Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

New advances in the diagnosis and treatment of pediatric tuberculosis

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Childhood tuberculosis (TB) remains a disease that causes substantial global morbidity and mortality. In the last decade, advances in immunologic and PCR-based testing have promised to improve our ability to prevent disease by identifying and treating latent TB infection and enabling earlier recognition of disease, respectively. Nevertheless, TB diagnostic and therapeutic research in children has been limited and poorly harmonized due to the lack of a standardized approach. Only recently has the scientific community arrived at consensus regarding a standard case definition of intrathoracic disease and developed guidelines to support a standardized approach for the evaluation of new TB diagnoses in children. These two events have set the stage for a new era in pediatric TB research. In this article we will summarize the performance of new diagnostics (IFN-γ release assays and GeneXpert®) in children, as well as review available treatment regimens for children with latent TB infection and disease.

Keywords: childhood tuberculosis • GeneXpert® TB/RIF • IFN-γ release assays • preventive therapy • tuberculin skin test

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Tuberculosis (TB), along with HIV and malaria, remains a modern day scourge. The decreased numbers of incident cases observed in industrialized countries over the last century has not been replicated in developing nations, where over 90% of the disease burden occurs. Approximately 9 million new cases, 12 million prevalent cases and 1.5 million deaths due to TB occur annually [1]. Of the 8.8 million new cases of TB disease in 2010, approximately 500,000 were diagnosed in children less than 15 years of age [1]. This conservative estimate is based on smear status, which is rarely defined in child TB cases. Despite the availability of effective therapy for drug-susceptible TB, long-term prospects for TB control remain dismal. In part, this is due to lack of a vaccine conferring long-term protection, increased rates of drug-resistant isolates, synergy between HIV and TB, and a massive reservoir of cases of future disease in the form of persons with latent TB infection (LTBI).

When a person is in contact with an individual with TB (termed the 'source case'), three options are possible (Table 1). TB exposure is defined as a patient who has no immunological, clinical or radiographic evidence of *Mycobacterium tuberculosis* (*M.tb*). LTBI is defined as a person who has evidence of immunologic recognition of *M.tb*, without having clinical or radiographic findings. Children with untreated LTBI are at risk for progression to TB disease; this risk is contingent on age and immune status. For example, infants with untreated LTBI have a 30–40% lifetime risk, HIV-infected patients have a 5–10% annual risk, and older immunocompetent children have a 5–10% lifetime risk of developing TB disease [2]. It is estimated that one-third of the global population has LTBI [1]. The final category are persons with TB disease, who have signs, symptoms or radiographic findings caused by *M.tb*.

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Table 1. Defining tuberculosis exposure, infection and disease.

Category	Age (years)	Physical examination	TST and/or IGRA	Chest radiograph	Culture
Exposure[†]	<5	Normal[‡]	Negative	Normal[†]	-
Infection	All ages	Normal[†]	Positive	Normal[†]	-
Disease	All ages	Variable; in pulmonary disease, radiographic findings often more striking than clinical findings; active surveillance in developing nations may lead to fewer symptoms at diagnosis	Variable; usually positive, but less commonly so for disseminated or CNS disease	Abnormal in most children with pulmonary TB[§] and many with extrapulmonary TB	+/-

[†]While TB exposure can occur throughout a person's lifetime, the category of TB exposure is used primarily to describe preschool aged children or other individuals who, by virtue of a suboptimal immune response, may be at a higher risk of progressing to TB disease during the window period for TST conversion.

[‡]Or no findings attributable to TB disease.

[§]Subclinical TB (culture-positive TB with a normal radiograph) has been described in HIV-infected persons and also in immunocompetent hosts at the time patients are transitioning from TB infection to disease.

IGRA: IFN-γ release assay; TB: Tuberculosis; TST: Tuberculin skin test.

There are many differences between adults and children in terms of TB diagnosis and treatment. Children tend to have lower organism burden (paucibacillary) disease, are less likely to form cavitary lesions and are less likely to expectorate sputum. Consequently, children contribute less to transmission than adults, decreasing their public health importance. The WHO has focused on identification and treatment of the most infectious patients: those with positive sputum smears, where *M.tb* is visualized under the microscope. While this is understandable from a public health standpoint and from a diagnostic standpoint in resource-limited settings where sputum smear may be the only diagnostic modality available, this approach systematically excludes children for the TB diagnostic discourse. The ramifications of this are widespread: the absence of evidence of childhood TB in many developing nations is misinterpreted as evidence of absence of disease [3]. This has decreased motivation for studies of diagnostics and therapeutics for childhood TB. In regard to diagnostics, the scientific community has made a recent push to include children in studies of diagnostic accuracy, resulting in an increase in the available evidence. Nevertheless, the majority of evidence-based guidelines still assign low grades to the quality of the evidence and rely heavily upon expert panel opinion. From the standpoint of TB treatment, children tolerate antituberculous therapy better than adults. In part, this may be due to the average child having less hepatic dysfunction than the average adult. However, it may also be a consequence of children metabolizing medications faster than adults [4], leading to decreased serum concentrations and toxicity, but potentially also to subtherapeutic drug levels. Much work remains to be done in order to accurately understand the pharmacodynamics and pharmacokinetics of TB medications in children.

Immunology

The development of pediatric TB is classically thought to involve three key pathophysiologic events: *M.tb* exposure; *M.tb* infection; and development of TB disease. In high-TB-burden countries, more than 60% of children <5 years of age with TB have documented household exposure [5,6]. After inhalation of droplet nuclei containing *M.tb,* infection is thought to occur when the organism is established intracellularly within the lung and associated lymphoid tissue. In the traditional model, macrophages present antigens from phagocytosed bacilli to T cells that have critical regulatory and effector roles [4]. After Th1 type antigen-activation, T cells secrete cytokines including IFN-γ, in turn activating macrophages, leading to more effective control of mycobacterial growth [5]. The host immune response is a determining factor of clinical outcomes following exposure to *M.tb* [7–10].

Following primary infection, most immune competent children develop asymptomatic LTBI which may be characterized by a positive test of infection including the tuberculin skin test (TST) or IFN-γ release assays (IGRAs) [7]. When occurring, disease has traditionally been thought to progress from no infection to asymptomatic infection to TB disease in a linear, unidirectional fashion. However, emerging evidence suggests a more complex, dynamic bi-directional continuum of responses exists leading to a spectrum of TB infection and disease states [10]. The risk of TB disease progression is greatest during the first year following infection [11] and is inversely related to age, suggesting an immature or inappropriate immune response favors disease progression. Among children with a TST ≥ 10mm, the estimated lifetime risk of TB disease is 10–20% and 5–10% in HIV-uninfected children ≤5 years of age and 6–15 years of age, respectively [12]; this risk increases dramatically

in the presence of depressed cellular immunity due to HIV or malnutrition [13–15]. Of HIV-uninfected infants <1 year of age, 50% will progress to TB disease following *M.tb* infection in the absence of isoniazid preventive therapy (IPT) [11]. Risk of disease progression increases to 20% again in adolescence [11]. Although increased exposure risk may contribute to the increased risk of TB in adolescence, there are likely other contributing causes, including pubertal changes in the immune response.

There are very limited data examining the pediatric immune response following *M.tb* exposure. One recent study compared T-cell repertoires of children with TB disease and infection [16]. This cross-sectional study compared IFN-γ production in response to *M.tb*-specific proteins ESAT-6 and CFP-10 by peripheral blood mononuclear cells and CD8+ T cells isolated from Ugandan children hospitalized with TB or healthy Ugandan children with household TB exposure. Among children <5 years of age, the magnitude of the CD8+ response and the proportion of positive CD8+ assays were greater in children with TB disease compared with children with contact. Among household-exposed children, the CD8+ response was greater in older compared with younger children. In contrast, the peripheral blood mononuclear cells (PBMCs) response did not differ between these groups. The authors postulated that the PBMC response is likely driven by a predominant CD4+ response, which may be associated with transient infection (more likely in younger children) while the CD8+ response may be associated with persistent infection (more likely in older children), or children with TB disease. The authors further postulated that the CD8+ response reflects mycobacterial load, but is not reflective of protective immunity. This study generated useful hypotheses that beg to be further studied in larger, longitudinal cohort studies designed to directly measure T-cell kinetics via production of a collection of cytokines and in response to a variety of antigens.

Diagnosis of infection

There is no accepted reference standard measure of *M.tb* infection. A growing number of studies have employed surrogate measures of infection to serve as the reference standard in studies of diagnostic accuracy for test of TB infection [17]. Although the majority of these studies have used simple dichotomous measures of exposure, a few key studies have illustrated that exposure may be quantified and support comparison of test of infection [18,19].

There are three commonly used immune-based tests of *M.tb* infection. The TST has well-recognized limitations including limited specificity in BCG-vaccinated populations. The identification of genes in the *M.tb* genome that are absent from *Mycobacterium bovis* BCG [20] and most non-tuberculous mycobacteria [21] supported the development of more specific assays that quantify the *in vitro* production of IFN-γ by T cells after stimulation with *M.tb*-specific antigens (ESAT-6, CFP-10 ± TB7.7) [22–24]. There are two commercially available IGRAs – the QuantiFERON®-TB (Cellestis, Australia) and the T-SPOT.*TB* (Oxford Immunotec, UK). The QuantiFERON (QFT) test incubates whole blood and measures IFN-γ production with an ELISA while the T-SPOT.TB measures the number of IFN-γ-producing PBMCs with an enzyme-linked immunosorbent spot (ELISPOT) assay. Since there is no gold-standard of infection, many studies have assessed the utility of IGRAs for the detection of *M.tb* infection using a cohort of TB-diseased children as the infected group. Systematic review of pediatric studies estimated the specificity of commercially approved IGRAs for detecting TB disease at 91% for the ELISA-based tests and 94% for the ELISPOT-based test, compared with 88% for the TST (positive defined as 10 mm) [17]. Estimates of test sensitivity were similar for the three tests: 83% (QFT assays), 84% (T-SPOT.TB*)*, and 84% (TST). Similarly, a second pediatric systematic review found the performance of the TST and QFT assays to be no different for the detection of TB disease [25]. Although a qualitative review of four pediatric studies concluded that the QFT assays were more specific in detection of *M.tb* infection in children compared with the TST [25], a larger pooled analysis was unable to demonstrate a statistically significant difference between these tests [17]. Like the TST, IGRAs cannot differentiate between *M.tb* infection and active TB.

Limited data are available regarding IGRA performance in HIV-infected children, in whom the performance of the TST is impaired [26–28]. Two studies have shown a non-commercial IFN-γ ELISPOT to have higher sensitivity for detecting TB compared with the TST in HIV-infected children [26,29]. A comparison of the QFT-gold assay and the TST for the detection of TB in 36 young, HIV-infected children with confirmed disease found comparable sensitivity in children with CD4+ count >200 cells/ml; indeterminate QFT-gold results were reported in 25% of children tested [30]. A single study has examined IGRAs for the detection of *M.tb* infection in 23 HIV-infected South African children [31]. This pilot study demonstrated high levels of discordant and indeterminate IGRA results and suggested that the T-SPOT.TB may have improved sensitivity for the detection of *M.tb* infection in HIV-infected individuals.

Due to the lack of definitive pediatric data and concerns regarding cost–effectiveness, the WHO has

recommended against the use of IGRAs in children living in low- and middle-income countries [32]. IGRAs are commonly used in children living in upper income countries and have influenced clinical decision [29].

Diagnosis of disease

M.tb, along with *M. bovis,* is one of several species in the *M.tb* complex. These species and other nontuberculous mycobacteria are pleomorphic weakly Gram-positive rods that retain certain dyes even after being exposed to alcohol and acidic solutions. This is the origin of the term 'acid-fast' in reference to mycobacteria. *M.tb* is the only mycobacteria with human-to-human transmission except for *Mycobacterium leprae*, the causative agent of leprosy. It is a fastidious, slow-growing bacterium. The most common culture modalities are egg and glycerin-impregnated solid agar (e.g., Löwenstein–Jensen), synthetic media (e.g., Middlebrook 7H9) or liquid media (e.g., BACTEC, MGIT). The latter have become increasingly common in high-burden nations due to its automated nature and self-contained system, resulting in increased through put and decreased infection control concerns, respectively. All three methods have excellent concordance for first-line TB drugs [33].

Children with TB disease are identified in one of two ways. First, in low-incidence, high-resource settings, many children may be identified through active surveillance. That is, an adult is identified and public health investigations identify children in contact with the individual. When these children are screened, a proportion may be asymptomatic despite radiographic anomalies [34]. In these instances, the children often have paucibacillary disease, culture confirmation is rare, and the prognosis is excellent. The second way children are identified is via passive surveillance. This is more common in resource-limited settings or in children lacking access to care even in industrialized nations. Here, children present once symptomatic. These children tend to have higher organism burden disease, are more likely to have microbiologic confirmation of TB disease, and may have higher morbidity and mortality from their disease.

Given the infrequency of culture-confirmation, the use of standardized clinical case definitions is crucial for diagnostic and therapeutic studies in childhood TB. A consensus definition for intrathoracic disease was recently established. These definitions provide a common language to describe the probability of having *M.tb*, disease location and severity [35,36]. Confirmed cases are those in which children have at least one sign or symptom suggestive of TB and at least one positive culture. Probable TB cases include children with at least one sign or symptom compatible with TB, a chest radiograph consistent with TB and at least one of the following: a positive response to antituberculous therapy; exposure to a person with TB disease; or immunological evidence of TB infection. Possible TB cases include children with at least one sign or symptom compatible with TB in association with either a positive chest radiograph or ancillary evidence of disease, but not both [36]. Intrathoracic disease is defined as disease involving pulmonary parenchyma, intrathoracic lymph nodes, or the pleural/pericardial spaces. Extrathoracic disease involves sites outside the thoracic cavity, most commonly peripheral lymph nodes and tuberculous meningitis. By convention, if a child has both intra- and extra-thoracic involvement, the disease is termed extrathoracic [36]. There exist no quantitative markers of *M.tb* organism load, contributing in part to the different paradigm of infection versus disease that is unique to TB. This has ramifications for diagnostic and therapeutic studies. A detailed definition for disease severity was recently proposed. Multifocal pulmonary disease, cavitary lesions, endobronchial disease, lymph node compression of airway, pericarditis, meningitis and osteomyelitis are considered severe [37]. This classification system is an alternative to the traditional classification of intrathoracic versus extrathoracic disease which, while anatomically easy to comprehend, does not take attendant morbidity and mortality into consideration.

The most common chest radiographic findings in childhood TB disease vary by age. Disseminated (miliary) TB is most common during infancy, intrathoracic lymphadenopathy is a common radiographic finding during the preschool years, and lobar infiltrates and pleural effusions become more common during later childhood. Cavitary lesions are uncommon findings in prepubertal children. While most children with TB disease are not contagious, any child with cavitary lesions, irrespective of age, should be treated as potentially contagious and all appropriate infection control precautions should be implemented. The vast majority of childhood TB has an intrathoracic component; even in children with extrapulmonary TB, the short timetable from inoculation to disease means that the lung portal of entry has not had a chance to resolve prior to dissemination. For example, over 90% of children with tuberculous meningitis have abnormal radiographs [38].

Accurate interpretation of radiographs is essential. Frontal radiographs are indicated for all children with suspected TB. The addition of a lateral view may be beneficial for young children, as recognition

of intrathoracic adenopathy may be facilitated by a lateral radiograph, especially in a young child with residual thymic tissue. The recent expert consensus panel to standardize case definitions of intrathoracic TB for research studies has also defined a standardized methodology for radiographic interpretation of childhood TB [36]. This strategy first evaluates adequacy of the radiograph (penetration, rotation and inspiratory versus expiratory). Second, the presence or absence of specific findings is documented. This includes evaluation for airway compression, soft tissue densities consistent with intrathoracic adenopathy, air space disease, nodular opacities, pleural effusion, cavities, calcification and bony changes.

In an immunocompromised child, there may be substantial symptomatic and radiographic overlap between TB disease and other opportunistic infections. In addition to the paucibacillary nature of childhood TB, children infrequently produce sputum, further complicating diagnostic evaluation. In high-prevalence settings, a child may have been exposed to more than one person with TB disease, potentially with differing drug susceptibility patterns. Consequently, obtaining cultures prior to initiation of therapy is critical. Unlike adults, who can expectorate sputum, young children often cannot produce adequate sputum specimens. Historically, children have been admitted to inpatient units for morning gastric aspiration performed serially over several days; sputum produced overnight is swallowed, and aspirated before the child eats breakfast. This methodology is costly (though for children already hospitalized with for TB, the incremental increase in cost is negligible), time-consuming and has relatively low yield. More recently, sputum induction has become well established. This procedure, which can be accomplished in the outpatient setting [39], involves administration of hypertonic saline by nebulization, which triggers coughing. The posterior oropharynx is then suctioned before the child can swallow secretions. One induced sputum has been shown to have the same smear and culture yield (10–30%) as three gastric aspirate specimens in some studies [40]. Sputum induction requires staff training, equipment and a facility in which to obtain specimens, which decreases risk of nosocomial transmission. Another way of obtaining respiratory specimens is through nasopharyngeal aspiration. Here, specimens are obtained by direct aspiration using a mucus trap connected to a suction device. Nasopharyngeal aspirations can be obtained with or without the assistance of tussive agents.

The most common acid-fast stains are Ziehl–Neelsen, used for respiratory specimens as a point-of-care test, and fluorescent stains such as auramine, a more sensitive test, especially in paucibacillary disease [41]. Children rarely are acid-fast bacilli (AFB) smear-positive [2]. As cultures can be intermittently positive, it is recommended that at least two specimens be acquired and sent for AFB stain and culture [42]. However, even with optimal specimen collection and processing, only 30–40% of children with pulmonary TB have *M.tb* isolated. This yield is higher for specimens obtained from extrapulmonary sites such as skeletal and peripheral lymph node biopsies [43]. The burden of childhood TB will be underestimated if culture-confirmation and/or AFB smear-positivity are used as standalone diagnostic criteria.

Drug susceptibility testing (DSTs) have traditionally been tested through agar proportions (comparing the number of colonies on media with and without antibiotic solution) or through growth in liquid media. These tests take several weeks for final results. DSTs are usually done sequentially. Testing for susceptibility to the first-line drugs of isoniazid (INH), rifampin (RIF) and ethambutol (EMB), in addition to streptomycin, is widely available. Testing for pyrazinamide susceptibility is more technically challenging and is not available in all settings. If DSTs indicate resistance to first-line medications, then expanded DSTs are indicated; however, these may not be available in all regions. With increasing recognition of multidrug-resistant (MDR)-TB (where the isolate is resistant to at least INH and RIF), emphasis on rapid identification of drug resistance has been prioritized. Molecular methods of identifying drug resistance (Xpert TB/RIF) have become more widely available (see below).

A number of nucleic acid amplification tests (NAATs) are now available to detect *M.tb*. The first generation NAATs were developed to help differentiate *M.tb* from non-TB mycobacteria in HIV-infected patients. PCR-based modalities have shown reduced sensitivity and specificity in children compared with adults (25–83% and 80–100%, respectively) [44]. While a positive PCR may help establish the diagnosis, a negative PCR does not preclude a child having TB. The next generation of NAATs detects not only *M.tb*, but specifically identifies mutations conferring drug resistance. MTBDR*plus* is an assay that can detect the most common mutations conferring resistance to INH (*katG*, *inhA* genes) and rifampin (*rpoB* gene); it has been shown to be very concordant with traditional DSTs and reduced time to detection of MDR-TB [45]. Xpert TB/RIF is a real-time PCR assay that detects RIF resistance. As RIF resistance is uncommon in isolation and is instead

commonly seen in the presence of INH resistance, Xpert TB/RIF therefore provides a mechanism for the rapid (90 min) detection of MDR-TB [46]. One pediatric study showed a sensitivity of 100% in HIV-infected patients and 66% in HIV-uninfected patients when Xpert TB/RIF compared to culture, whereas AFB smear had a sensitivity of 39% [47]. Per-sample analysis of Tanzanian children demonstrated that GeneXpert had a similar sensitivity (54.7% [95% CI: 42.7–66.2%]) compared with culture methods and detected threefold more confirmed TB cases than smear microscopy, but with equal rapidity [48]. In addition to providing rapid results, Xpert TB/RIF is a self-contained system with advantages in terms of the need for less laboratory capacity and infection control infrastructure. However, it does not replace traditional culture, as traditional DSTs are necessary to identify optimal drugs for use in children and adults with MDR-TB.

Treatment

Decisions as to how many and which antituberculous drug(s) (Table 2) to administer to a child depend on a number of factors including classification (exposure, infection, disease); site of disease (e.g., meningitis), DST results for the child or source case, if known; method of administration (self/family administered or health department administered); potential medication interactions; and, medical comorbidities. Therapeutic regimens for TB exposure, infection and disease are summarized in Table 3.

■ Post-exposure preventive treatment

Children with exposure or LTBI are generally treated with monotherapy. The philosophy is that for low organism loads, the mathematical probability of having a spontaneous mutation conferring resistance is very low. However, when children have TB disease, they have a higher organism burden, and the chance of having such mutations is no longer negligible. For these children, multidrug therapy is initiated; the theory here is that, for example, INH will kill the RIF-resistant bacteria and vice versa.

In settings with low burden of TB, immune competent children under 5 years of age with TB exposure are usually treated with a single drug until the definitive TST has been placed and interpreted. This definitive TST is generally placed 8–10 weeks after contact with the source case has been broken, either physically (child is no longer in contact with the source case) or microbiologically (the source case has been deemed noninfectious based upon sputum results). The most commonly used drug is INH, unless the source case is known to have an INH-resistant isolate. Prophylaxis may be stopped if the definitive TST is <5 mm. If the definitive TST is ≥5 mm, therapy for TB infection is continued to complete a course of therapy (most commonly, 9 months of INH or 6 months of RIF) [49]. IPT used in hyperendemic areas for high-risk individuals, may be used irrespective of TST results (please see section below). The monotherapy options recommended for the treatment of LTBI include 9 months (US recommendation) or 6 months (WHO recommendation) of INH or 6 months of RIF [50,51]. Due to cost considerations and the potential for drug–drug interactions, the latter is generally reserved for cases where the source case has INH-monoresistance or the child is intolerant of INH. Both options are quite effective, but adherence with regimens can be quite low (30–64%) [52], due in part to the duration of therapy. Consequently, shorter course regimens are desirable. In some European countries, 3–4 month courses of INH + RIF are used, appearing as effective and well-tolerated as INH, with better adherence [53]. More recently, a 12-dose combination regimen of INH and rifapentine (a long-acting RIF derivative) was found to be well-tolerated and effective for children over 11 years old [54]; this regimen should be administered under supervision. Insufficient data were reported for the latter study to inform clinical decision making for younger children. IPT has been difficult to operationalize internationally, despite WHO guidelines recommending post-exposure IPT in all children <5 years of age and all HIV-infected children regardless of TST availability [55]. Despite emerging evidence demonstrating the cost–effectiveness of post-exposure IPT in high-burden TB settings [56], a growing body of evidence consistently demonstrates low uptake and limited infrastructure to support the delivery of post-exposure IPT in high-burden TB countries [57–60].

■ Pre-exposure preventive treatment

The WHO has developed guidelines regarding the use of IPT in HIV-affected persons [61]. Extensive evidence from population-based studies dating back to the 1940s supports the effectiveness of post-exposure IPT in children. HIV-infected children of any age with known TB exposure are treated with a full course of post-exposure prophylaxis. Although post-exposure IPT is universally accepted, much discourse continues regarding the effectiveness of pre-exposure IPT in HIV-infected children. The two large pediatric trials published to date have shown conflicting results. The first randomized, double-blind, placebo-controlled trial studied the effect of adding IPT or placebo to a co-trimoxazole preventive treatment in children

Table 2. First- and second-line tuberculosis medications for children <40 kg[†].

Agents	Daily dose [71]		Thrice-weekly dose [55]		Formulations	Drug interactions	Toxicities	CNS penetrance[‡]	Monitoring parameters
	mg/kg/day	Maximum dose	mg/kg/day	Maximum dose					
Isoniazid	10 (10–15)	300 mg	10 (8–12)	900 mg	100, 300 mg scored tablets; 10 mg/ml suspension[§], im., iv.	+	Hepatitis, peripheral neuropathy	++	[¶]
Rifampin	15 (10–20)	600 mg	10 (8–12)	600 mg	150, 300 mg capsule; iv.; extemporaneous preparation of oral solution	++	Hepatitis	+	[¶]
Pyrazinamide	35 (30–40)	-	35 (30–40)	-	500mg scored tablet	-	Arthralgia, rash, hepatitis	++	[¶]
Ethambutol	20 (15–25)	-	30 (25–35)	-	100, 400 mg tablets	-	Optic neuritis	-	[¶]
Agents for drug-resistant tuberculosis									
Streptomycin	15 (12–18)	1 g	Few data are available regarding the efficacy of intermittent administration of second-line medications		1 g vials for im., iv.	-	Nephro- and oto-toxicity	-	Baseline and monthly creatinine and audiology evaluation
Other injectables[#]	15–30	1 g			500 mg–1 g vials for iv.	-	Nephro- and oto-toxicity	-	Baseline and monthly creatinine and audiology evaluation
Ethionamide[††]	15–20	1 g	Few data are available regarding the efficacy of intermittent administration of second-line medications		250 mg tablet	-	Hepatotoxicity, hypothyroidism, neuropathy, optic neuritis, vomiting	++	Consider baseline ALT, TSH
Levofloxacin	7.5–10	1 g			250, 500, 750 mg tablet; iv.	+	Arthropathy	++	-
Cycloserine	10–15	1 g			250 mg capsule	-	Rash, seizures, psychosis	++	Serum concentrations, monthly neuro-psychiatric evaluation

[†]Dosing for children ≥ 40 kg follows adult guidelines [70]. Doses for children 0–3 months are the same as older children. Recommendations regarding dosing for individual countries may vary from WHO guidelines [55,71].

[‡]Penetration across uninflammed meninges.

[§]The isoniazid suspension available in the USA is highly sorbitol-based, resulting in osmotic diarrhea. Consequently, this formulation should be used only for young infants who are not even receiving pureed foods; thereafter, children should receive tablets, which can be crushed and mixed with food.

[¶]Routine laboratory evaluation is not necessary except in children with underlying hepatic disease; however, should children develop any side effects (abdominal pain, nausea/vomiting, jaundice), medication(s) should be stopped and laboratory evaluation undertaken.

[#]Other injectable agents include amikacin, capreomycin and kanamycin; the three agents are grouped given the similarities in drug dosing, interactions and toxicities.

[††]Ethionamide is often substituted for ethambutol in children with tuberculous meningitis, since ethionamide achieves high levels in cerebrospinal fluid.

ALT: Alanine aminotransferase; BID: Twice-daily; im.: Intramuscular; iv.: Intravenous; TID: Three-times daily; TSH: Thyroid-stimulating hormone.

Table 2. First- and second-line tuberculosis medications for children <40 kg[†] (cont.).

Agents	Daily dose [71]		Thrice-weekly dose [55]		Formulations	Drug interactions	Toxicities	CNS penetrance[‡]	Monitoring parameters
	mg/kg/day	Maximum dose	mg/kg/day	Maximum dose					
Paraamino salicylic acid	200–300 divided BID or TID	10 g	Few data are available regarding the efficacy of intermittent administration of second-line medications		4 g packets	+	Hepatotoxicity, hypothyroidism	+	Baseline and monthly TSH

[†]Dosing for children ≥ 40 kg follows adult guidelines [70]. Doses for children 0–3 months are the same as older children. Recommendations regarding dosing for individual countries may vary from WHO guidelines [55,71].
[‡]Penetration across uninflammed meninges.
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[¶]Routine laboratory evaluation is not necessary except in children with underlying hepatic disease; however, should children develop any side effects (abdominal pain, nausea/vomiting, jaundice), medication(s) should be stopped and laboratory evaluation undertaken.
[#]Other injectable agents include amikacin, capreomycin and kanamycin; the three agents are grouped given the similarities in drug dosing, interactions and toxicities.
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(median age 25 months) with advanced HIV disease [62]. The study was halted early due to increased mortality in the placebo group. The mortality reduction in the treatment group was confined to the initial 3 months of treatment raising concerns that sub-clinical TB was present at enrollment. A subsequent observational cohort study of the survivors of this trial, who were eventually started on antiretroviral therapy (ART), reported that IPT provided additional benefit to ART in protecting against TB [63]. However, findings from this cohort are limited as the epidemiologic context, that is, HIV-infected children not receiving ART with advanced symptomatic illness and a high risk of early mortality, are thankfully less common today. The second randomized, double-blind, placebo-controlled trial studied the effect of primary INH prophylaxis against TB in 3–4 month old HIV-infected and HIV-uninfected children exposed to HIV during the perinatal period. INH or placebo was added to ART that was initiated early in life [64]. Children were monitored for TB exposure and received post-exposure IPT immediately following exposure. The study was halted early as there was no significant difference between protocol-defined TB or death occurring in the INH group and the placebo group. Among HIV-uninfected children, there was no significant difference in the combined incidence of TB infection, TB disease, or death between the INH group and the placebo group. This study suggests that primary isoniazid prophylaxis does not improve TB-disease-free survival among HIV-infected children effectively monitored for TB exposure and provided with post-exposure IPT. Nevertheless, the value of pre-exposure IPT in the absence of careful monitoring for exposure remains unclear. The WHO childhood TB subgroup will soon release new guidelines that further address the use of pre-exposure prophylaxis in HIV-infected children.

■ Treatment for disease

Children with TB disease receive multiple drugs and these medications would optimally be administered through directly observed therapy (see below). The duration of therapy typically is at least 6 months for non-meningeal or non-miliary TB in an immunocompetent host. Children with tuberculous meningitis or miliary disease often receive 9–12 months of therapy; the WHO recommends 12 months of therapy for meningitis and osteoarticular disease. Therapy courses of 6 month for patients with persistent cavitary disease or HIV infection have been associated with higher relapse rates than those seen with longer courses of therapy [65,66].

Antituberculous drugs can be administered in several ways. For children with TB disease or children

Table 3. Commonly utilized therapeutic regimens.

Category	Agent and duration		Notes
	Initial regimen	Continuation regimen	
Exposure			
	First-line: INH; second-line: RIF, treat until definitive TST is performed	N/A	Definitive TST: 8–10 weeks after child either 1) broke contact with the source case or 2) source case ceased being infectious, as evidenced by sputum smear results If definitive TST is ≥5mm, treat as TB infection (see below) For children <6 months of age, therapy often is extended until a TST can be done at or after they are 6 months of age
Infection			
	First-line: INH for 9 months	N/A	WHO recommendation: 6 months of INH
	Second-line: RIF for 4–6 months	N/A	Used if the child is INH-intolerant or if the source case has an INH-resistant isolate
	Alternative: INH + RIF for 3 months	N/A	Offers increased adherence given shorter course of therapy and may provide additional benefit if child was infected with an *M.tb* isolate with monoresistance to one of the drugs
	Alternative: INH + rifapentine weekly for 12 doses (via DOPT)	N/A	Not recommended for children < 12 years of age
	If source case has MDR-TB, attempt to find two oral agents to which the isolate is susceptible	N/A	Recommendation based upon expert opinion
Disease			
	Always administer multiple medications, ideally via DOT		Use of monotherapy will select for resistant isolates Non-DOT administered therapy may result in nonadherence and select for resistant isolates
Uncomplicated pulmonary and non-meningeal extrapulmonary TB	INH, RIF, PZA, EMB for 2 months	INH + RIF for 4 months	A regimen of INH + RIF for 6 months has been used for children with isolated hilar adenopathy† Inability to use PZA should extend therapy to 9 months total EMB may be stopped if the isolate is found to be pan-susceptible The presence of a cavity at the end-of-therapy radiograph (6 months) should prompt consideration of extending therapy to 9 months total to decrease the risk of relapse
Meningitis	INH, RIF, PZA + ethionamide or injectable for 2 months	INH + RIF for 7–10 months	Injectable agents may be started while a child is hospitalized; the child may then be transitioned to ethionamide prior to discharge
HIV-infected, pulmonary disease	INH, RIF, PZA, EMB for 2 months	INH + RIF for 7 months	6 months of therapy for disease in HIV-infected adults was associated with higher rates of relapse

†Isolated hilar adenopathy would be an uncommon finding except in areas where active surveillance for TB enables early identification of children.

DOPT: Directly observed preventive therapy; DOT: Directly observed therapy; EMB: Ethambutol; INH: Isoniazid; MDR-TB: Multidrug-resistant tuberculosis; M.tb: Mycobacterium tuberculosis; PZA: Pyrazinamide; RIF: Rifampin; TB: Tuberculosis; TST: Tuberculin skin test.

Table 3. Commonly utilized therapeutic regimens (cont.).

Category	Agent and duration		Notes
	Initial regimen	Continuation regimen	
Disease			
HIV-infected, extrapulmonary disease	INH, RIF, PZA, EMB (or ethionamide if meningitis) for 2 months	INH + RIF for 10 months	Includes skeletal, miliary or CNS involvement
Isolated INH resistance	RIF, PZA, EMB for 6–9 months	N/A	Fluoroquinolones may be added for children with extensive disease
Isolated RIF resistance	INH, PZA, EMB, levofloxacin for 2 months	INH, EMB, luoroquinolones for 10–16 months	Isolated RIF resistance is quite uncommon An injectable agent may be used in the first 2–3 months for children with extensive disease
MDR-TB	3–5 drugs to which the isolate is susceptible	If injectable is used, try to stop it by 6 months	Optimal duration of therapy is not established Expert consultation should be sought

[†]Isolated hilar adenopathy would be an uncommon finding except in areas where active surveillance for TB enables early identification of children.

DOPT: Directly observed preventive therapy; DOT: Directly observed therapy; EMB: Ethambutol; INH: Isoniazid; MDR-TB: Multidrug-resistant tuberculosis; M.tb: Mycobacterium tuberculosis; PZA: Pyrazinamide; RIF: Rifampin; TB: Tuberculosis; TST: Tuberculin skin test.

in industrialized nations identified through contact investigations with TB exposure or disease, directly observed therapy (DOT) is commonly implemented. Here, children have medications provided to them and administration supervised by a dispassionate third party (e.g., not the family). In industrialized nations, this third party is often a local health department, whereas in other settings, community health workers or leaders can assume this responsibility. DOT increases adherence [67] and removes barriers to care such as purchasing of medications. Where DOT is not feasible, particularly in high-prevalence settings, the family can administer medications.

■ Antituberculous agents

INH is a bactericidal drug that, along with rifampin, serves as the mainstay of therapy for drug-susceptible TB disease and LTBI therapy (Tables 2 & 3). INH has excellent tissue penetration into all tissues, including the brain, and is available in a number of formulations. Its major side effects include hepatotoxicity and peripheral neuropathy. The most common hepatic manifestation is an asymptomatic, transient rise in serum transaminases. Jaundice and hepatic failure are exceedingly rare [68], occurring far less frequently than in adults. INH-induced peripheral neuropathy is due to pyridoxine depletion; this is an uncommon occurrence in children. Pyridoxine (B6) supplementation is recommended for exclusively breast-fed infants, children with minimal milk or meat intake, HIV-infected children, and children at risk for malabsorption. In the USA, where INH suspensions are sorbitol-based, osmotic diarrhea is a common occurrence. As this side-effect is not seen with the tablet formulation, practitioners may consider changing infants to tablet formulations, which may be crushed and mixed with food, when pureed foods are introduced.

Rifampin (RIF) and other rifamycins, such as INH, are bactericidal drugs with excellent tissue penetration. Cost considerations, potential for drug–drug interactions, and a preponderance of data for INH use for LTBI have resulted in minimal use of RIF monotherapy. RIF is recommended for LTBI for children who are intolerant of INH or for children whose source cases have INH-monoresistance. More recently, a long-acting rifamycin, rifapentine, has been combined with INH for a 12-dose regimen (one dose/week) administered via directly observed preventive therapy; this regimen has been recommended for children 12 years of age and older [54]. Rifabutin is recommended in lieu of RIF for HIV-infected children receiving protease inhibitor-based ART. All rifamycins are hepatically metabolized and have a number of drug interactions; these include altering levels of azole antifungal drugs, certain antiepileptics and oral contraceptives. In addition to warning caregivers of potential drug interactions, it is essential that families be notified of the orange discoloration of the urine that is almost universally seen with RIF administration. Some children also have discoloration of feces, tears or sweat.

Pyrazinamide (PZA) facilitates killing of *M.tb* within macrophages and diffuses well into most body

tissues. PZA seems to exert its maximum effect within the first 2 months, and is therefore not recommended for continuation therapy, unless a patient's isolate has drug resistance. As with all first-line agents except EMB, PZA is hepatically metabolized. It can also increase serum uric acid and is the first-line drug most associated with rash, joint pain and hepatotoxicity. If PZA is omitted from the initial stages of therapy, extension of therapy from 6 to 9 months is indicated for uncomplicated forms of TB.

EMB is a bacteriostatic drug that in recent years has been added to empiric therapy for disease to prevent the emergence of resistance. If a child is known (based upon their own cultures or those of a close contact) to have drug-susceptible TB, EMB may be omitted from the initial regimen. EMB achieves very low levels in cerebrospinal fluid, making it a suboptimal selection for tuberculous meningitis [69]. EMB is the only first-line drug that is renally metabolized and may be used in conjunction with second-line medications as part of a liver-sparing regimen for children with hepatic impairment. The main side effect of EMB is ocular toxicity (optic neuritis, difficulty with red–green color discrimination). While this has decreased uptake of EMB for preverbal children in whom visual acuity screening is challenging, children metabolize EMB much faster than adults [69]. The resultant decrease in serum levels likely contributes to the rarity of ocular complications in children. The WHO and Centers for Disease Control and Prevention (CDC) recommend that EMB can be used in children of all ages [70,71] .

Other antituberculous agents are considered second-line drugs due to toxicity profiles, route of administration, reduced efficacy in comparison with first-line drugs, or some combination thereof (Table 2). The primary use for second-line drugs is for the treatment of drug-resistant TB. The reduced killing efficiency of many of these drugs results in the long treatment duration needed for MDR-TB. A second use of these drugs may be for children who are intolerant of first-line medications due to side effects or to underlying organ system dysfunction. For example, a liver-sparing regimen may include EMB, a fluoroquinolone, and an injectable agent. The most common second-line medication is ethionamide, which is often used in combination with INH, RIF and PZA in children with tuberculous meningitis. There is substantial cross-resistance between INH and ethionamide. Fluoroquinolones have a major role in the treatment of MDR-TB [70]. In this case, the risk of tendonopathy is far outweighed by the benefit of a bactericidal drug with excellent tissue penetrance. The use of second-line medications should prompt referral to a specialist in TB.

Therapeutic modifications

Routine laboratory monitoring is not indicated for otherwise healthy children receiving first-line medications. However, if a child were to have any side effects, medications should be halted until the child can be seen and, if necessary, laboratory evaluation performed. This entails careful counseling of families regarding possible medication side effects and clear communication between the family, physician and health department. Maximizing safety for children receiving second-line medications often requires baseline and serial laboratory evaluation and, in the case of injectable agents, audiologic testing.

Some children will have a paradoxical worsening of TB symptoms while on effective therapy. This is termed immune reconstitution inflammatory syndrome (IRIS). IRIS is defined by new or enlarging lymph nodes or tuberculomas, serositis or worsening radiographic findings [72]. While most commonly described in HIV-infected patients, IRIS can also be seen in immunocompetent hosts. One recent study indicated that IRIS was seen in 15% of HIV-seronegative children, and was more common in malnourished children [73]. Most cases of IRIS can be managed with conservative therapy with nonsteroidal anti-inflammatory agents, while more severe reactions (e.g., lymph node compression on airways, expanding tuberculomas) may require a course of corticosteroids [72].

Future perspective

TB diagnostic and therapeutic research in children has been limited and poorly harmonized. The lack of a standardized approach has made it difficult to compare often conflicting results and impossible to systematically pool data as is done in systematic reviews [17,74]. Only recently has the scientific community arrived at consensus regarding a standard case definition of intrathoracic disease [36] and developed guidelines to support a standardized approach for the evaluation of new TB diagnostic in children [35]. These two events have set the stage for a new era in pediatric TB research.

The WHO's goal to eliminate TB by the year 2050 is dependent upon reducing the time to diagnosis of TB disease in high-burden TB countries. Reduction in the time to diagnosis has the potential to not only improve outcome for individuals, but also improve community outcomes by decreasing the number of secondary cases resulting from the original source case. In order to have a global impact, new TB diagnostics must be true point-of-care tests that accurately and rapidly identify both children and adults with drug-resistant and drug-sensitive TB disease while differentiating those with *M.tb* infection. This point-of-care test must also be highly cost

effective given the magnitude of the TB epidemic and its predominance in low- and middle-income countries. Global TB elimination can only be achieved by addressing the epidemic in high-risk populations such as children and HIV-infected. Given the paucibacillary nature of disease in these groups, new point-of-care diagnostics must also have the ability to perform accurately on a variety of specimens, including urine and blood in addition to sputum. Finally, once developed, the impact of any new diagnostic is dependent on its rapid and broad global introduction and uptake to ensure steady decreases in market-price that support sustainability of the technology in low-income, high-burden TB countries.

Global TB elimination is also dependent upon reducing the time-to-treatment and improving treatment adherence among children and adults living in high-burden TB countries. Current TB disease and infection treatment regimens are far too long for both children and adults. Shorter, effective, well-studied treatment regimens for both TB disease and infection are urgently needed in children. Recent consensus opinions on a pediatric case definition have set the stage to support this much-needed research. Large multinational treatment consortiums, such as the International Maternal–Pediatrics–Adolescent AIDS Clinical Trial (IMPAACT) group, have recently expanded their scope to address childhood TB. The inclusion of young and HIV-infected children in future clinical trials is of paramount importance as these children carry the highest burden of disease. Finally, without health systems strengthening in high-burden TB countries, decreased time-to-diagnosis will not lead to a decreased time-to-treatment and shorter, effective treatment regimens will not have an impact due to poor adherence.

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Executive summary

Background
- Pediatric tuberculosis (TB) is underappreciated due to diagnostic difficulties in isolating the bacterium from young patients.
- The risk of disease progression is highest in infants, where risk is three- to eight-times higher than HIV-infected persons.
- Latent TB infection is defined as a patient with immunological evidence of *Mycobacterium tuberculosis* who lack symptoms, physical examination findings and radiographic anomalies.

Immunology
- Both the tuberculin skin test (TST) and IFN-γ release assays (IGRAs) measure immune response to *M. tuberculosis*.
- IGRAs evaluate immune response to antigens predominantly found in *M. tuberculosis* and offer increased specificity over the TST.

Diagnosis of infection
- There is no reference standard for latent TB infection; both the TST and IGRAs are used.
- Limited data on IGRA test performance exist for HIV-infected and other populations of immunocompromised children.

Diagnosis of disease
- As children rarely are acid-fast bacilli (AFB) sputum smear or culture positive, the diagnosis of TB disease in a child often is contingent upon compatible clinical and/or radiographic findings, epidemiological link with a known case, and immunological evidence of response to TB antigens (either the TST or IGRA).
- Reliance upon microbiologic confirmation to diagnose TB disease will result in severe underdiagnosis of disease and withholding of therapy for children.
- Nucleic acid amplification tests, including those that specifically evaluate for common mutations conferring antibiotic resistance, can accelerate TB diagnosis.

Treatment
- Children tolerate TB medications much better than adults.
- Children with TB exposure and infection are often treated with monotherapy, while children with TB disease receive multidrug therapy, preferably via directly observed therapy.
- Routine laboratory monitoring is not needed for otherwise healthy children receiving first-line therapy for TB disease. However, should symptoms arise, clinicians should have a low threshold for checking hepatic transaminases.

Future perspectives
- Consensus definitions are now available to standardize clinical research studies.
- Sensitive point-of-care testing allowing for detection of both *M. tuberculosis* and drug resistance offers the possibility of rapid diagnosis and initiation of effective therapy.

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