v) of the lower bacillary load in children which is often smear negative (Detjen et al., 2007) (vi) confirmation by culture of Mycobacterium tuberculosis, usingthe gold standard of diagnosis in adult TB, rarely exceeds 30–40% sensitivity (although it may be considerably higher in children with advanced disease) (Hesseling et al., 2002) even when using gastric aspirates, induced sputum, liquid media, and polymerase chain reaction (PCR) (Edwards et al., 2007); vii) distinguishing between recent primary infection and active disease is highly difficult (Gomez-Pastrana et al., 2001); viii) gastric aspirates continue to be the best specimens for testing for suspected pulmonary TB in children (Ling et al., 2011) with 30–40% sensitivity (Hesseling et al., 2002).

Bacteriologic confirmation, the accepted gold standard, is of limited use in children because of the paucibacillary nature of their disease and poor bacteriologic yields. Sputum smear microscopy, often the only diagnostic test available in endemic areas, is positive in less than 10 to 15% of children with probable tuberculosis (Schaaf et al., 2006). However, the yield is high in children with adult-type disease and sputum smear microscopy has definite diagnostic value in older children (more than 10 year of age) (Cegielski and McMurray, 2004). Culture yields are also low; reported yields in children with probable tuberculosis are less than 30 to 40% (Schaaf et al., 2006). However, the bacteriologic yield depends on the specific intrathoracic disease manifestation. A study from South Africa reported a yield of 77% in children with advanced intrathoracic disease, whereas the yield in those with uncomplicated hilar adenopathy was only 35% (odds ratio, 6.3; 95% confidence interval, 3.2–12.8) (Graham et al., 2001). This indicates the potential value of sensitive bacteriology-based diagnostic approaches, particularly in endemic areas where children frequently present with advanced disease.

Most children with TB are classified as smear-negative pulmonary TB (PTB) for the reasons mentioned above, which is an inappropriate term as a smear or culture has not usually been done. This leads to difficulties in determining the true extent of PTB in children in different areas and circumstances. Extrapulmonary TB (EPTB) accounts for up to 20–30% of the total caseload of TB in children, and the diagnosis is usually easier than PTB because of the characteristic clinical features like lymphadenopathy with or without scrofula, spinal deformity, disseminated disease, meningitis, effusions (pleural or pericardial), or painless ascite (Lewinsohn et al., 2004). The isolation of Mycobacterium tuberculosis takes several weeks. Consequently, the diagnosis of TB in children is often supported only by epidemiological, clinical, and radiographic findings in the presence of a positive tuberculin skin test (López Ávalos and Montes de Oca, 2012).

The value of the classic diagnostic is based on: (1) exposure to an adult index case; (2) chronic respiratory symptoms that do not respond to broad-spectrum antibiotics; (3) documented weight loss or failure to thrive; (4) a positive tuberculin skin test (TST); (5) the presence of suggestive signs on the chest radiograph (CXR), which is greatly reduced in endemic areas where exposure to and/or infection with M. tuberculosis is common (Marais et al., 2006). These criteria are less helpful in endemic areas where a positive TST result is common and exposure to M. tuberculosis is often undocumented (Hesseling et al., 2002). For all these reasons, many

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children with TB are never diagnosed or registered as cases of TB (Nelson and Wells, 2004). Furthermore, the consequences of missed diagnosis in children are severe, as untreated children have a high probability of developing active TB, usually within two years of infection (López Ávalos and Montes de Oca, 2012).

The difficulty to obtain samples for TB diagnosis in children has led researchers to create smart approaches as "la cuerda dulce" (sweet string), reported by Chow et al., (2006). They provide a technique which consists of a coiled nylon string inside a gel capsule. The string unravels through a hole in the end of the weighted capsule as it descends into the stomach and the capsule then dissolves in it, allowing the string to become coated with gastrointestinal secretions containing whatever pathogens are present. After about four hours, the capsule is passed in the feces. This methodology is well tolerated by children and is less invasive than the gastrointestinal lavage (López Ávalos and Montes de Oca, 2012).

In addition to poor bacteriologic yields, the collection of bacteriologic specimens is often problematic. Two or three fasting gastric aspirates collected on consecutive days, usually requiring hospital admission, are routinely performed in young children who cannot cough up phlegm. A retrospective study from California compared the bacteriologic yield achieved in gastric aspirates collected from hospitalized and nonhospitalized children. Although the yield in hospitalized children was higher (percentage of positive cultures, 48 compared to 37%), this difference was not statistically significant, which suggests that hospitalization may not be a prerequisite for the collection of a good gastric aspirate specimen. Bronchoalveolar lavage, using flexible fiberoptic bronchoscopy, has additive value when used in combination with gastric lavage, but this technique is highly specialized and is unavailable in most endemic areas. In a study from Peru, midmorning nasopharyngeal aspiration was compared with early morning gastric aspiration; gastric aspiration provided a slightly better yield than nasopharyngeal aspiration (38 compared to 30%), but the results were comparable (Nelson et al., 2004). Nasopharyngeal aspiration is minimally invasive, does not require hospitalization or fasting, and can be performed any time of the day. A study from South Africa demonstrated that a single specimen, using hypertonic saline–induced sputum collection, may provide the same yield as three gastric aspirate specimens (Corbett et al., 2003). However, the overall yield in this study remained poor (15% with one and 20% with three induced sputum specimens) and the technique has not been used outside the hospital setting. Additional studies are awaited to confirm the feasibility and diagnostic value of collecting induced sputum specimens in primary health care settings.

Because of the difficulty in achieving bacteriologic confirmation, the diagnosis of childhood tuberculosis in non- endemic areas is usually based on (1) known contact with an adult index case (frequently within the household), (2) a positive tuberculin skin test (TST), and (3) suggestive signs on the chest radiograph. This triad provides a fairly accurate diagnosis in settings where exposure to M. tuberculosis is rare and well documented. However, its diagnostic accuracy is greatly reduced in endemic areas where exposure to M. tuberculosis is common and often undocumented, as exposure frequently occurs outside the household. Despite reservations about the specificity of the TST response after Bacille Calmette-Guérin (BCG) vaccination and/or exposure to environmental mycobacteria, a positive TST reaction

remains a fairly accurate measure of M. tuberculosis infection in immune-competent children. Current U.S. guidelines recommend the use of three different cutoff points to define a positive TST reaction. In endemic areas a positive TST is not uncommon in randomly selected healthy children (Jeena et al., 2002), which limits its diagnostic value. Consequently, the diagnosis of tuberculosis in children from endemic areas depends mainly on clinical features and the subjective interpretation of the chest radiograph (Marais et al., 2007). However, chest radiography is unavailable in many endemic areas and it has well-known limitations that may result in both under- and overdiagnosis of disease (Brent et al., 2007). Despite these limitations it provides an accurate diagnosis in the majority of symptomatic children with tuberculosis and the interpretation of the chest radiograph remains the most widely used diagnostic criterion in clinical practice (Palme et al., 2002).

Various clinical scoring systems have been developed. A critical review of these clinical scoring systems concluded that they are limited by a lack of standard symptom definitions and adequate validation (Walls and Shingadia, 2007). Developing standard symptom definitions through consensus of expert opinion is a difficult and subjective exercise; better guidance may be provided by objectively measuring the potential diagnostic value of different symptom definitions. A community-based survey demonstrated that the poorly defined symptoms traditionally associated with tuberculosis (such as a cough greater than 3 weeks in duration) are frequently reported in a random selection of healthy children (Bryce et al., 2005). Of 1,397 children without tuberculosis, 253 (26.4%) reported a cough during the preceding 3 months and 66 (6.9%) reported a cough greater than 3 weeks in duration (Bryce et al., 2005). In addition, nearly 50% of children with visible hilar adenopathy on the chest radiograph (diagnosed with tuberculosis) reported no symptoms at all (Bryce et al., 2005). These observations demonstrate the limited diagnostic value of poorly defined symptoms and the need for improved symptom and case definitions. In a follow-on study the use of well-defined symptoms with a persistent, nonremitting character showed greatly improved diagnostic accuracy (Scot et al., 2008). However, the potential diagnostic value offered by the use of these well-defined symptoms requires further validation in a prospective, community-based study that includes children from all relevant risk groups. It is expected that symptom-based diagnostic approaches would have less value in high-risk children (less than 3 years of age and/or immune compromised) where disease progression may occur rapidly, emphasizing the need for preventive chemotherapy and early diagnosis of disease in this group (Chintu, et al., 2002). Other diagnostic modalities may hold promise, but have not shown convincing results to date (WHO, 2006).

Serologic tests are currently unable to diagnose childhood tuberculosis with accuracy, and sputum-based polymerase chain reaction (PCR) tests have shown variable results and limited utility (WHO, 2007). Good results were reported with the use of a heminested PCR technique in Peru, but the study used uninfected children as the control group and therefore could not evaluate the ability of this novel PCR-based test to differentiate latent infection from active disease (Upham et al., 2006), which is important, as specific concerns have been raised regarding the specificity of PCR-based tests.

The diagnostic dilemma is even more pronounced in HIV-infected children. The specificity of symptom-based diagnostic approaches is reduced by the presence of chronic HIV-related

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symptoms, while the potential window for symptom-based diagnosis is limited by the rapidity with which disease progression may occur. Chest radiograph interpretation is complicated by HIV-related comorbidity and atypical disease presentation. These difficulties increase the potential diagnostic value of sensitive bacteriology-based approaches, to identify HIV-infected children with tuberculosis (Upham et al., 2002). However, as HIV-infected children are in the high-risk group the detection of M. tuberculosis infection is also highly relevant. Disease progression may occur soon (less than 12 months) after primary or reinfection, or latent infection may be reactivated at a later date because of a decline in immunity. The traditional TST has poor sensitivity to detect M. tuberculosis infection in HIV-infected children; 50% or less of HIV-infected children with bacteriologically confirmed tuberculosis are TST positive, despite using an induration size of at least 5 mm (Upham et al., 2002). This is a major limitation and development of a more reliable measure of infection will be valuable to identify HIV-infected children who may benefit from preventive chemotherapy; it may also provide supportive evidence to establish a diagnosis of active tuberculosis.

*3.5.2. Challenges presented by diagnosis of latent infection in children*

LTBI, in children as in adults, lacks a diagnostic gold standard. The diagnosis is usually pursued after a documented household exposure, or to evaluate if chemoprophylactic therapy is indicated in the context of immunosuppression. In this setting, pre-existing MTB specific host immune responses are measured to confirm previous infection. Data in adults have confirmed that IGRA are more sensitive and specific than the TST (Pai et al., 2004; Ferrara et al., 2006) in this context. Preliminary data suggest IGRA also perform better in children but age-related data are still sparse. Longitudinal studies assessing their positive predictive value for the development of active TB are required in both TB-endemic and low-incidence countries, as the continued exposure in TB endemic settings might yield very different results, compared to the "one-off" exposure more typically encountered in non-endemic countries.

### 3.6. Challenges presented by drug resistance

There were an estimated 0.5 million adult cases of MDR-TB in 2007. By the end of 2008, 55 countries and territories had reported at least 1 case of extensively drug-resistant TB (WHO Report, 2009). Latest research reports published in The Lancet at the end of August 2012, indicate that researchers have found rates of both multi drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) higher than previously thought and that they are threatening global efforts to curb the spread of TB. They contend that most international recommendations for TB control have been developed for MDR-TB prevalence of up to around 5 percent but that now the world faces a prevalence up to 10 times higher in some places, where almost half of the patients are transmitting MDR strains. The Researchers who studied rates of the disease in Estonia, Latvia, Peru, the Philippines, Russia, South Africa, South Korea, and Thailand are reported to have found that almost 44 percent of cases of MDR TB were also resistant to at least one second-line drug outline goes here (Dalton et al., 2012)

Comprehensive studies on resistance to anti-TB drugs in children are lacking, because they are not included in global surveys. Surveillance of anti-TB drug resistance during 1995–2007

among children from South Africa showed a significant increase in resistance to INH or RIF from 6.9% to 15.1% and an increase in multidrug resistance from 2.3% to 6.7% (Schaaf et al., 2009). Drug resistance among children has been documented in clinical trials of both pulmonary and extrapulmonary TB (Rekha and Swaminathan, 2007). Management of MDR-TB is a challenge, because it requires prolonged treatment for 24 months with second-line drugs, which are more toxic and expensive than first-line drugs. According to the 2006 WHO guidelines for programmatic management of MDR-TB, an optimal regimen should include a fluoroquinolone, an injectable (capreomycin, kanamycin, or amikacin), and at least 2 of the following drugs: cycloserine, thiomides, para-amino salicylic acid, and first-line agents other than INH and RIF (WHO, 2008). Experience with second-line TB drugs in children is limited; 38 children in Peru were treated with supervised, individualized regimens consisting of 5 drugs in the national program. Despite half of these children being anemic and malnourished, treatment was well tolerated and resulted in a 95% cure rate (Drobac et al., 2006).

There is little published information on optimal treatment of latent TB infection in children in contact with patients with MDR-TB. In a 30-month follow-up of contacts of patients with MDR-TB, 5% of children who received appropriate chemoprophylaxis and 20% of those who did not receive prophylaxis developed disease (Schaaf et al., 2007). Regimens used included INH, PZA, and ethionamide or EMB. Currently, the best approach may be to perform a complete risk assessment and clinical evaluation and to individualize therapy, while keeping these children under close observation. Multicentric trials are urgently required to determine the most effective drug combinations and optimal duration of chemoprophylaxis for contacts of patients with MDR-TB.

TB is often not considered in the differential diagnosis in children, especially in low endemic settings. TB can mimic many common childhood diseases, including pneumonia, generalised bacterial and viral infections, malnutrition and HIV. However, the main impediment to the accurate diagnosis of active TB is the paucibacillary nature (containing just a few bacilli) of the disease in children. Younger children also produce smaller amounts of sputum, which is usually swallowed rather than expectorated. Bacteriological samples may be collected by conducting early morning gastric washings, a fairly unpleasant procedure that requires hospital admission and overnight-fast for up to three consecutive nights. Consequently bacteriological confirmation is the exception rather than the rule with only 10-15 % of sputum samples revealing acid fast bacilli (AFB) and culture remaining negative in around 70% of cases with probable TB (Zar et al., 2005). Without a definitive diagnosis treatment is therefore often initiated on clinical judgment, aided by algorithms based on exposure history, clinical features, chest x-ray (CXR) and TST (Marais et al., 2006). Several approaches have been taken to improve the diagnosis (Marais and Pai, 2007).

### 3.7. Improving bacteriological detection and rapid resistance analysis

Recent advances in bacteriological and molecular methods for the detection of MTB in patient samples aim to identify drug-resistance in parallel with detection of MTB. These include the Microscopic Observation Drug Susceptibility assay (MODS) (Moore et al., 2006), more sensitive PCR techniques (Sarmiento et al., 2003) or phage-based tests such as FASTPlaque

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(Kalantri et al., 2005). This represents laudable progress, particularly in the context of increasing drug resistance. Calorimetric culture systems such as the TK medium (Kocagoz et al., 2004) and electronic-nose technology (Fend et al., 2006) are also under investigation. Among adults MODS appears to be at least as sensitive as gold standard liquid culture methods (Moore et al., 2006), Data comparing its performance in children is more limited, but MODS has been evaluated in a paediatric hospital setting and found to be more sensitive than solid media in one study (Oberhelman et al., 2006). Data validating other new methods in paediatric specimens are also lacking, yet performance may be affected by the paucibacillary nature of childhood TB. The lowest limit of detection of TB by the electronic nose for example has been reported to be 104 CFU/ml of sputum for example which is just within the range of the expected bacillary burden in paediatric specimens (Fend et al., 2006). Validation of these assays on paediatric samples is a research priority (López Ávalos G G and Montes de Oca, 2012). The introduction of GeneXpert which includes use of integrated DNA extraction and amplification systems and utilizes real-time PCR (rt-PCR) technology to both diagnose TB and detect rifampicin resistance, has given a ray of hope with paediatric TB diagnosis and rifampicin resistance (Gordetsov et al., 2008).

## 4. Diagnosis and treatment: Current state of affairs

### 4.1. Classical diagnosis

#### *4.1.1. Clinical symptoms approach*

The use of well-defined symptoms improves diagnostic accuracy of pulmonry tuberculosis (PTB) (Imaz et al., 2001). With clinical symptoms approach only, the status can be classified in two; suspected TB or probable TB. Two situations lead the clinician to suspect that a child has tuberculosis. The first is a history of chronic illness with clear symptoms: cough and/or fever, weight loss or failure to thrive, an inability to return to normal health after measles or whooping cough, fatigue, and wheezing; second, is when one or more of the following: malnutrition, lymphadenopathy, chest signs, hepatomegaly and/or splenomegaly, meningeal signs, and/or ascites is/are observed. For probable TB, in addition to suspected TB, the child presents with a positive TST, a suggestive radiological chest appearing as pleural effusion, caseation of biopsy material, poor response to 2 weeks of antibiotic treatment, and/or favourable response to antituberculous treatment (weight gain and loss of signs) (Hesseling et al., 2002).

In pediatric TB, the most common symptoms are pulmonary parenchymal disease and intrathoracic adenopathy accounting for 60–80% of all cases. Among extrapulmonary manifestations, lymphadenopathy is the most common (67%), followed by central nervous system involvement (13%), pleural (6%), miliary and/or disseminated TB (5%), and skeletal TB form (4%). Disseminated disease and TB meningitis are usually found in very young children who are below the age of 3 years, and/or HIV-infected children (Nelson et al., 2004). TB meningitis occurs when the child has contact with a suspected or confirmed case.

In general, there is a sense of skepticism regarding the potential diagnostic value of symptom-based approaches but nevertheless, the natural history of childhood tuberculosis demonstrates that symptoms may have diagnostic value if appropriate risk stratification is applied. Marais et al., (2005), evaluated whether well-defined symptoms have a diagnosis value in children and a standard symptom-based questionnaire was completed and reported symptoms were individually characterized. A tuberculin skin test (TST) and chest radiograph (CXR) were performed in all children. In this study,well-defined symptoms had excellent diagnostic value.

*4.1.2. Radiologic studies*

Radiography became available after the First World War, and since that time, PTB detection became easier (Marais et al., 2004). Evidence of pulmonary TB in chest radiographs varies, but usually radiographs show enlargement of hilar, mediastinal, or subcarinal lymph nodes and lung parenchymal changes with hilar lymphodenopathy with or without a focal parenchymal lesion. The most common findings are segmental hyperinflation then atelectasis, alveolar consolidation, interstitial densities, pleural effusion, and, rarely, a focal mass. Cavitation is rare in young children but is more common in adolescents, who may develop reactivation disease similar to that seen in adults (Marais. and Pai, 2007). High-resolution computed tomography is the most sensitive tool currently available to detect hilar adenopathy and/or early cavitation (Hesseling et al., 2002).

*4.1.3. Diagnostic algorithms*

These are point-scoring systems to make diagnostic classifications. Diagnostic algorithms were developed to deal with these diagnostic difficulties and provide the health care worker with a rational, stepwise tool to identify children in need of treatment. They are very helpful and very easy to use in countries with restricted technology, but only few of them are available especially in resource limited countries (Edwards et al., (2007). Although the natural history of tuberculosis (TB) in children follows a continuum, the American Thoracic Society (ATS) definition of stages is useful (Blumberg, et al., 2003). According to the ATS the stages are as follows:

Stage 1: Exposure has occurred, implying that the child has had recent contact with an adult who has contagious TB. The child has no physical signs or symptoms and has a negative tuberculin skin test (TST) result. Chest radiography does not reveal any changes at this stage. However, not all patients who are exposed become infected, and the TST result may not be positive for 3 months. Unfortunately, children younger than 5 years may develop disseminated TB in the form of miliary disease or TB meningitis before the TST result becomes positive. Thus, a very high index of suspicion is required when a young patient has a history of contact.

Stage 2: This second stage is heralded by a positive TST result. No signs and symptoms occur, although an incidental chest radiograph may reveal the primary complex.

Stage 3: In stage 3, TB disease occurs and is characterized by the appearance of signs and symptoms depending on the location of the disease. Radiographic abnormalities may also be seen.

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Stage 4: Stage 4 is defined as TB with no current disease. This implies that the patient has a history of previous episodes of TB or abnormal, stable radiographic findings with a significant reaction to the TST and negative bacteriologic studies. No clinical findings suggesting current disease are present.

Stage 5: TB is suspected, and the diagnosis is pending. Any patient with pneumonia, pleural effusion, or a cavitary or mass lesion in the lung that does not improve with standard anti-bacterial therapy should be evaluated for tuberculosis (TB). Also, patients with fever of unknown origin, failure to thrive, significant weight loss, or unexplained lymphadenopathy should be evaluated for TB (Marais et al., 2006).

*4.1.4. Mycobacterial detection and isolation*

Microbiological confirmation of TB in young children is not routinely attempted in many high burden settings due to the difficulty in obtaining samples and the poor performance of smear microscopy (Nicol and Zar, 2011). Diagnosis of TB still relies primarily on examination of Acid-Fast Bacilli- (AFB-) stained smears from clinical specimens in adults, however, children with pulmonary TB usually do not cough up voluntarily, either because they do not produce sputum or because it produces discomfort. When sputum samples cannot be obtained, gastric aspirate samples are used for detection and isolation of M. Tuberculosis. Most of the current TB diagnostic methods were developed over a century ago. In 1898, Neunier became the first person to culture stomach contents for the evidence of tuberculosis in children (Marais and Pai, 2007; Lalvani and Millington, 2007), so even with this method, fewer than 20% of children with TB have a positive AFB smear of sputum or gastric aspirate.

For many years, the collection of three consecutive early morning gastric lavages or gastric aspirate samples has been the accepted method for attempting microbiological confirmation even as the yield is very low and that in many populations cannot be performed due to the lack of infrastructure. In addition low pH is known to kill tuberculous bacilli, indicating that stomach pH may inhibit TB survival for subsequent culture (Marais. and Pai, 2007). More recently, a number of less invasive alternative methods have been proposed, including induced sputum (administration of an inhaled bronchodilator followed by nebulized hypertonic 3–5% saline and then collecting nasopharyngeal aspiration or expectoration of mucus from lower respiratory tract). In the nasopharyngeal aspiration, a cannula elicits a cough reflex and the sweet string test mentioned above (Nicol and Zar, 2011). One of the methods that can be used to collect samples for microbiological analysis is the string test. This is a non-invasive collection method and is reported to be well tolerated by children as young as 4 years (Chow et al., 2006). Inducing sputum after hypertonic saline nebulization has also been shown to be feasible for young children, although the most widely used procedure is still the early-morning gastric aspiration or lavage. However, all these procedures involve hospitalization, trained personnel, and attention to infection control.

All of these alternative ways of sampling have been made to increase yield because a positive culture is regarded as the "gold standard test" to establish a definitive diagnosis of TB in a symptomatic child (Hesseling et al., 2002). If culture is negative, diagnosis is made on the basis of a positive TST. With clinical and radiographic findings suggestive of TB, and history of

contact with an adult source case, the child may be diagnosed with positive TB based on symptomatology. This measure was taken because the yields in children are less than 50%. Zar et al., (2000), investigated whether sputum induction could be successfully performed in infants and young children with and without HIV and determined the utility of salbutamol-induced sputum compared to gastric lavage (GL) for the diagnosis of pulmonary tuberculosis. They concluded that sputum induction can be effectively performed and is well tolerated and safe even in infants and this induction is better than GL for the isolation of M. tuberculosis in both HIV-infected and uninfected infants and children.

Although culture on Lowenstein-Jensen medium is considered to be the gold standard, liquid culture systems (commercial and non commercial) offer the possibility of more rapid and more sensitive diagnosis of active TB and drug susceptibility but are not widely available in resource-poor settings (Brittle et al., 2009) compared mycobacterial yields and time to detection in pediatric clinical samples with use of mycobacterial growth-indicator tubes with those with use of solid Lowenstein-Jensen slants and found that the yield was substantially higher with use of mycobacterial growth-indicator tubes (11% compared to 1.6%). Furthermore, the mean time to detection could be reduced from 18.5 days to 12.4 days with use of a nutrient broth supplement; newer approaches, such as the colorimetric culture systems and phage-based tests are of interest, but limited data are available for children.

#### *4.1.5. Smear microscopy*

Advances have been done in the performance of smear microscopy for the rapid detection of MTB, for example, the concentration of specimens by centrifugation or the change of the staining of carbol fuchsin (Ziehl-Neelsen or Kinyoun) for a fluorescent dyes (auramine-rhodamine), which both increases sensitivity and reduces the time for screening (Bakir et al., 2008). However, even under optimal circumstances, the sensitivity of smear microscopy for the diagnosis of childhood TB remains less than 15%, except in older children with adult-like disease (Nicol and Zar, 2011).

#### *4.1.6. Tuberculin skin test (TST)*

This is one of the major classes of tests that are currently used to detect Latent TB. Tuberculin which is also called purified protein derivative or PPD is a standardised killed extract of cultured TB, which is injected into the skin to estimate an individual's immune response to TB. There are three methods of testing: the Mantoux test, the Heaf test and the Tine test but not all of them are currently available for use and some countries prefer one over the other. The Heaf test is no loner available because its continued manufacture was not economically viable.

The Tuberculin skin test, or Mantoux TST, is based on the detection of a cutaneous delayed-type hypersensitivity response to purified protein derivative, a poorly defined mixture of antigens present in M. tuberculosis, Mycobacterium bovis Bacille Calmette-Guerin (BCG) and several nontuberculous mycobacteria (Nicol et al., 2011). TST is the standard method for detecting infection by M. tuberculosis. The reaction is measured as millimeters of induration

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after 48 to 72 hours. This test was the only method available for the diagnosis of latent tuberculosis infection (LTBI) until very recently.

The Heaf test uses what is called a Heaf gun which uses disposable single-use heads, each head having six needles arranged in a circle. The device has standard heads and pediatric heads - the standard head being used on all patients aged 2 years and older while the pediatric head is for infants under the age of 2. For the standard head, its needles protrude 2 mm when the gun is actuated while for the pediatric heads, the needles protrude only 1 mm. Before application, the skin is cleaned with alcohol, then 100,000 units/ml (equivalent to about 0.1 ml) of tuberculin is evenly smeared on the skin and the gun applied to the skin and fired. The excess of the solution is then wiped off and a waterproof ink mark is drawn around the injection site as an indicator of the site of administration and the test read 2 to 7 days later. The results of the test are interpreted as follows:

Grade 0: no reaction, or induration of 3 or less puncture points;Grade 1: induration of four or more puncture points; Grade 2: induration of the six puncture points coalesce to form a circle; Grade 3: induration of 5 mm; or more and Grade 4: induration of 10 mm or more, or ulceration.

There is not much difference between the Heaf and Mantoux test, but the two tests can be related as follows: Heaf grade 0 and 1 approximately equivalent Mantoux less than 5 mm; Heaf grade 2 approximatley equivalent to Mantoux 5–14 mm and Heaf grade 3 & 4 being approximately equivalent to Mantoux 15 mm or greater, To avoid cases of false positives and false negatives, the tuberculin used for Heaf tests is 1000 times more concentrated than that used for Mantoux tests. In countries where both tests are used, use of the correct concentration avoids false positive and false negative results.

The recommended Tuberculin Skin Test (TST), which has now been standardised by the WHO to contain 0.1 ml of tuberculin (100 units/ ml), is the Mantoux test (CDC, 2010). The dosage of 0.1 ml containing 5 tuberculin units [TU] of purified protein derivative (PPD) should be injected intradermally into the volar aspect of the forearm using a 27-gauge needle. A detergent called Tween 80 to prevent loss of efficacy on contact and adsorption by glass stabilizes the PPD. A wheal should be raised and should measure approximately 6-10 mm in diameter. Skilled personnel should always read the test 48-72 hours after administration. Measure the amount of induration and not erythema. This should be measured transverse to the long axis of the forearm. Multiple puncture tests such as Tine test and Heaf test lack sensitivity and specificity and hence are not recommended in this situation (Marais et al., 2006).

Subcutaneous injection should be avoided because it results in false negative results. The site of administration is indicated by a water-proof ink mark drawn around the site of injection to serve as an indicator for the site. The reading, which is done two to seven days involves measuring area of induration transversely (left to right) across the forearm and recorded to the nearest millimetre. It should be borne in mind that the induration (dermal thickening causing the cutaneous surface to feel thicker and firmer) should not be confused with erythema (redness of the skin) caused by hyperemia of the capillaries in the lower layers of the skin.

If a patient who has previously had a negative tuberculin skin test develops a positive tuberculin skin test at a later date, tuberculin conversion is said to have occurred. When such

a reaction occurs, it provides strong evidence for significant exposure to TB. Different countries have different standards about the time interval between tests. The UK recommendation is that the two tests have to be done at least six weeks apart; while in the U.S. the recommendation is that the two tests can be done one week apart.

Another phenomenon associated with tuberculin skin test is what is called boosting, which occurs when people who have had some traces of infection with M. tuberculosis and/or previous exposure to BCG vaccination against tuberculosis, are given repeated tuberculin skin tests. In these cases, the first test revives or primes the immune response so that on repeat testing, the response is much stronger and the patient now appears to have a positive reaction. The second tuberculin skin test result is what is taken to be the correct one. Again, the guidelines on how to approach the phenomenon of boosting are different in different countries with the U.S. guidelines emphasising that, ignoring previous immunisation with BCG would lead to a person showing the phenomenon of boosting, being falsely described as a tuberculin converter. On the other hand, UK guidelines advocate two tuberculin skin tests one week apart, if boosting is suspected, taking the result of the second test as being the true result. The phenomenon of boosting can occur up to two years after the first Mantoux test.

According to the American Academy of Pediatrics (AAP) immediate skin testing is indicated for the following children: 1)Those who have been in contact with persons with active or suspected TB; 2) Immigrants from TB-endemic countries or children with travel histories to these countries; 3) Those who have radiographic or clinical findings suggestive of TB. 4) Children who are infected with human immunodeficiency virus (HIV) or those living in a household with persons infected with HIV and; 5) Incarcerated adolescents.

Testing at 2-year to 3-year intervals is indicated if the child has been exposed to high-risk individuals including those who are homeless, institutionalized adults who are infected with HIV, users of illicit drugs, residents of nursing homes, and incarcerated adolescents or adults. Testing when children are aged 4-6 years and 11-16 years is indicated for the following children:1) Children without risk factors residing in high-prevalence areas; 2) Children whose parents emigrated from regions of the world with a high prevalence of TB or who have continued potential exposure by travel to the endemic areas and/or household contact. Performing an initial TST before the initiation of immunosuppressive therapy is recommended in any patient (AAP, 1996).

Wit regard to administering the TST to previous recipients of the Bacille Calmette-Guérin (BCG) several problems are encountered when it comes to interpreting the results of the test. Immunization with BCG is not a contraindication to the TST but differentiating tuberculin reactions caused by vaccination with BCG versus reactions caused by infection with M tuberculosis is difficult. History of contact with a person with contagious TB or emigration from a country with a high prevalence of TB suggests that the positive results are due to infection with M tuberculosis. However, multiple BCG vaccinations may increase the likelihood that the positive TST result is due to the BCG vaccination. The positive reactivity caused by BCG vaccination generally wanes with the passage of time. With the administration of TST, this positive tuberculin reactivity may be boosted. However, previous BCG vaccination does

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not affect interpretation of a TST result for a person who is symptomatic or in whom TB is strongly suspected (Marais et al., 2006).

For the UK the guidelines for interpreting tuberculin skin tests are formulated according to the Heaf test. For patients who have had BCG previously, latent TB is diagnosed if the Heaf test is grade 3 or 4 and have no signs or symptoms of active; if the Heaf test is grade 0 or 1, then the test is repeated and, in patients who have not had BCG previously, latent TB isiagnosed if the Heaf test if grade 2, 3 or 4, and have no signs or symptoms of active TB. Repeat Heaf testing is not done in patients who have had BCG of the phenomenon of boosting.

The Centers for Disease Control and Prevention (CDC) and the AAP provided recommendations regarding the size of the induration created by the TST that is considered a positive result and indicative of disease [http://www.cdc.gov/tb/]. The TST is interpreted on the basis of 3 "cut points": 5 mm, 10 mm, and 15 mm. Induration of 5 mm or more is considered a positive TST result in the following children: 1) Children having close contact with known or suspected contagious cases of the disease, including those with household contacts with active TB whose treatment cannot be verified before exposure; 2) Children with immunosuppressive conditions (such as HIV) or children who are on immunosuppressive medications; 3) Children who have an abnormal chest radiograph finding consistent with active TB, previously active TB, or clinical evidence of the disease.

Induration of 10 mm or more is considered a positive TST result in the following children: 1) Children who are at a higher risk of dissemination of TB disease, including those younger than 5 years or those who are immunosuppressed because of conditions such as lymphoma, Hodgkin disease, diabetes mellitus, and malnutrition; 2) Children with increased exposure to the disease, including those who are exposed to adults in high-risk categories (such as homeless, HIV infected, users of illicit drugs, residents of nursing homes, incarcerated or institutionalized persons); 3) those who were born in or whose parents were born in high-prevalence areas of the world; and those with travel histories to high-prevalence areas of the world. Induration of 15 mm or more is considered a positive TST result in children aged 5 years or older without any risk factors for the disease.

False-positive reactions and false-negative results are common and can be due to various causes. False-positive reactions are often attributed to asymptomatic infection by environmental non-TB mycobacteria (due to cross-reactivity). False-negative results, on the other hand, may be due to vaccination with live-attenuated virus, anergy, immunosuppression, immune deficiency, or malnutrition. In cases of anergy, a lack of reaction by the body's defence mechanisms when it comes into contact with foreign substances, the tuberculin reaction will occur weakly, thus compromising the value of Mantoux testing. For example, anergy is present in AIDS, a disease which strongly depresses the immune system. Therefore, anergy testing is advised in cases where suspicion is warranted that it is present. However, routine anergy skin testing is not recommended. Other factors that may cause a false-negative result include improper administration (such as subcutaneous injection, injection of too little antigen), improper storage, and contamination. PPD has been recognized to have an initial false-negative rate of 29% (Marais et al., 2006).

With a TST, it is not possible to assert or deny the presence of TB, but it only indicates infection with a mycobacterium. In a child who has not been BCG-vaccinated, a TST has been defined as positive when the diameter of skin induration is greater than 10 mm, and in a BCG-vaccinated child, when the diameter of induration is greater than 15 mm. A negative TST does not exclude TB and some induration (5–14 mm) could be supportive if the clinical features and contact history are suggestive (Lewinsohn et al., 2004). Furthermore, the utility of this conventional test is hampered by technical and logistical problems: potential for false-positive and false-negative results; problems in administration and interpretation; difficulty in separating true infection from the effects of prior BCG vaccination, infection due to nontuberculous mycobacteria (Dogra et al., 2007). In children with debilitating or immunosuppressive illnesses, malnutrition, or viral (as HIV) and certain bacterial infections, the yield is unknown, but it is certainly higher than 10%. Moreover, false-positive reactions to TST are often attributed to asymptomatic infection by environmental nontuberculous mycobacteria (Nicol et al., 2011).

Given that the US guidelines recommend that previous BCG vaccination be ignored in the interpretation of tuberculin skin tests, false positives are possible. People who have previously had BCG, will falsely appear to be tuberculin converters and this may lead to treating more people than necessary, with the possible risk of those patients suffering adverse drug reactions. However, considering the fact that BCG vaccine is not 100% effective, and that it is less protective in adults than pediatric patients, not treating these patients could lead to a possible infection which tennds to justify the current US policy. The U.S. guidelines also allow for tuberculin skin testing in immunosuppressed patients whereas the UK guidelines recommend that tuberculin skin tests should not be used for such patients because it is unreliable

### 4.2. New approaches in TB diagnostics

#### *4.2.1. Polymerase chain reaction (PCR)*

Diagnostic PCR is a technique of in vitro DNA amplification that uses specific DNA sequences (oligonucleotides) as effective fishhooks for the DNA/cDNA of microorganisms. In theory, this technique can detect a single organism in a lot of specimens such as sputum, gastric aspirate, pleural fluid, cerebrospinal fluid, blood, and urine. Various PCR assays, mostly using the mycobacterial insertion element IS6110 as the DNA marker for M. tuberculosis-complex organisms, have a sensitivity and specificity greater than 90% for detecting pulmonary TB in adults. This is a rapid, sensitive, specific, and reasonable-cost (Montenegro et al., 2003) method for the detection of M. tuberculosis in clinical samples. The PCR may be used to (a) diagnose tuberculosis in difficult samples with negative microscopic examination, negative culture, or with scarce sample; (b) determine if the organisms in the sample are M. tuberculosis or atypical mycobacteria; (c) identify the presence of genetic variations like a mutations or deletions known to be associated with resistance to some antimycobacterial agents (Marais et al., 2005).

Studies in children have obtained better sensitivity by PCR than by culture. In 2001, Gomez-Pastrana et al., (2001) reported a comparison between sensitivity of culture and PCR showing higher sensitivity for the latter. PCR may have a special role in the diagnosis of extrapulmonary TB and pulmonary TB in children since sputum smears are usually unrevealing in these cases.

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However, these tests are not performed correctly in all clinical laboratories. The cost involved, the need for sophisticated equipment, the limitations in their specificity, the need to obtain multiple samples to optimize yield and scrupulous technique to avoid cross-contamination of specimens preclude the use of PCR techniques in many developing countries (Montenegro et al., 2003).The sensitivity of PCR of gastric lavage/bronchoalveolar lavage has been found to be 56.8% in children with clinically active disease. Authors conclude that nested PCR is a rapid and sensitive method for the early diagnosis of TB in children. Additionally, other unique sequences of M. tuberculosis have been suggested as diagnostic test for TB, because they are absent in M. africanum, M. microti, M. bovis, and M. bovis BCG (Liang et al., 2008).

*4.2.2. In-house nucleic acid amplification (NAA) assays*

These assays are highly dependent on the operator's skills. Performance is also influenced by the choice of target sequence and DNA extraction method. Interpretation of the performance of these assays in pediatric TB suspects is hindered by the lack of a sensitive and specific reference standard. When compared with culture, the sensitivity of NAA for the diagnosis of childhood TB is typically low (40–83%). However, it appears, at least from some reports, that NAA identified a group of children who are clinically diagnosed with TB but in whom mycobacterial culture is negative. This means that with a proper technique it could be done efficiently (Nicol and Zar, 2011 ).

*4.2.3. Adenosine deaminase*

Adult studies have shown increased levels of adenosine deaminase (ADA) in pleural TB and TB-caused meningitis, both paucibacillary forms of TB, and have advocated for its use in diagnosis. Due to this evidence, a serum ADA has already been evaluated in a childhood population with a very high sensitivity (100%) and specificity (90.7%) for pulmonary TB. This study demonstrated the great potential of this technique because it has significant difference in serum ADA levels between children with disease and infection. However, there were several weaknesses in the study design, including unclear case definition, exclusion of nontuberculous patients, and a relatively small TB patient population (20 with active disease) (Marais. and Pai, 2007).

In the case of extrapulmonary TB, ADA measurement can be helpful, but its sensitivity and specificity varies widely and has been lower than multiplex PCR using primers for IS6110, dnaJ, and hsp65. Specifically, a meta-analysis of 63 studies of ADA in tuberculous pleuritis reveals that the sensitivity of the test is of 0.92 (95% CI 0.90–0.93) and specificity of 0.90 (95% CI 0.89–0.91) (Lawn and Nicol, 2011).

*4.2.4. Serology and antigen detection*

In absence of good diagnostic method for tuberculosis, the interest in serodiagnosis has been increased (Marais et al., 2005). Serological tests vary in a number of features, including antigen composition (38 kDa, Ag 60, and lipoarabinomannan, LAM), antigen source (native or recombinant), chemical composition (protein or lipid), extent of antigen(s) purification, and

immunoglobulin detected. The majority are based on the enzyme-linked immunosorbent assay (ELISA) rapid versions and use various immunochromatographic formats, with lateral flow being the most popular.

A recent review of serological tests concluded that commercial antibody detection tests for extrapulmonary TB have no role in clinical care or case detection (Steingart et al., 2007). The search for novel biomarkers in blood or urine that can reliably distinguish active from latent TB in children with and without other co-infections remains an important global goal. Well-defined cohorts of paediatric patients in TB-endemic and non-endemic settings will be essential for initial screening and future validation of such potential markers. In the meantime, the diagnosis of TB in children in resource-poor countries continues to rely on practical algorithms, which lack standard symptom definitions and adequate validation (Marais et al., 2006). This poses an increased challenge in the context of HIV infection

Imaz et al., (2001) reported the importance of the recombinant 16-kDa antigen (re-Ag16) of M. tuberculosis in the serodiagnosis of tuberculosis (TB) in children measuring the values of IgA, IgM, and IgG and an increased mean antibody response to reAg16 was observed in contact children compared with nonmycobacterial disease patient with a 95% of specificity. A combining result of the IgG and IgA assays led to 43% positivity in children with active TB (Trilling. et al., 2011).

Mycobacterial antigen detection has been evaluated in adults, but rarely in children. Serology has found little place in the routine diagnosis of tuberculosis in children, even though it is rapid and does not require specimen from the site of disease. Sensitivity and specificity depend on the antigen used, gold standard for the diagnosis of tuberculosis, and the type of tubercular infection. Though most of these tests have high specificity, their sensitivity is poor because several factors can alter the results such as age, exposure to other mycobacteria, and BCG vaccination (Marais et al., 2005).

#### *4.2.5. In vitro interferon-γ (IFN-γ) release assays (IGRAs) and antigen-testing*

In addition to the traditional TST, which is known to lack both sensitivity and specificity, blood based assays have recently become available. These T-cell assays rely on stimulation of host blood cells with MTB specific antigens and measure production of IFN-γ. Numerous published studies compare the two available commercial assays, T Spot TB (Oxford Immunotec) and Quantiferon-Gold IT (Cellestis), with the TST for both detection of active disease and LTBI (Ferrara et al., 2006). T-cell assays have proven to be more specific than the TST, (Arend et al., 2007) but they are still unable to distinguish between active disease and LTBI. Interpretation therefore remains dependent on the clinical context. Some few studies have presented paediatric data but none have provided an assessment of age-related performance of these assays, and reservations remain regarding their performance in very young children and in immunocompromised populations, such as those with HIV (Clark et al., 2007).

There is still a lot of on going research aimed at establishing the proper role of gamma interferon tests and the guidelines ar still under constant review. The interferon-γ release assays (IGRAs) currently commercially available include QuantiFERON-TB Gold (QFT-G),

Quanti FERON-TB Gold In-Tube and T-SPOT.TB. These tests are aimed at he body's response to specific TB antigens not present in other forms of mycobacteria and BCG (ESAT-6). The tests are not affected by prior BCG vaccination, and despite their being new, these are now becoming available globally and CDC recommends that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential-testing surveillance programs for infection control such as those for health-care workers. Health Protection Agency (HPA) recommends the use of IGRA testing in health care workers, if available, in view of the importance of detecting latently infected staff that may go on to develop active disease and come into contact with immunocompromised patients and the logistical simplicity of IGRA testing.

*4.2.6. GeneXpert MTB/RIF system*

GeneXpert includes the development of integrated DNA extraction and amplification systems. This requires minimal manipulation of sample and operator training. It utilizes real-time PCR (rt-PCR) technology to both diagnose TB and detect rifampicin resistance. The test amplifies a region of the rpoB gene of M. tuberculosis. Mutations of this region give rise to 95% of rifampicin resistance. Resistant strains contain mutations localized within the 81 bp core region of the bacterial RNA polymerase rpoB gene, which encodes the active site of the enzyme. In addition, the rpoB core region is flanked by Mycobacterium tuberculosis-specific DNA sequences. Thus, it is possible to test for M. tuberculosis and for rifampicin resistance simultaneously. The simplicity for the user makes this an assay that could feasibly be widely implemented outside centralized laboratories and potentially impacts on TB control (Gordetsov et al., (2008). The Xpert system has some advantages over the cultivation, mainly in specificity and a shorter time to get results (Imaz. et al., 2001).

Recently, Nicol et al., (2011), reported the application of this method in 452 hospitalized children from South Africa, with or without HIV, with a median age of 19.4 months, and suspected of having TB. Two Xpert tests doubled the case detection rate compared with smear microscopy (76% versus 38%), identifying all smear-positive and 61% of smear-negative cases, the specificity was 98.8%. The sensitivities for smear-negative TB were 33.3% and 61.1% when testing one or two samples, respectively. The samplings were induced sputum and they detected three quarters of culture-confirmed tuberculosis with very high specificity; the yield of this method was twice that of smear microscopy. This could suggest the possibility of replacing the microscopy for this type of methodology which has greater sensitivity especially with a second sample (Rachow et al., 2011).

*4.2.7. Gas sensor array electronic nose (electronic nose)*

The potential to detect different Mycobacterium species in the headspaces of cultures and sputum samples is another innovative approach that is currently in development. The array uses 14 sensors to profile a "smell" by assessing the change in each sensor's electrical properties when exposed to a specific odour mixture. In an initial study using sputum samples from patients with culture-confirmed tuberculosis and those without tuberculosis, the E-Nose correctly predicted 89% of culture-positive patients with a specificity of 91% (Imaz. et al.,

2001). In a further development applying advanced data extraction and linear discriminant function analysis, obtained sensitivities were of 68% and 75%, and specificities of 75% and 67% for Rob and Walter electronic noses, respectively (Imaz. et al., 2001). Further applications of this test, including its potential value in the diagnosis of child tuberculosis, are needed.

### 4.3. Diagnosing congenital TB

Congenital TB is rare but symptoms typically develop during the second or third week of life and include poor feeding, poor weight gain, cough, lethargy, and irritability. Other symptoms include fever, ear discharge, and skin lesions. The principles in place are that for one to make a definitive diagnosis of congenital TB, the infant should have proven TB lesions and that it should have at least one of the following: 1) skin lesions during the first week of life, including papular lesions (ulcerated areas of the skin) or petechiae (bleeding into the skin); 2) documentation of TB infection of the placenta or the maternal genital tract; 3) presence of a primary complex in the liver and 4) the possibility of postnatal transmission should be ruled out. Signs of congenital TB include failure to thrive, icterus (jaundice or yellow skin), hepatosplenomegaly (enlargement of both the liver and spleen), tachypnea (rapid breathing), and lymphadenopathy (involving inflammation of lymphnodes) (Marais et al., 2006). Patients with asymptomatic infection have a positive tuberculin skin test (TST) result, but they do not have any clinical or radiographic manifestations. Children with asymptomatic infection may be identified on a routine healthy-child physical examination, or they may be identified subsequent to TB diagnosis in household or other contacts ( for example, children who recently have immigrated or adopted children). Primary TB is characterized by the absence of any signs on clinical evaluation. As discussed above, these patients are identified by a positive TST result. Tuberculin hypersensitivity may be associated with erythema nodosum and phlyctenular conjunctivitis (Marais et al., 2006).

Endobronchial TB with lymphadenopathy, which is the disease with enlargement of lymph nodes, is the most common variety of pulmonary TB. Symptoms are the result of impingement on various structures by the enlarged lymph nodes. Enlargement of lymph nodes and persistent cough may result in signs suggestive of bronchial obstruction or hemi-diaphragmatic paralysis, whereas difficulty in swallowing may result from esophageal compression. Vocal cord paralysis may be suggested by hoarseness or difficulty breathing and may occur as a result of local nerve compression. Dysphagia (swallowing problems) due to esophageal compression may also be observed. Pleural effusions due to TB may also occur and usually occur in older children and are rarely associated with miliary disease. The typical history reveals an acute onset of fever, chest pain that increases in intensity on deep inspiration, and shortness of breath. Fever usually persists for 14-21 days. Signs include: tachypnea, respiratory distress, decreased breath sounds, and, occasionally, features of mediastinal shift (moving of the tissues and organs that comprise the mediastinum) (Marais et al., 2006).

Progression of the pulmonary parenchymal component of TB leads to enlargement of the caseous area (caseated = cheese-like necrotised tissue) and may lead to pneumonia, atelectasis (collapse of lung tissue), and air trapping. This is more likely to occur in young children than in adolescents. The child usually appears ill with symptoms of fever, cough, malaise, and

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weight loss. This condition presents with classic signs of pneumonia, including tachypnea, nasal flaring, grunting, dullness to percussion, egophony or egobronchophony (increased resonance of voice sounds, with a high-pitched bleating quality, heard especially over lung tissue compressed by pleural effusion); decreased breath sounds, and crackles (Marais et al., 2006). Reactivation of TB disease usually has a sub-acute presentation with weight loss, fever, cough, and, rarely, hemoptysis (coughing up of blood or bloody sputum from the lungs or airway). This condition typically occurs in older children and adolescent and is more common in patients who acquire TB at age 7 years and older. Physical examination results may be normal or may reveal post-tussive crackles (Marais et al., 2006).

### 4.4. Diagnosis of extrapulmonary TB

In this case the clinical picture is used to get an indication of the diagnosis. The diagnosis at any site should be confirmed by obtaining specimens for bacteriology wherever possible. This means that fluid aspirated or biopsies taken should be placed in a medium such as saline which will not kill the bacteria. Too often still biopsy specimens are placed in formalin so that bacteriological confirmation including sensitivity testing cannot be done. Miliary TB may manifest sub acutely with low-grade fever, malaise, weight loss, and fatigue. A rapid onset of fever and associated symptoms may also be observed. History of cough and respiratory distress may be obtained. Physical examination findings include lymphadenopathy, hepatosplenomegaly, and systemic signs including fever. Respiratory signs may evolve to include tachypnea, cyanosis, and respiratory distress. Other signs, which are subtle and should be carefully sought in the physical examination, include papular, necrotic, or purpuric lesions on the skin or choroidal tubercles in the retina (Marais et al., 2006).

Patients with lymphadenopathy (scrofula or deposits in subcutaneous lymphatic ganglia) may have a history of enlarged nodes. Fever, weight loss, fatigue, and malaise are usually absent or minimal. One of the most severe complications of TB is TB meningitis, which develops in 5-10% of children younger than 2 years; thereafter, the frequency drops to less than 1%. A very high index of suspicion is required to make a timely diagnosis because of the insidious onset of the disease. A sub-acute presentation usually occurs within 3-6 months after the initial infection. Nonspecific symptoms such as anorexia, weight loss, and fever may be present. After 1-2 weeks, patients may experience vomiting and seizures or alteration in the sensorium (the part of the cerebral cortex that receives and coordinates all the impulses sent to individual nerve centers which includes auditory, gustatory, olfactory, somatosensory and visual centers). Deterioration of mental status, coma, and death may occur despite prompt diagnosis and early intervention.

Three stages of TB meningitis have been identified. Stage 1 is defined by the absence of focal or generalized neurologic signs. Possibly, only nonspecific behavioral abnormalities are found. Stage 2 is characterized by the presence of nuchal rigidity (inability or discomfort during neck flexion), altered deep tendon reflexes, lethargy (abnormal lack of energy), and/or cranial nerve palsies. TB meningitis most often affects the sixth cranial nerve due to the pressure of the thick basilar inflammatory exudates on the cranial nerves or to hydrocephalus; this results in lateral rectus palsy. The third, fourth, and seventh cranial nerves may also be affected. Funduscopic

changes may include papilledema (swelling of the optic disc from increased intracranial pressure) and the presence of choroid tubercles (chroid plexus = vascular proliferation of the cerebral ventricles that serves to regulate intraventricular pressure by secretion or absorption of cerebrospinal fluid).which should be carefully sought. Stage 3, the final stage, comprises major neurologic defects, including coma, seizures, and abnormal movements such as choreoathetosis (irregular involuntary movements that may involve the face, neck, trunk, extremities, or respiratory muscles, giving an appearance of restlessness), paresis (slight or incomplete paralysis), paralysis of one or more extremities. In the terminal phase, decerebrate (elimination of cerebral brain function) or decorticate posturing, opisthotonus (a type of spasm in which the head and heels arch backward in extreme hyperextension and the body forms a reverse bow), and/or death may occur. Patients with tuberculomas or TB brain abscesses may present with focal neurologic signs. Spinal cord disease may result in the acute development of spinal block or a transverse myelitis–like syndrome (an abnormal condition characterized by inflammation of the spinal cord with associated motor or sensory dysfunction). A slowly ascending paralysis may develop over several months to years.

### 4.5. Treatment

#### *4.5.1. General treatment overview*

Each of the first-line drugs makes a specific contribution during different periods of drug action (assuming complete drug susceptibility and the absence of significant immune compromise). Period 1 lasts 2 to 3 days (van der Weert et al., 2006), during which time fast-growing extracellular bacilli, comprising the vast majority of the organism load, are killed, mainly by the excellent bactericidal activity of isoniazid (INH) (Kampmann et al., 2005). Period 2 lasts 4 to 8 weeks. Slower growing extracellular bacilli are killed (van der Weert et al., 2006) and the rate of killing is determined more by the physiological state of the bacilli and less by the bactericidal activity of the drug. During this period, the bactericidal activity of rifampin (RIF) is important and pyrazinamide (PZA) contributes by killing extracellular bacilli that persist in acidic areas of inflammation (van der Weert et al., 2006). Period 3 lasts 4 to 6 months. Persistent intracellular bacilli are eradicated mainly by RIF, although INH will continue to offer protection against the development of resistance and may assist with organism eradication, especially in fibrocaseous tissue with poor drug penetration. Host immunity plays an important role throughout, but is of particular importance to effect organism eradication and prevent disease relapse, as indicated by the high relapse rate in HIV-infected children.

Practical operational issues are extremely important for effective public health intervention. Operational issues include access to early and accurate diagnosis, the uninterrupted provision of quality-assured drugs and appropriate treatment regimens, as well as the establishment of systems to ensure good treatment adherence. Fixed-dose combinations should be used whenever possible to reduce the risk of drug resistance and to improve simplicity and adherence, but quality assurance is essential to ensure optimal bioavailability of all the constituent drugs (Dekker and Lotter, 2003). With proper implementation, the World Health Organization's directly observed therapy, short-course (DOTS) strategy addresses most of the

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important operational issues. However, the predominant emphasis of the DOTS strategy on sputum smear–positive disease excludes the vast majority of children. There is a desperate need to improve service delivery to children with tuberculosis, particularly in endemic areas with limited resources (Starke, 2002).

*4.5.2. Preventive chemotherapy*

Chemoprophylaxis refers to preventive treatment given after exposure (without proof of infection), whereas treatment of latent infection implies that infection (indicated by a positive TST) was documented. The term preventive chemotherapy is preferred because it is more inclusive and incorporates both chemoprophylaxis and treatment of latent infection. The TST is a fairly accurate measure of infection after exposure in immune-competent children, although TST conversion, which reflects a sufficiently strong delayed-type hypersensitivity response, may be delayed for up to 3 months (Marais et al., 2004). Therefore, household exposure, particularly involving high-risk children, should be treated as infection until the absence of infection can be convincingly demonstrated. In immune-competent children this can be done by repeating the TST 3 months after exposure ended (American Thoracic Society, 2000). In immunocompromised children the TST is not a sufficiently reliable test to exclude M. tuberculosis infection and children with documented exposure should receive preventive chemotherapy as if they are infected (Marais et al., 2006).

The reality on the ground is that most endemic areas do not have the capacity to follow current World Health Organization guidelines regarding the use of preventive chemotherapy in children, which advise active tracing and screening of all children less than 5 years old in household contact with a sputum smear–positive adult source case. This results mainly from the huge burden of adult tuberculosis and resource constraints that limit the ability to perform TST and chest X-ray screening tests. Because the TST and chest X-ray are regarded as prerequisite screening tests, screening of exposed children and the provision of preventive chemotherapy are not even attempted in most resource-constrained areas. Access to preventive chemotherapy in these settings may be improved by employing symptom-based screening, although the benefits and risks of such a simplified approach require further evaluation. A study from an endemic area indicated that symptom-based screening may identify those children who require further investigation to exclude active tuberculosis (Marais et al., 2006), thus allowing asymptomatic household contacts, especially those who are at high risk to progress to disease, immediate access to preventive therapy despite the inability to perform TST and chest X-ray–based screening (Marais et al., 2006).

Another consideration is that in some endemic areas the majority of disease transmission, particularly in children greater than 2 to 3 years of age, occurs outside the household (Verver et al., 2004). In endemic areas, narrowing the focus of contact tracing to those children who are at highest risk to progress to disease after exposure or infection (less than 3 years of age and/or immune compromised) will decrease the burden placed on already overstretched health care systems, while still ensuring access to preventive chemotherapy for the children who need it most (Van Zyl et al., 2006). In older (greater than 3 years of age), immune-competent children the risk of tuberculosis after exposure is low and disease progression is

usually indicated by the presence of persistent, slowly progressive symptoms. Therefore, passive case finding together with adequate diagnostic vigilance seems appropriate in this low-risk group.

In non endemic areas where resources permit and where the risk of future reinfection is low, it seems warranted to extend preventive chemotherapy to low-risk children as well, to eliminate the reservoir of latent infection within the community. INH monotherapy for 6 to 9 months is the best-studied chemoprophylactic regimen and it reduces the tuberculosis risk in exposed children by at least two-thirds; probably by more than 90% with good adherence. However, poor adherence is a major concern, particularly in endemic areas (Van Zyl et al., 2006).

In real life the effectiveness of a preventive chemotherapy regimen is determined first by its efficacy and second by adherence to the prescribed regimen. Because of documented poor adherence to 6–9 months of unsupervised INH monotherapy, consideration should be given to alternative preventive strategies with comparable efficacy but with improved adherence. Theoretically the addition of RIF has important advantages; RIF has strong sterilizing activity to eradicate latent bacilli and its addition will shorten the duration of treatment required (Mitchison, 2005). It will also improve efficacy in settings where INH mono-resistance is prevalent. The use of a 3-month INH and RIF regimen for preventive chemotherapy is well established and trials have shown equivalence to 6 to 9 months of INH alone, although the evidence is not as comprehensive as that for INH monotherapy (Ena and Valls, 2005).

PZA is another important sterilizing drug and in theory the combination of RIF and PZA represents the treatment of choice for latent infection. This combination has proven efficacy in animal but adverse reactions in adults have limited the initial enthusiasm (Priest, 2004). However, these adverse reactions have not been observed in children, in whom the three-drug combination of INH, RIF, and PZA is generally well tolerated (Marais et al., 2006). Adherence may be improved by shortening the duration of treatment, but consideration may also be given to the provision of supervised preventive therapy. Creative approaches will be required to achieve this, particularly in places where health care services are already overburdened. With curative treatment, intermittent (two or three times weekly) therapy during the continuation phase is as effective as daily therapy to achieve organism eradication, once the organism load has been sufficiently reduced (Al-Dossary et al., 2002). The same principle would apply to the treatment of latent infection, where the organism load is low. Targeting high-risk children for short-course, supervised intermittent preventive therapy seems achievable, but defining optimal preventive therapy regimens remains a fertile and important area for future research (Marais et al., 2006).

Vaccination with BCG is the most widely used preventive strategy, although its efficacy remains controversial and studies have shown that it contributes to this variable protection: variations in strain-specific immunogenicity, timing and technique of vaccine administration, genetic factors, the presence or absence of environmental mycobacteria, and the effect of multiple re-infection events as may occur in highly endemic areas. It is generally accepted that BCG vaccination offers significant protection against disseminated disease in young children (below 2 years), but that it offers little or no protection against adult-type tuberculosis.

However, reports have documented significant protection against the development of adult-type tuberculosis when BCG was administered to TST-negative adolescents in locations with a low prevalence of environmental mycobacterial exposure (Bjarveit et al., 2003).

In addition, a report from Turkey indicated that contrary to the prevailing theory, BCG may also protect against M. tuberculosis infection as based on a positive enzyme-linked immunospot result. An even more controversial area is the risk versus benefit that BCG provides to HIV-infected children. There is a definite risk for HIV-infected infants to develop severe forms of BCG disease after neonatal BCG vaccination (Hesseling et al., 2006), but it remains poorly quantified. As the risk:benefit ratio has not been determined, the World Health Organization still advises BCG vaccination of asymptomatic HIV-exposed infants in tuberculosis endemic areas. Establishing the risk: benefit ratio of BCG vaccination in HIV-infected infants and the development of novel vaccines with improved efficacy and safety, remain major research challenges (Marais et al., 2006).

*4.5.3. Curative treatment*

The main variables that influence the success of chemotherapy, apart from primary drug resistance, are the bacterial load and the anatomic distribution of bacilli. Cavitary disease indicates a high bacterial load, as demonstrated by the frequency with which these patients are sputum smear–positive, which implies an increased risk for random drug resistance against individual drugs. Disseminated disease may signify penetration of bacilli into the central nervous system (CNS) (Van den Bosch et al., 2004) implying that adequate drug penetration across the blood–brain barrier is an important requirement for the treatment of disseminated disease (Marais et al., 2006).

From a public health perspective the challenge is to develop a pragmatic classification of childhood tuberculosis that incorporates the diverse spectrum of disease, but focuses primarily on treatment relevance. The main variables that influence the success of chemotherapy identify three groups of children with tuberculosis: (1) those with sputum smear–negative disease, (2) those with sputum smear–positive (often cavitary) disease and (3) those with disseminated disease. The discussion reflects current treatment guidelines for these three groups as well as the new regimens to consider on the basis of established treatment principles (Marais et al., 2006).

As a guide for individual patient classification and management five simple questions have been formulated: (1) Is the child exposed to or infected with M. tuberculosis? (2) Does the child have active tuberculosis? (3) If the child is exposed or infected, but does not have active tuberculosis, is preventive chemotherapy indicated? (4) If the child has active tuberculosis, what is the appropriate treatment regimen? (5) Are there any special circumstances such as HIV infection, retreatment, or exposure to a drug-resistant source case to consider? The underlying rationale is universally applicable irrespective of diagnostic or resource constraints; although areas with access to advanced technology may achieve improved levels of diagnostic certainty.

Sputum smear–negative disease is usually paucibacillary and therefore the risk of acquired drug resistance is low. Drug penetration into the anatomic sites involved is good and the success of three drugs (INH, RIF, and PZA) during the 2-month intensive phase, and of two drugs (INH and RIF) during the 4-month continuation phase, is well established. In the presence of extensive radiographic disease with or without cavitation, and/or suspicion of INH resistance, the use of ethambutol (EMB) in addition to the three drugs during the intensive phase should be contemplated. After completion of the intensive phase, successful organism eradication may be achieved with intermittent (two or three times weekly) therapy during the continuation phase (Al-Dossary et al., 2002). The efficacy of shorter treatment durations for HIV-uninfected immune-competent children with sputum smear–negative disease requires further evaluation, as a 4-month regimen of INH and RIF may be an acceptable therapy for some adults with sputum smear– and culture-negative tuberculosis.

Sputum smear–positive disease implies a high organism load and an increased risk for random drug resistance against individual drugs. Selecting drug-resistant mutants is a particular concern where INH mono-resistance is prevalent, as this increases the likelihood of selecting multidrug-resistant (MDR) organisms. The use of four drugs (INH, RIF, PZA, and EMB) during the 2-month intensive phase should reduce this risk. Once the organism load is sufficiently reduced, intermittent (two or three times weekly) therapy with INH and RIF during the 4-month continuation phase is sufficient to ensure organism eradication (Al-Dossary et al., 2002). However, caution should be exercised when initial treatment response has not been optimal and in HIV-infected patients. The use of long-acting rifamycins together with INH is discouraged (Rieder et al., 2001).

Disseminated disease is frequently associated with CNS involvement (Donald et al., 2005). It is therefore essential to consider the cerebrospinal fluid (CSF) penetration of drugs used in the treatment of disseminated disease. INH and PZA penetrate the CSF well. RIF and streptomycin penetrate the CSF poorly, but may achieve therapeutic levels in the presence of meningeal inflammation. The value of streptomycin is limited by poor CSF penetration and intramuscular administration. EMB hardly penetrates the CSF, even in the presence of meningeal inflammation, and has no demonstrated efficacy in the treatment of TBM. Ethionamide shows good CSF penetration and has been used successfully as a fourth drug in the treatment of TBM. The fact that RIF penetrates the CSF poorly in the absence of meningeal inflammation reduces its sterilization value and may warrant the inclusion of PZA during the continuation phase, to assist with CNS sterilization.

Several reports have illustrated the efficacy of short-course regimens in the treatment of TB meningitis, but the risk of CNS relapse is rarely reported. In two of these studies a relapse was documented despite the completion of 6 months of treatment with INH and RIF with an initial 2 months of PZA. Therefore, it seems prudent to include a fourth drug with good CNS penetration (such as ethionamide) for the treatment of disseminated disease, at least during the intensive phase, and to consider PZA for the full 6 months of treatment to reduce the risk of CNS relapse. CNS relapse is rare in the United States, where PZA is routinely discontinued after 2 months, but the total treatment duration is 9 to 12 months. Current fixed-dose combination tablets provide 4 to 6 mg of INH per kilogram. This dose may be suboptimal, particu-

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larly in settings where the majority of the bacterial population rapidly acetylates INH (Schaaf et al., 2005). In addition, the serum level achieved with a similar dose of INH per kilogram is lower in children than in adults, increasing the risk for suboptimal dosing in children (Schaaf et al., 2005). The majority of new INH resistance encountered in endemic areas is of an intermediate or low level, which underscores the importance of optimal INH dosing (Donald et al., 2004). A standard INH dose of 10 mg/kg seems appropriate in children, as even doses up to 20 mg/kg are well tolerated (Schaaf et al., 2005); children are less susceptible to the toxic effects of INH than are adults.

In general, adverse events are less common in children than in adults. The most severe adverse event is the development of hepatotoxicity, which can be caused by INH, RIF, PZA, or ethionamide. An elevation of liver enzymes (less than five times normal values) is not an indication to stop treatment, but the occurrence of liver tenderness, hepatomegaly, or jaundice should prompt the immediate stopping of all potentially hepatotoxic drugs. Jaundice is often preceded by a period of days or weeks of malaise and nausea. Hepatic reactions usually occur in the first weeks of therapy, but may happen at any time during the treatment period. Drug-related hepatic toxicity is usually caused by a single drug, but rarely a combination of drugs, which individually cause no problem, may cause hepatic toxicity. Children should be screened for other causes of hepatitis, as in many cases the anti-tuberculosis drugs are not the cause of liver function derangement. In South Africa, hepatitis A infection is frequently responsible for non–drug-related liver function derangement in children receiving anti-tuberculosis treatment. Potentially hepatotoxic drugs should be reintroduced only after liver functions have normalized. Non-hepatotoxic drugs should be used in the interim and expert opinion should be sought.

Ethambutol is usually not advised in children less than 7 years as visual acuity cannot be evaluated. However, its use may be warranted in children with hepatotoxicity, cavitary disease, or resistance to first-line drugs; it seems safe at recommended dosages. Ethionamide frequently causes vomiting, but this can usually be overcome by dividing the daily dose and by a slow increase up to the full dose during the first week or two of therapy. Recommended dosages for the various first- and second-line drugs are reflected in the publication by Marais et al., (2006) as indicated in the Table 1 below:

Despite significant symptomatic improvement radiographic disease resolution may take many months; persistent radiographic signs are not an indication to change treatment if there is clinical improvement. Paradoxical exacerbation of symptoms or signs may also occur after anti-tuberculosis therapy is initiated. This results from immune reconstitution with increased inflammation, particularly surrounding diseased lymph nodes or tuberculomas, that may follow nutritional rehabilitation (Marais et al., 2004), and/or antiretroviral therapy. The release of bacterial toxins after successful anti-tuberculosis treatment may also contribute.

Treatment should be continued unaltered, although the temporary addition of corticosteroids may be considered. Such adjunctive therapy may be helpful in a number of disease manifestations where the host inflammatory response contributes to disease pathology such as CNS involvement, severe lymph node compression of the airways, and pericardial effusion. There

| | | Maximum Dosage (mg/kg/dose) | |
|---|---|---|---|
| | **Mode of Action** | **Daily** | **Two or Three Times/wk** |
| **First-line drugs** | | | |
| Isoniazid | Bactericidal | 10–15 (300 mg) | 20–30 (900 mg) |
| Rifampin | Bactericidal and sterilizing | 10–20 (600 mg) | 10–20 (600 mg) |
| Pyrazinamide | Sterilizing | 20–40 (2,000 mg) | 50 (2,000 mg) |
| Ethambutol | Bacteriostatic | 15–25 (1,200 mg) | 30–50 (2,500 mg) |
| **Second-line drugs** | | | |
| Ethionamide or prothionamide | Bactericidal | 15–20 (1,000 mg) | NA |
| Streptomycin | Bacteriostatic | 20–40 (1,000 mg) | NA |
| Fluoroquinolones | Bactericidal | | NA |
| Ciprofloxacin | | 20–40 (1,500 mg) | |
| Aminoglycosides | Bacteriostatic | | NA |
| Kanamycin | | 15–30 (1,000 mg) | |
| Amikacin | | 15–30 (1,000 mg) | |
| Capreomycin | | 15–30 (1,000 mg) | |
| Cycloserine or terizidone | Bacteriostatic | 10–20 (1,000 mg) | NA |
| Para-aminosalicylic acid | Bacteriostatic | 200–300 (10 g) | NA |

NA = not applicable. Source: Marais et al., (2006).

**Table 1.** First- And Second-Line Antituberculosis Drugs And Recommended Dosages In Children

is insufficient evidence to demonstrate whether steroids are effective in tuberculous pleural effusion.

### *4.5.4. Retreatment*

Anti-tuberculosis treatment rarely fails in children and, if it does, every effort should be made to find the most likely cause. In settings where the prevalence of drug resistance is low the commonest cause is failure to properly take the medications, which can occur even during DOT, if supervision is not complete. It is important to remember that non-adherence has a differential diagnosis; there are psychologic, sociologic, religious, economic, and practical reasons why people are non-adherent and one must deal with all these issues for chemotherapy to be successful. With treatment interruption the child may be restarted on the original treatment regimen while ensuring adequate supervision, as the risk of developing drug resistance is small in children with paucibacillary disease. If an immune-competent child presents with a new episode of tuberculosis more than 6 months after completing treatment

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for a previous episode, then it most likely represents re-infection disease and standard first-line treatment is appropriate. In the case of genuine treatment failure (absence of clinical response to supervised treatment) drug susceptibility testing is of paramount importance. If an adult source case is identified with drug-resistant tuberculosis, the child should be treated according to the drug susceptibility pattern of the source case's strain (Marais et al., 2006).

#### *4.5.5. Treatment of paediatric TB/HIV co-infection*

The high risk of HIV-infected children to progress to disease after infection justifies the use of preventive chemotherapy in children who are latently infected. However, the difficult issue in endemic areas is how to deal with the ever-present risk of undocumented re-infection within the community. The prevention or reversal of severe immune compromise by using highly active antiretroviral therapy (HAART) should preclude the need for repeated or continuous preventive chemotherapy, although the risk for tuberculosis probably remains higher than in HIV-uninfected children. The cellular immune response assists with organism eradication and therefore it is not unexpected that disease relapse has been documented in HIV-infected children. The value of prolonging the treatment duration from 6 to 9 months, to ensure organism eradication in HIV-infected children, is under investigation. During a repeat episode both relapse and reinfection should be considered and every effort should be made to establish a culture-confirmed diagnosis and to do drug susceptibility testing (Marais et al., 2006).

When initiating treatment (curative treatment or RIF-containing preventive therapy) in HIV-infected children already receiving HAART or for whom HAART is contemplated, it should be appreciated that the rifamycins, especially RIF, and some of the nonnucleoside reverse transcriptase inhibitors and/or protease inhibitors may cause significant drug interactions. HIV-infected children may also develop particularly pronounced paradoxical reactions after the institution of HAART, because of immune reconstitution inflammatory syndrome. Recommendations on optimal drug combinations are frequently revised. The most recent recommendations can be obtained from the Centers for Disease Control and Prevention website, at [http://www.cdc.gov/nchstp/tb/].

Latest WHO recommendations advise starting antiretroviral therapy (ART) once anti-TB therapy (ATT) is established (after a period of 2-8 weeks) for all WHO clinical Stage Four HIV-infected children and Stage Three children with advanced or severe immunosuppression. For children in WHO clinical stage with mild or no immunosuppression, ART may be deferred until 6 months of ATT are completed (WHO, 2006). On-going prospective trials involving adults and children in TB/HIV endemic countries might provide future guidelines for the ideal timing of the initiation of anti-retroviral therapy (ART) in patients with HIV receiving TB therapy. There is already evidence from prospective trials that shows that high mortality is associated with TB in advanced stages of HIV-disease in children who do not receive ART promptly. Further research is required to improve our understanding of immune reconstitution disease (IRD) in children (Walters et al., 2006). Also, therapeutic drug monitoring (TDM), where available, should be undertaken when children are receiving concomitant ART and ATT. TDM data from ethnically similar children in resource-rich countries may in the future inform dosing recommendations in resource-poor settings where TDM is not available.

*4.5.6. Treatment of extrapulmonary paeditric PTB*

Treatment is as for pulmonary disease, with isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampicin for four months, except for CNS disease when treatment should be continued for a full year. Steroids may be used in pericardial and meningeal disease. Surgery is usually unnecessary, especially where lymph glands and abscess are present, as long term discharging sinuses may result. Surgery is sometimes necessary in spinal TB where there is instability and may be needed to overcome strictures in genito-urinary or gastro-intestinal disease. Occasionally pericardectomy may be required when pericardial disease causes tamponade.

*4.5.7. Treatment of latent paediatric TB infection*

Treatment of LTBI, also known as chemoprophylaxis, is important to prevent future disease activation. The fact that over 50% of hospitalized children with culture-confirmed TB have a reported close TB contact and do not receive chemoprophylaxis, is an indication of the important missed opportunities using existing public health interventions. For the last 20 years the WHO guidelines recommended all children under 5 years in close contact with an infectious (usually smear positive) case receive 6 months isoniazid. Once active disease has been excluded, isoniazid monotherapy for 6-9 months has been proven to reduce the TB risk in exposed children by over 90% with good adherence. More recent studies suggest that 3 months of combined isoniazid and rifampicin are equally effective (Ena and Valls, 2005). In a recent study with very short follow-up, continuous isoniazid prophylaxis for HIV-infected children without documented evidence of latent infection, but living in an environment of high exposure, has also been shown to reduce overall morbidity and mortality from TB and other infections (Zar et al., 2007). Further trials in HIV-infected children receiving ART are ongoing.

Recommendations for chemoprophylaxis will continue to differ in TB-endemic and non-endemic settings, because of the perceived risk of exposure. Whilst most paediatricians in Europe and North America would advocate chemoprophylaxis for HIV infected, TB-exposed children only, this needs to be interpreted with caution if the exposure is potentially ongoing or recurrent, and the ability to distinguish LTBI from active disease is limited. In this context, many practitioners in TB-endemic settings are reluctant to place children on chemoprophylaxis because of the potential emergence of resistant strains, if indeed the child has active disease instead of LTBI.

*4.5.8. Treatment of drug resistant paediatric TB*

Acquisition of resistance rarely occurs in children due to the paucibacillary nature of their disease but overall, children may also be subject to less selection pressure from anti TB therapy. Thus most resistance in children is due to primary transmission of a resistant organism, and MDR /XDR-TB rates in children reflect community transmission rates. Diagnosis requires a high index of suspicion as the culture yield in children makes definitive microbiological confirmation difficult. Resistance should be suspected if an index case has known resistant TB; the child shows initial improvement on anti-TB therapy and then deteriorates; or there is no

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response to initial treatment. Acquired resistance is well described in HIV co-infected adults previously treated for TB, possibly due to malabsorption of anti-TB drugs (Wells, et al., 2007). The presence of acquired resistance in the paediatric population is reported and in particular children with TB/HIV co-infection should be closely monitored (Soeters et al., 2005).

Although the principles of DOTS Plus have been put forward for the management of MDR TB, at the moment, there is no consensus for any regimen or optimal treatment that should be used for persons with known exposure to MDR-TB. The recommendation by CDC is a combination of pyrazinamide and ethambutol, with either pyrazinamide or a fluoroquinolone and that immunocompetent contacts should be treated for 6 months while immunocompromised contacts should be treated for 12 months. Current guidelines recommend using at least four drugs to which the patient is naïve, including an injectable and a fluoroquinolone, in an initial phase of at least 6 months; followed by at least three of the most active and best tolerated drugs in a 12-18 month continuation phase.

Standardised regimens have been developed for settings where drug susceptibility testing is not available (WHO, 2006). Six classes of second-line drugs (SLDs) are available (WHO, 2003) but experience in children is limited for the majority and multi-centre paediatric trials are needed. Under optimum circumstances MDR-TB responds well to appropriate therapy. However delays in diagnosis and treatment, adherence issues, and a lack of child-friendly formulations and strategies for DOTS all frequently complicate management and contribute to a high morbidity and mortality (Drobac et al., 2006).

The WHO currently recommends avoidance of chemoprophylaxis in cases of contact with known MDR-TB and to observe for 2 years if clinically asymptomatic. Children with latent MDR-TB infection become the reservoir for future transmission following disease reactivation in adulthood, emphasizing the need to further research and improved management of MDR-TB infection in children, both at the clinical and operational level.

According to the European Centre for Disease Prevention and Control (ECDC) 2012 Guidelines, there are two valid options to consider for the management of MDR TB and XDR TB contacts; preventive treatment or follow-up by careful clinical observation. The purpose of preventive therapy is to prevent the progression of LTBI to TB disease in an individual who has been exposed to MDR/XDR TB. The concept of preventive therapy has been shown to be effective for LTBI after contact with drug-susceptible TB but corresponding evidence for preventive therapy of MDR TB and XDR TB contacts is very scarce. Although for children there are indications of a positive effect of preventive therapy, for other groups of contacts, the necessary body of evidence has yet to be generated, and there are ongoing studies to collect evidence in support of the use of preventive therapy in contacts of MDR TB cases.

There is currently no evidence available on the optimal follow-up time in contacts of MDR TB or XDR TB with regard to patient benefits and costs of the intervention. In young children under five years of age the majority ( over 90%) of TB disease will develop within 12 months of infection. Infants and children under five years of age, immunocompromised individuals due to HIV infection or TNF-antagonist treatment are at increased risk of progression from LTBI to TB disease. These individuals as well as other

identified risk groups require special attention as part of the individual risk assessment (WHO, 2007; Salgado and Solovic et al., 2010).

The optimal duration of MDR-TB treatment in children is not known. World Health Organization guidelines recommend treatment until 18 months after the first negative culture (24 months in XDR-TB). As children often have paucibacillary disease, documenting a culture conversion is usually difficult. Thus, the same duration as in adults would apply. The duration of the intensive phase of treatment (when an injectable drug is given) should be at least 6 months. Surgical resection should be considered when the patient has localized lesions and has persistently positive smear or culture results inspite of aggressive chemotherapy (Shah, 2012).

*4.5.9. BCG vaccination and HIV infection*

Approaches for prevention of TB include prevention of infection (through immunization) or of progression from latent infection to disease (chemoprophylaxis). Bacille Calmette-Guérin (BCG) vaccine, a live attenuated vaccine derived from Mycobacterium bovis that was developed in the 1920s, is administered to children at birth in many countries. WHO guidelines recommend administration of BCG soon after birth to all infants in countries with a high TB prevalence. Current WHO guidelines advise that all children below 5 years of age, who are in close contact with a sputum smear-positive index patient, should be actively traced, screened for TB, and provided preventive chemotherapy after active TB has been excluded (Marais et al., 2004).

Although this is good policy, implementation is fraught with challenges, including difficulty in diagnosing latent TB in a highly BCG-vaccinated population, ruling out incipient active disease, and the lack of procedures for documentation and follow-up of contact screening and chemoprophylaxis in national programs. Because the majority of transmission in children below 3 years of age occurs in the household and they are also the group at highest risk of progression to disease after primary infection, this activity should be given higher priority in national infection-control programs. Moreover, active tracing and screening of household contacts at high risk would allow children with disease to receive a diagnosis earlier, thus reducing complications.

Furthermore, additional protection by revaccination with BCG has not been demonstrated (Rodrigues et al., 2005). To date, the efficacy of the BCG vaccination has not been determined in HIV infected individuals in whom the immune responses to BCG may be reduced, (Hesseli et al., 2007) although this is the subject of ongoing trials. Due to the risk of disseminated BCG disease which may rarely complicate use of this live vaccine in immunocompromised individuals, BCG vaccination is no longer recommended in children known to be HIV-infected (Hesseling et al., 2007). In practice, this has had little impact in HIV-endemic countries, where the HIV-status of the baby is rarely established at birth, the usual time of BCG vaccination.

A large trial in southern India that included over 350,000 participants aged above 1 year concluded that BCG vaccine did not offer protection against the development of adult pulmonary TB (WHO, 2006). However, BCG vaccine has been shown to be protective against

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disseminated forms of TB in young children, with a protective estimate ranging from 67%–79% against TB meningitis and 58%–87% against miliary disease. A theoretical model estimated that a universal BCG vaccine program would have a beneficial impact in settings with prevalence rates of greater than 30 sputum smear-positive cases/100,000 population (WHO, 2007). However, there is no evidence of any BCG-induced protective effect in HIV-infected children. On the contrary, studies have documented BCG-induced disseminated disease and adverse reactions. Therefore, the WHO recommendations have been revised, making HIV infection a contraindication for BCG vaccination, even in settings where TB is highly endemic. Strategies required for effective implementation of this policy change include high uptake of maternal HIV testing coupled with implementation of proven strategies to prevent mother-to-child HIV transmission, including maternal treatment with HAART and early virological diagnosis of HIV infection in infants, followed by treatment.

The revised recommendations present a dilemma for national programs. Although the benefits of BCG vaccine far outweigh the risk among HIV-uninfected children living in high areas with a high prevalence of TB, the risk is higher among HIV-infected infants with or without symptoms at the time of vaccination. National recommendations will need to consider a variety of factors, including the prevalence of TB in the population, the prevalence of HIV infection, the availability of HIV testing and facilities for prevention of mother-to-child transmission during pregnancy, the capacity to conduct follow-up of vaccinated children, and the availability of early infant diagnosis of HIV infection. Abandoning the use of BCG vaccine before newer vaccines become available may put millions of young children at risk of TB. There is an urgent need for operational research in TB endemic countries to determine the best way to manage this issue programmatically.

## 5. On-going research targeting paediatric TB

### 5.1. New vaccine pipelines

The global commitment of the WHO and the Stop TB (WHO, 2005) campaign has spurred on the efforts of the international research community to develop a more effective anti-TB vaccine by the year 2015. In view of the proven efficacy of existing BCG vaccine in preventing disseminated TB in children and reducing child mortality (Roth et al., 2006) two conceptually different strategies have been pursued: firstly, the development of 'priming vaccines', which, it is hoped, will replace BCG by providing better and longer protection; secondly, the design of 'booster vaccines' to boost pre-existing BCG-derived immunity. Novel vaccines currently under development all use a "booster-strategy" after priming with BCG in infancy (Doherty et al, 2007). As the current candidates are progressing through phase I and II trials, including studies in HIV-infected individuals and age-de-escalation, it is most likely that more than one vaccine will progress into phase III.

The most advanced vaccine candidate is MVA- 85A, currently in phase II under a prime-boost strategy with BCG. Four products are in phase I (72f, Hybrid 1, Aeras 402, rBCG-UreC-Hly), each stemming from PPPs. Many of the candidates are results from the EU FP6 projects, i.e.

TBVAC and Muvapred, where valuable progress has been achieved. Several other candidates are still in the pre-clinical phase. For example, mutation of virulence genes produced a TB strain potentially conferring greater protection with fewer side effects than BCG. In addition, an improved, recombinant BCG vaccine with a higher efficacy and a better safety profile moving into phase I clinical trials is a possible prospect.

New research is directed at the development of a multistage TB vaccine containing latency antigens, an attractive concept, which is actively being pursued (Andersen, 2007). Such a vaccine could be used as a booster vaccine with the goal of preventing new infections in those uninfected with MTB and to prevent reactivation in those with LTBI. Unfortunately, the lack of reliable correlates of protective immunity currently remains a major obstacle to predict vaccine efficacy in all TB vaccine trials for both adults and children.

## 6. Existing research gaps

### 6.1. Research needs

Tracing of MDR TB contacts is important to prevent TB disease and further transmission. Priority studies needed include those to identify the most effective contact-tracing procedures for close contacts and the most effective follow-up procedures in healthcare workers constantly exposed to MDR TB. As part of the management of MDR TB contacts, studies on specific groups are needed, for example on children below the age of five years, children with HIV infection and other immunocompromised states. In particular, studies are needed: 1) for treated contacts: (randomised) clinical trials: 2) to determine which drugs and which drug combinations and dosages are optimal for preventive therapy; 3) to determine the duration of preventive therapy; 4) to assess the effectiveness of preventive therapy in conjunction with antiretroviral treatment; 5) to assess the risk of development of new drug resistance in contacts receiving (inadequate and adequate) preventive therapy; 6) for untreated contacts, and healthcare workers constantly exposed to MDR TB: 7) to identify the optimal follow-up period for different groups of individuals; and 8) to identify the optimal frequency of testing for LTBI during the follow-up period.

In order to increase adherence to treatment of MDR/XDR TB contacts (and reduce the risk of development of new drug resistance in contacts), studies are needed: 1) to identify new drugs with less adverse events and to explore possible (positive and negative) interactions between combined drugs; 2) to identify biomarkers indicating the risk of progression from LTBI to TB disease and 3) to assess operational management to shorten preventive therapy. Since the provision of preventive therapy has economic and logistic implications at the national and community level, cost-effectiveness and cost-benefit studies are also needed. These studies are particularly valuable because they can help to inform the decision on intervention policies..

A substantial amount of funding has been injected into research on various aspects of TB but there are still many issues that require additional research especially in the area of childhood tuberculosis. The most salient ones include:1) accurately quantifying the global burden of

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childhood tuberculosis especially in the endemic areas; 2) improving the understanding of the disease interactions with the immune system and re-evaluating the role of BCG and the new vaccine candidates in protecting children and adults against TB; 3) defining the diagnostic contribution of novel T-cell–based assays in endemic and non-endemic areas especially with regard to diagnosis of paediatric tuberculosis; 4) identifying new ways of diagnosing childhood tuberculosis in HIV negative and in TB/HIV co-infection in children, particularly in resource-limited settings; 5) carrying out operational research aimed at improving the access of children in endemic areas to preventive therapy and treatment, using the existing DOTS/DOTS Plus frameworks; 6) evaluating the efficacy of new short-course intermittent preventive chemotherapy regimens especially those aimed at childhood TB; 7) exploring shorter durations of treatment in immune-competent children with smear-negative disease; 8) defining the optimal treatment regimen and treatment duration in children with TB/HIV co-infection; 9) monitoring the impact of MDR and XDR tuberculosis on children and evaluating regimens for effective MDR/XDR disease prevention and treatment; 10) developing and evaluating new drugs that may shorten the treatment duration and/or assist with the treatment of MDR/XDR disease and emphasizing case finding and reporting as some of the strategies to combat the escalation of XDR-TB [http://ec.europa.eu/research/research-eu].

### 6.2. Need for more specific diagnostic tests

In the field of diagnosis, there is an urgent need to replace sputum microscopy the current gold standard test, with more sensitive tests that are applicable at point of care. Despite the fact that the technique can only pick up 60% of cases, it has been in use for over a hundred years. Furthermore, sputum culture is not suitable for extrapulmonary TB and for paediatric TB since children can not produce sputum. On the other hand, the newer immunological based tests such as IGRAs are not well suited for use in TB/HIV co-infection and in high burden TB areas, where they cannot be accurately used to distinguish active from latent TB. Since the majority of the infected people never actually develop the disease, there is need to have a diagnostic tool which is able to distinguish latent from active disease and help to identify healthy individuals from diseased ones. Improved diagnostics are critical to TB care and control.

The need for serious investment in the critical areas especially in new TB diagnostic tools, drug susceptibility testing, and development of new biomarkers to enable health providers detect TB disease activity and to determine follow up treatment outcomes cannot be over emphasised. The fact that a number of new diagnostic tools are in the pipeline, including culture-based tests to identify M. tuberculosis and those used to determine drug resistance based on molecular assays and immune response is good news. However, there is still need to ensure that the new tests can be availed world-wide and be used at the point-of-care even in resource-poor settings, where there may be limited technical expertise and the necessary equipment. [http://ec.europa.eu/research/research-eu]

### 6.3. Newer biomarkers for TB disease activity, cure and relapse

There are three major reasons that can be used to justify the need for new TB biomarkers : 1) a diagnostic test which is able to differentiate between healthy individuals with a latent TB

infection and patients with active dsease is needed; 2) a prognostic test which can be able to predict the risk of latent TB becoming active needs to be established; 3) there is need for a diagnostic test that can be used to serve as a surrogate endpoint of disease which can be used for monitoring drug and vaccine trials in TB. It is envisaged that the basis for these novel diagnostic measures will be biomics, comprising metabolomic, proteomic and transcriptomic profiles in custom-made biosignature. Identification of non invasive biomarkers, especially the molecular assay of M. tuberculosi fragments in urine and the measurement of volatile biomarkers of volatile organic compounds generated by Mycobacteria TB in the breath or the oxidative stress resulting from infection is one step in the right direction. [http://ec.europa.eu/research/research-eu]

### 6.4. Need for post-exposure vaccines and those effective against all M. tuberculosis strains

The Bacille Calmette-Guerin (BCG) vaccine is currently the only vaccine in use against tuberculosis. The efficacy of this vaccine is limited to prevention of severe forms of tuberculosis among children and there are lots of problems in cases of TB/ HIV coinfection.The current vaccine candidates are being developed for pre-exposure administration but, considering the fact that one third of the world's population is already infected, there is a serious need for a post-exposure vaccine to prevent re-activation. Another shortcoming with the vaccines in the pipeline is that they can delay clinical TB but cannot achieve sterile eradication. There is need for combination vaccines that can combine the effects of booster vaccines with another generation of vaccines that can act to effect sterilisation at the post-exposure stage. Focus should also be put on the development of a vaccine that can afford protection against the wide range of M. tuberculosis strains, to ensure universal effectiveness.To avoid complications in clinical and epidemiological research, the evaluation of all the vaccines should also deal with confounding factors such as prior BCG vaccination or HIV status and there should be well worked out guidelines for use of these vaccines in children [http//www.ec.europa.eu/research/research-eu]

### 6.5. Development of new drugs

When it comes to the current status of clinical, diagnostic and therapeutic strategies, childhood TB has been grossly neglected and there is, therefore need for better and standardized treatment strategy. Although there is a reasonable number of candidates in the discovery and pre-clinical pipeline, there are still gaps between the different stages of TB drug development, and drugs specically targeting paediatric TB are still needed. Most of the drugs in the pipeline use the same mechanisms with the majority aimed at boosting efficacy or shortening the duration, with very few targeingt dormant stages of the bacillus and, therefore, not suitable for eradication of latent infections. Thus urgency for serious research into the development of new drugs and treatment regimens aimed at achieving this therapeutic objective cannot be over emphasised. Dealing with TB/HIV co-infection is another gap that needs serious attention: there is need to develop drugs that can prevent dormant mycobacteria from re-activating in HIV-positive individuals [http://ec.europa.eu/research/research-eu.

## 7. Future prospects

### 7.1. Reducing the burden of childhood tuberculosis

TB in children presents a number of difficult challenges which will only be solved by a shift in research priorities. Advances in paediatric TB research will provide wider insights and opportunities for TB control. While development of a new vaccine to prevent TB should be the ultimate goal, development of better diagnostics represent one of the most important steps towards improving individual case management and also providing a more robust case definition for much needed drug and vaccine trials for studies on TB epidemiology and correlates of protective immunity in childhood. Data on the epidemiology of childhood TB may in turn help to inform public health policy by providing a window on current transmission and the effectiveness of control strategies and by identifying children with LTBI for chemoprophylaxis to limit the future propagation of the epidemic. Emphasis should be placed on reducing the vulnerability of the community because successful control of the tuberculosis epidemic is the most effective way to reduce the burden of childhood tuberculosis However, this will require a holistic approach with sustainable poverty alleviation as a key element [http://www. ec.europa.eu/research/research-eu].

### 7.2. Involvement of funders and industry

Important resources are required for the exploration of pathways leading to TB diagnostics-oriented basic science in pathogen biology, biomarker discovery, systems biology and point of care test development. The current priorities for TB vaccine development are: i) new vaccine candidates that achieve sterile eradication, and that can progress into phase II and phase III trials; ii) vaccine testing in a naïve stage on M. tuberculosis uninfected individuals; iii) vaccines which can achieve post-exposure prophylaxis to those who are already infected (currently 2 billion people); iv) strategies on how to get vaccines from the research bench to the bedside and into the community. Apart from the protective effect of novel vaccine candidates, priority should be given also to their delivery route, formulation (storage, shelf-life and distribution) and utility for HIV-infected individuals, particularly children.

### 7.3. Back to basic research

As we talk about nano technology and pin point delivery of drugs it is a shame that 130 years after the Robert Koch's discovery of M. Tuberculosis we still have huge gaps in our understanding of the biology, immunology and pathophysiology of the bacillus. We are, as yet, not able to explain fully the molecular, biochemical and immunological mechanisms that enable TB infection to go on for years and cause severe disease and death. With the availability of state-of-the-art molecular research tools such as functional genomics and metabolomics a paradigm shift towards empahsising basic research could help provide answers to some of the unanswered questions about M.tuberculosis in general, and paediatric TB in particular. The currently available knowledge has proved insufficient when it comes to the rational design of vaccines or other control tools and this has resulted in a lot of trial-and-error approaches. With

the HIV virus still elusive, defeating the dual alliance of TB/HIV co-infection has added another diamension in the already complicated war against the resistant strains of M. tuberculosis. Promoting basic research by providing the necessary resources and involving stakehoolders on the political front can provide solutions to some of the outstanding problems if not solving all of them. Where there is will, there is a way [http//ec.europa.eu/research/research-eu].

### 7.4. Concluding remarks

Recent developments for TB diagnostics seem promising fields due to the fact they are fast and minimally invasive, but they have drawbacks of not being validated in diverse populations and improved according to the patient's needs. Refinement of existing tools and development and testing of new tools are urgently required to improve diagnosis and treatment of TB in children. Higher global priority and funding will be required to improve on childhood nutrition and promote improvement in the socioeconomic and environmental condition of communities if we are to have a significant impact on TB transmission to children. [Expand +Clinical Infectious Diseasescid.oxfordjournals.org

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