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New approaches and emerging technologies in the diagnosis of childhood tuberculosis

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EDUCATIONAL AIMS

- To sketch the historical background of childhood tuberculosis (TB).
- To introduce important disease concepts such as risk assessment.
- To discuss new approaches to contact screening and the diagnosis of active TB.
- To provide an overview of new tools and emerging technologies in the diagnosis of intrathoracic TB in children.

KEYWORDS
child TB;
diagnostic advances

Summary Childhood tuberculosis (TB) has long been neglected by TB control programmes, as children tend to develop sputum smear-negative disease and rarely contribute to disease transmission. However, children suffer severe TB-related morbidity and mortality in areas with endemic TB and carry a significant proportion of the global disease burden. Apart from improved control of the global TB epidemic, access to accurate diagnosis and effective treatment is essential to reduce the disease burden associated with childhood TB. Access to child friendly anti-TB treatment is improving, but establishing an accurate diagnosis remains a challenge. This review provides an overview of recent advances in the diagnosis of childhood TB, focusing on bacteriological, immunological, radiological and symptom-based approaches. It is possible to establish a fairly accurate diagnosis of either latent infection or active TB in immunocompetent children, even in resource-limited settings, but establishing an accurate diagnosis of TB in HIV-infected (immunocompromised) children remains a major challenge.

National tuberculosis (TB) control programmes in many countries with endemic TB previously neglected childhood TB, as children rarely develop sputum smear-positive disease and contribute little to disease transmission within the community. However, there is increased awareness of the severe TB-related morbidity and mortality suffered by children and the fact that children carry a significant proportion of the global TB disease burden.[1–3]

Access to accurate diagnosis and effective treatment in countries with endemic TB is essential to reduce the morbidity and mortality associated with childhood TB. This has been facilitated by recent landmark developments: the World Health Organization recently published guidelines

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KEY POINTS

- It is possible to screen child TB contacts in resource-limited settings.
- It is possible to accurately diagnose the majority of child TB cases, even in resource-limited settings.
- The diagnosis of TB in HIV-infected children remains difficult.

for national TB programmes on the management of TB in children,[4] and for the first time the Global Drug Facility has made child-friendly drug formulations available to deserving countries. These positive developments focus attention on the tenacious problem of establishing an accurate diagnosis of TB in children, particularly in TB-endemic settings with limited resources that carry the brunt of the disease burden.

HISTORICAL BACKGROUND

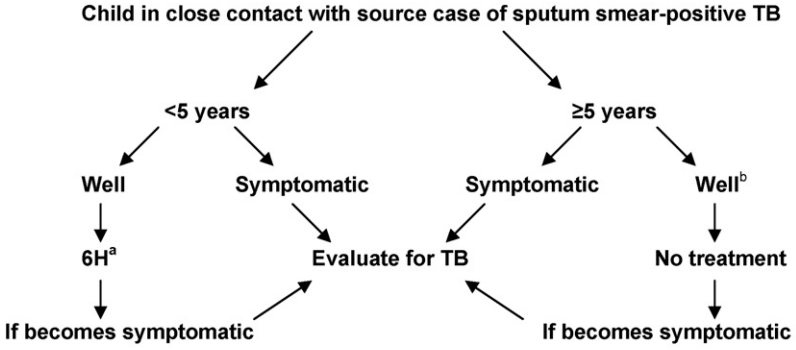
Robert Koch (1843–1910) discovered *Mycobacterium tuberculosis*, providing irrefutable proof that TB was a transmissible disease caused by a biological agent. However, it was soon recognised that although *M. tuberculosis* causes TB, infection with the organism as indicated by a positive tuberculin skin test was not at all uncommon.[5] It remains an intriguing and largely unexplained observation that only a small minority of people infected with *M. tuberculosis* ever progress to active disease. This observation explains why the diagnostic challenge is more pronounced in countries with endemic TB where TB infection is exceedingly common, thereby increasing the need to differentiate latent TB infection (LTBI) from active disease.

SCREENING FOR LTBI

Current World Health Organization guidelines advise that all children under 5 years of age in close contact with a sputum smear-positive index case should be actively traced, screened for TB and provided with preventive chemotherapy once active TB has been excluded (Fig. 1).[4] The International Standards for TB Care have made the same recommendation.[6] A TST and chest radiograph (CXR) are no longer regarded as prerequisite screening tests in settings where these tests are not readily available. It has been recognised that symptom-based screening may have considerable value to improve the access of children in resource-limited settings to preventive therapy.[4,7] In settings where the TST is used for screening purposes, it is important to remember that TST conversion may be delayed for up to 3 months. In fact, the contribution made by the TST in routine TB contact screening is limited as infection can only be reliably excluded in non-anergic children 3 months or more after exposure has occurred.

The new guidelines indicate that any 'close contact' with a sputum-smear positive source case is important, even if this occurs outside the household. It also acknowledge that HIV-infected (immunocompromised) children should be regarded as high-risk contacts, irrespective of their age. However, the guidelines fail to address the frequency with which children in settings where TB/HIV co-infection is common are exposed to adults with sputum smear-negative pulmonary TB and the transmission risk that this exposure poses within the household. In most areas with endemic TB, health systems are already overburdened with the provision of curative treatment and are reluctant and/or unable to perform contact screening and provide preventive therapy to large numbers of children. In these settings, it is of particular importance to focus preventive therapy interventions on those children who are at highest risk of progressing to active disease following documented TB exposure and/or infection. Fig. 2 presents a simplified screening approach that takes these considerations into account.

The rationale is completely different in low-burden countries with adequate resources where TB eradication



TB, tuberculosis.
[a]Isoniazid 5 mg/kg daily for 6 months.
[b]Unless the child is HIV-infected (in which case isoniazid 5 mg/kg daily for 6 months is indicated).

Figure 1 Suggested approach (World Health Organization, 2006) to contact management when chest X-ray and tuberculin skin testing are not readily available. (From reference 4 with kind permission.).

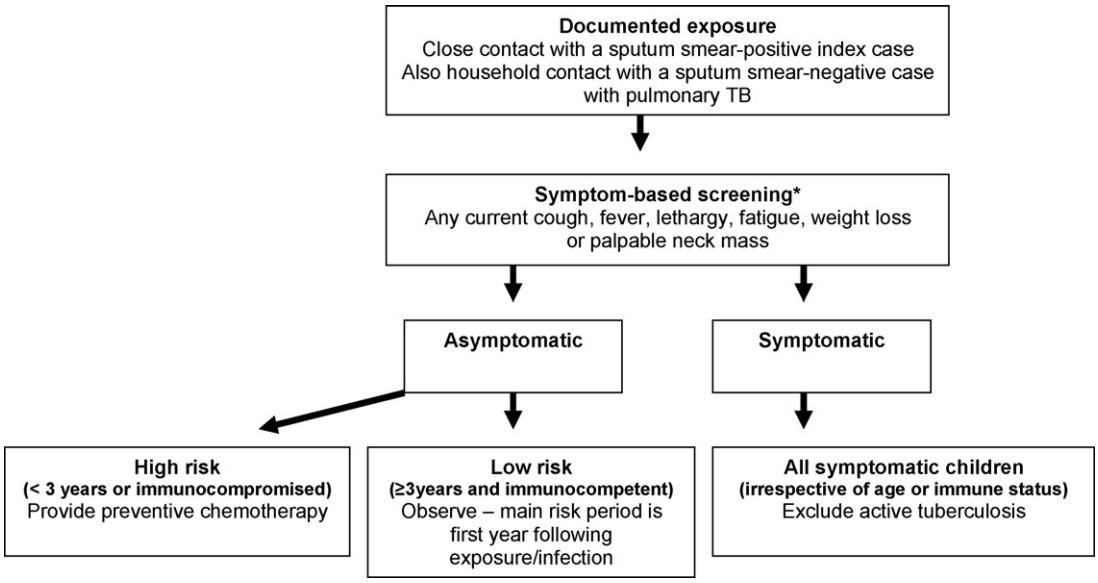


Figure 2 Proposed algorithm to screen children with documented exposure to a confirmed tuberculosis (TB) index case in resource-limited settings.

is an achievable goal. In these settings, the provision of preventive chemotherapy to everyone with documented TB infection seems justified because resource constraints are not a major consideration, eradicating the pool of LTBI will prevent future reactivation disease and the risk of reinfection is low.

New T-cell-based interferon-γ assays

New T-cell assays measure the interferon-γ released after stimulation by *M. tuberculosis*-specific antigens. Two assays are currently available as commercial kits: the T-SPOT.*TB* (Oxford Immunotec, Oxford, UK) and the QuantiFERON-TB Gold assay (Cellestis Limited, Carnegie, Victoria, Australia). In general, these tests are regarded as more specific and potentially more sensitive than the traditional TST.[8–12] However, although the Centers for Disease Control recommended the use of these assays in children, there is little evidence to support this;[13] paediatric studies were small and inconsistent, and inadequate evidence exists to make clinical recommendations at this time.[14–20]

In the absence of symptoms or radiological signs, T-cell assays such as the TST fail to make the crucial distinction between LTBI and active disease. The main application of these assays would be the screening of high-risk groups (close contacts and HIV-infected children) and the provision of preventive therapy to infected individuals. If these assays are shown to be more predictive of active TB than the TST, their application may expand exponentially, with the potential to revolutionise our approach to the diagnosis and treatment of TB. A sensitive test for *M. tuberculosis* infection may also provide supportive evidence to establish or refute a diagnosis of active TB, particularly in HIV-infected children. Further research is needed to define the role of these new T-cell assays as 'rule-out' tests for active TB in children. Finally, there is an opportunity to improve the existing TST by replacing the non-specific antigens currently used (purified protein derivative) with *M. tuberculosis*-specific antigens. This should result in a more specific skin test that may be a feasible and more cost-effective alternative for developing countries.

DIAGNOSING ACTIVE DISEASE

Establishing a definitive diagnosis of childhood TB remains a challenge. Sputum-smear microscopy is positive in less than 10–15% of children with TB, and culture yields are generally low (30–40%),[21,22] although it may be considerably higher in children with advanced disease.[23] In low-burden countries, the triad of (1) known contact with an infectious source case, (2) a positive TST, and (3) a suggestive CXR is frequently used to establish a diagnosis of childhood TB.[24] This provides a reasonably accurate diagnosis in non-endemic settings, but it has limited value in endemic areas where exposure to and/or infection with *M. tuberculosis* is common and often undocumented.[24] The diagnosis of TB in children is further complicated by the great diversity of disease manifestations.[25–27]

Symptom-based approaches

Owing to the diagnostic limitations mentioned and the difficulty of obtaining a CXR in areas with endemic TB and limited resources, a variety of clinical scoring systems have been developed to diagnose active TB. A critical review of these scoring systems concluded that they are severely limited by the absence of standard symptom definitions and inadequate validation.[28] An accurate definition of

symptoms is important to differentiate TB from other common conditions, as poorly defined symptoms (such as a cough of over 3 weeks' duration) have poor discriminatory power.[29]

However, the diagnostic use of well-defined symptoms with a persistent, non-remitting character holds definite promise in low-risk children (immunocompetent children aged over 3 years) in whom TB is usually a slowly progressive disease.[30,31] The most helpful symptoms include (1) persistent, non-remittent coughing or wheezing, (2) documented failure to thrive despite food supplementation (if food security is a concern), and (3) fatigue or reduced playfulness; clinical follow-up is also a valuable diagnostic tool, particularly in children who are at low risk of rapid disease progression.[31]

The most common extrathoracic manifestation of TB in children is cervical lymphadenitis. A simple clinical algorithm that identified children with a persistent (longer than 4 weeks) cervical mass of 2 × 2 cm or more, without a visible local cause or response to first-line antibiotics, showed excellent diagnostic accuracy in an area with endemic TB.[32] However, this approach would be less accurate in non-endemic areas and in areas where alternative diagnoses, such as Burkitt's lymphoma, occur more commonly. In non-endemic settings, a positive TST result is generally more specific for active TB than it is in areas with endemic TB, but it may fail to differentiate TB lymphadenitis from disease caused by non-TB mycobacteria. In fact, cervical lymphadenitis is mainly caused by non-TB mycobacteria in non-endemic areas, and it has been demonstrated that in non-endemic areas, in the absence of known TB exposure, a positive TST is generally indicative of disease caused by non-TB mycobacteria.[33] However, establishing a definitive tissue and/or culture diagnosis remains preferable, and this can be done in a minimally invasive fashion using fine needle aspiration.[32]

Radiology-based approaches

The diagnosis of TB in endemic areas depends predominantly on the subjective interpretation of the CXR.[34] Despite its many limitations, the CXR remains the most practical and helpful test in everyday practice. It usually provides an accurate diagnosis, at least in HIV-uninfected children with suspicious symptoms, if evaluated by an experienced clinician.[24] High-resolution computed tomography is the most sensitive tool currently available to detect hilar adenopathy and/or early cavitation.[35] This technique (if available) has definite application in rare problem cases, but the natural history of disease demonstrates that particular caution is required when interpreting the relevance of these findings[24] as a limited degree of hilar adenopathy is common following recent primary infection. These signs are usually transient and not indicative of disease in the absence of symptoms. Therefore, it is important to evaluate the presence of other clinical signs and symptoms, and not to base a diagnosis of TB solely on the radiographic findings.

Immune-based approaches

Immune-based diagnosis is complicated by the wide clinical disease spectrum (ranging from subclinical latent infection to various manifestations of active disease) and other factors that influence the immune response such as BCG vaccination, exposure to environmental mycobacteria and HIV co-infection, all of which are particularly prevalent in areas with endemic TB.[36]

No serological assay is currently accurate enough to replace microscopy and culture. A recent systematic review showed that currently available serological, antibody-based tests have highly variable sensitivity and specificity.[37] However, various serological tests are marketed as diagnostic tests despite the absence of evidence, particularly in countries with endemic TB where regulatory control is less well established.[36] The use of new T-cell assays (T-SPOT.*TB* and QuantiFERON-TB Gold) and the ability of these tests to diagnose LTBI have been discussed. Like the TST, current versions of these interferon-γ release assays fail to differentiate *M. tuberculosis* infection from active disease. Identifying new ways of differentiating LTBI from active TB, and finding the correct application for these tools in areas with endemic TB remains a top priority for future research.

Another innovative approach measures the immune response (a delayed-type hypersensitivity response similar to the TST) to the transdermal application of the MPB-64 antigen.[36] In initial pilot studies, the MPB-64 skin patch test successfully distinguished active TB from LTBI (88–98% sensitivity, 100% specificity).[38] This test is currently still in development (Sequella Inc, Rockville, MD, USA). The results of more extensive field trials and the technique's ability to detect active TB in children are awaited.

Organism-based approaches

A positive culture is regarded as the 'gold standard test' to establish a definitive diagnosis of TB in a symptomatic child. It is, however, limited by the fact that organisms may be isolated from non-diseased (asymptomatic) children shortly after primary infection, during the initial period of organism multiplication and/or occult dissemination. In addition, traditional culture methods are limited by suboptimal sensitivity, slow turnaround times, excessive cost (automated liquid broth systems) and the low bacteriological yields achieved in children with active TB. It is important to point out that adolescent children (over 10 years of age) frequently develop sputum smear-positive disease that may be diagnosed using traditional methods.[39]

New culture-based approaches include TK Medium, a simple colorimetric system with reduced turnaround times but its accuracy and robustness in field conditions has not been reported.[36] The Microscopic Observation Drug Susceptibility assay uses an inverted light microscope to rapidly detect mycobacterial growth in liquid growth media. It is an inexpensive method that has demonstrated excellent per-

Table 1 Traditional and new diagnostic approaches: potential applications and perceived problems and/or benefits. (From reference 10 with kind permission.)

	Application	Problems/benefits	Validation
Traditional diagnostic approaches			
TB culture using solid or liquid broth media	Bacteriological confirmation of active TB	Slow turnaround time; too expensive for most poor countries; poor sensitivity in children	Accepted gold standard
Chest radiography	Diagnosis of probable active TB	Rarely available in endemic areas with limited resources; accurate disease classification important	Marked inter- and intraobserver variability; reliable in expert hands and in the presence of suspicious symptoms
Symptom-based approaches	Diagnosis of probable active TB	Limited by poor symptom definition	Not well validated
TST	Diagnosis of *Mycobacterium. tuberculosis* infection	Rarely available in endemic areas with limited resources; does not differentiate LTBI from active disease; not sensitive in immunocompromised children; simple to use and less expensive than blood-based tests	Various cut-off points advised in different settings
New diagnostic approaches			
Organism-based			
Colorimetric culture systems (e.g. TK-Medium)	Bacteriological confirmation of active TB	Simple and feasible, limited resources required; potential for contamination in field conditions	Not well validated in children
Phage-based tests (e.g. FASTPlaque-TB)	Diagnosis of probable active TB and detection of resistance to rifampicin	Requires laboratory infrastructure; performs relatively poorly when used on clinical specimens	Not well validated in children
Microscopic observation drug susceptibility (MODS) assay	Diagnosis of probable active TB and detection of drug resistance	Simple and feasible, limited resources required	Not well validated in children
PCR-based tests	Diagnosis of probable active TB and detection of resistance to rifampicin	Rarely available in endemic areas; sensitivity poor in paucibacillary TB; specificity a concern in endemic areas where LTBI is common, except if specimen collected from a "sterile" source; requires adequate quality control systems	Extensively evaluated, but evidence not in favour of widespread use
Antigen-based assays			
LAM detection assay	Diagnosis of probable active TB	Simple, point-of-care testing; limited clinical data on accuracy	Not well validated
Immune-based			
Antibody-based assays	Diagnosis of probable active TB	Simple, point-of-care testing, variable accuracy and difficulty in distinguishing LTBI from active TB	Not validated
MPB-64 skin test	Diagnosis of probable active TB	Simple, point-of-care testing, requires a second visit to read the result	Not sufficiently validated No studies in children

Table 1 (*Continued*)

	Application	Problems/benefits	Validation
T-cell assays	Diagnosis of LTBI	Limited data in children, inability to differentiate LTBI from active TB; blood volume required (3–5 ml); expensive; may have particular relevance in high-risk children, where LTBI treatment is warranted	Not well validated in children
Symptom-based			
Symptom-based screening	Screening child contacts of adult TB cases	Limited resources required; should improve access to preventive chemotherapy for asymptomatic high-risk contacts in endemic areas	Additional validation preferable
Refined symptom-based diagnosis	Diagnosis of probable active TB	Limited resources required; should improve access to chemotherapy in resource-limited settings; poor performance in HIV-infected children	Additional validation preferable

LAM, lipoarabinomannan; LTBI, latent tuberculosis infection; PCR, polymerase chain reaction; TB, tuberculosis; TST, tuberculin skin test.

formance under field conditions (in both adults and children),[40–42] being more sensitive than standard liquid broth or solid culture media systems. The test is not widely available at present and it remains labour intensive and operator dependant; some biosafety concerns have been raised but are currently being addressed.

The phage amplification assay utilises bacteriophages to infect live *M. tuberculosis* and is commercially available as FASTPlaque-TB; a variant (FASTPlaque-TB Response) was designed for the rapid detection of resistance to rifampicin. The phage assay has a turnaround time of only 2–3 days but is less sensitive than traditional culture methods, and no information exists on its utility in children.[36] The test has proven ability to differentiate rapidly between rifampicin-susceptible and potential multidrug-resistant cases.[43] A new broth-based colorimetric method for detecting phage replication has shown high concordance with the traditional phage assay and gene mutation analysis for rifampicin resistance; it may offer a convenient test that could be automated and used even in resource-limited settings.[44]

Polymerase chain reaction-based tests amplify nucleic acid regions specific to the *M. tuberculosis* complex; these tests have so far shown highly variable results and limited utility in children.[36] However, such tests may have value in the confirmation of extrathoracic TB,[11] species identification (confirming the presence of *M. tuberculosis* complex), molecular epidemiology and the rapid detection of mutations associated with drug resistance. Apart from new phage-based assays, the use of the polymerase chain reaction to detect drug-resistant mutations offer another option to rapidly detect drug resistance, but its application in a clinical setting has not been reported.

Other innovative organism-based approaches include the detection of TB-specific antigens; preliminary results from a new antigen-capture assay that detect lipoarabinomannan in sputum and/or urine samples look promising.[45] The potential of a gas sensor array ('e-nose') to detect a specific 'odour mixture' associated with the presence of different mycobacteria has been established. In an initial proof-of-principle study, the e-nose correctly predicted 89% of culture-positive patients with a specificity of 91% (79), after training of the neural network.[46] Further applications of this test, including its potential value in the diagnosis of childhood TB, are under investigation. Table 1 summarises the traditional and novel diagnostic approaches, their potential application and the perceived problems and/or benefits of each.

Sample collection

Collecting an adequate sample presents a significant challenge, particularly in small children who cannot produce a good sputum specimen. In young children (less than 7–8 years of age), the routine specimens collected are two or three fasting gastric aspirates. The collection of two or three fasting, early morning gastric aspirate specimens is cumbersome and usually requires hospitalisation. The collection of a single hypertonic saline-induced sputum specimen seems to provide the same yield as three gastric aspirate specimens,[47] but the value/risk of the technique has not been tested outside the hospital setting, and sputum induction may pose a nosocomial transmission risk if adequate infection control measures are not in place.[48]

Table 2 Specimen collection methods: perceived problems and/or benefits. (From reference 48 with kind permission.)

Specimen collection method	Problems/benefits	Potential clinical application
Sputum	Not feasible in very young children; assistance and supervision may improve the quality of the specimen	Routine sample to be collected in children >7 years of age (all children who can produce a good-quality specimen)
Induced sputum	Increased yield compared with gastric aspirate; no age restriction; specialised technique, which requires nebulisation and suction facilities; use outside hospital setting not studied; potential risk of transmission	To be considered in the hospital setting on an in- or outpatient basis
Gastric aspirate	Difficult and invasive procedure; not easily performed on an outpatient basis; requires prolonged fasting; sample collection advised on 3 consecutive days	Routine sample to be collected in hospitalised children who cannot produce a good-quality sputum specimen
Nasopharyngeal aspiration	Less invasive than gastric aspiration; no fasting required; comparable yield to gastric aspiration	To be considered in primary health-care clinics or on an outpatient basis
String test	Less invasive than gastric aspiration; tolerated well in children >4 years of age; bacteriological yield and feasibility require further investigation	Potential to become the routine sample collected in children who can swallow the capsule but cannot produce a good-quality sputum specimen
Bronchoalveolar lavage	Extremely invasive	Only for use in patients who are intubated or who require diagnostic bronchoscopy
Urine/stool	Not invasive; Excretion of *Mycobacterium tuberculosis* well documented	To be considered with new sensitive bacteriological or antigen-based tests
Blood/bone marrow	Good sample sources to consider in the case of probable disseminated TB	To be considered for the confirmation of probable disseminated TB in hospitalised patients
Cerebrospinal fluid	Fairly invasive; bacteriological yield low	To be considered if there are signs of tuberculous meningitis
Fine needle aspiration	Minimally invasive using a fine 23-guage needle; excellent bacteriological yield, minimal side-effects	Procedure of choice in children with superficial lymphadenopathy

TB, tuberculosis.

The string test is another non-invasive collection method that has been used with great success to retrieve *M. tuberculosis* from sputum smear-negative HIV-infected adults, demonstrating superior sensitivity compared with induced sputum.[49] The test is also well tolerated by children as young as 4 years of age.[50] Fine needle aspiration is a robust and simple technique that provides a rapid and definitive diagnosis in children with superficial TB lymphadenitis; the use of a small 23-gauge needle is well tolerated and associated with minimal side-effects.[32] Table 2 provides a summary of various specimen collection methods and the perceived problems and/or benefits of each.

HIV INFECTION

HIV-related immune compromise is one of the main risk factors that increases the vulnerability to developing active TB following infection, which explains why sub-Saharan Africa, the region worst affected by HIV, reports the highest TB incidence rate in the world.[51] Children get TB where adult source cases spread the infection, implying an increased TB exposure in these high-burden areas.[52] Both HIV-infected and HIV-uninfected children are exposed. Unlike the adult epidemic, the majority of children diagnosed with TB in areas endemic for HIV are not infected with HIV; this is because relatively few children are infected with HIV and very young children are highly vulnerable to developing TB following exposure, irrespective of their HIV status.[53] Among HIV-infected children, however, TB is a major cause of morbidity and mortality and is often underdiagnosed in resource-limited settings.[53]

For several reasons the diagnostic challenge is most pronounced in these HIV-infected children. First, HIV-infected children who live with HIV-infected adults are more likely to be exposed to an adult index case at home. HIV-infected adults often have sputum smear-negative pulmonary TB, and although they may be regarded as less infectious, they do pose a significant risk of transmission (about 20–40% of the risk posed by a sputum smear-positive patient). In addition, adult patients with sputum smear-negative TB often experience prolonged diagnostic delay, which increases the transmission risk posed to children within the household; this risk is often not appreciated by health-care workers.

Second, the TST has a low sensitivity in HIV-infected children. Although the sensitivity is influenced by the degree of immune compromise present, it is positive in the minority of HIV-infected children with bacteriologically

confirmed TB despite using a reduced cut-off point for induration size of 5 mm or more.[54]

Next, chronic pulmonary symptoms from other HIV-related conditions such as gastro-oesophageal reflux and bronchiectasis are not uncommon, and failure to thrive is a typical feature of both TB and HIV, which greatly reduces the specificity of symptom-based diagnostic approaches. Rapid disease progression may also occur, thereby reducing the sensitivity of diagnostic approaches that focus on persistent, non-remitting symptoms.[31]

Finally, interpretation of the CXR is complicated by HIV-related co-morbidity such as bacterial pneumonia, lymphocytic interstitial pneumonitis, bronchiectasis, pulmonary Kaposi sarcoma and the atypical presentation of TB in immunocompromised children.[55]

Immune reconstitution inflammatory syndrome has emerged as an important complication to consider after the introduction of highly active anti-retroviral therapy in HIV-infected, immunocompromised patients.[52] This temporary exacerbation of TB-associated symptoms and signs is mainly ascribed to the effects of improved immune function, although a 'hypersensitivity' reaction to antigens released by killed TB bacilli may also contribute. It does not indicate treatment failure and should subside spontaneously, although severe cases may require treatment with corticosteroids.[52]

CONCLUSION

Although childhood TB used to be a neglected orphan disease, the tide has changed and treatment is now more readily available. The challenge is to provide practical management guidelines that are applicable in areas with limited resources. Two groups require access to anti-TB therapy in order to reduce the severe TB-related morbidity and mortality suffered by children in areas with endemic TB: (1) high-risk, exposed and/or infected children require access to effective preventive therapy; and (2) all children with active disease require access to effective treatment. Fig. 3 presents a flow diagram that summarises the most important practice points and guides individual patient management, based on answering five simple questions, irrespective of the resources available in an particular setting:[24]

1. Is the child exposed to or infected with *M. tuberculosis*?
2. Does the child have active TB?
3. If the child is exposed or infected and active TB has been excluded, is preventive chemotherapy indicated?

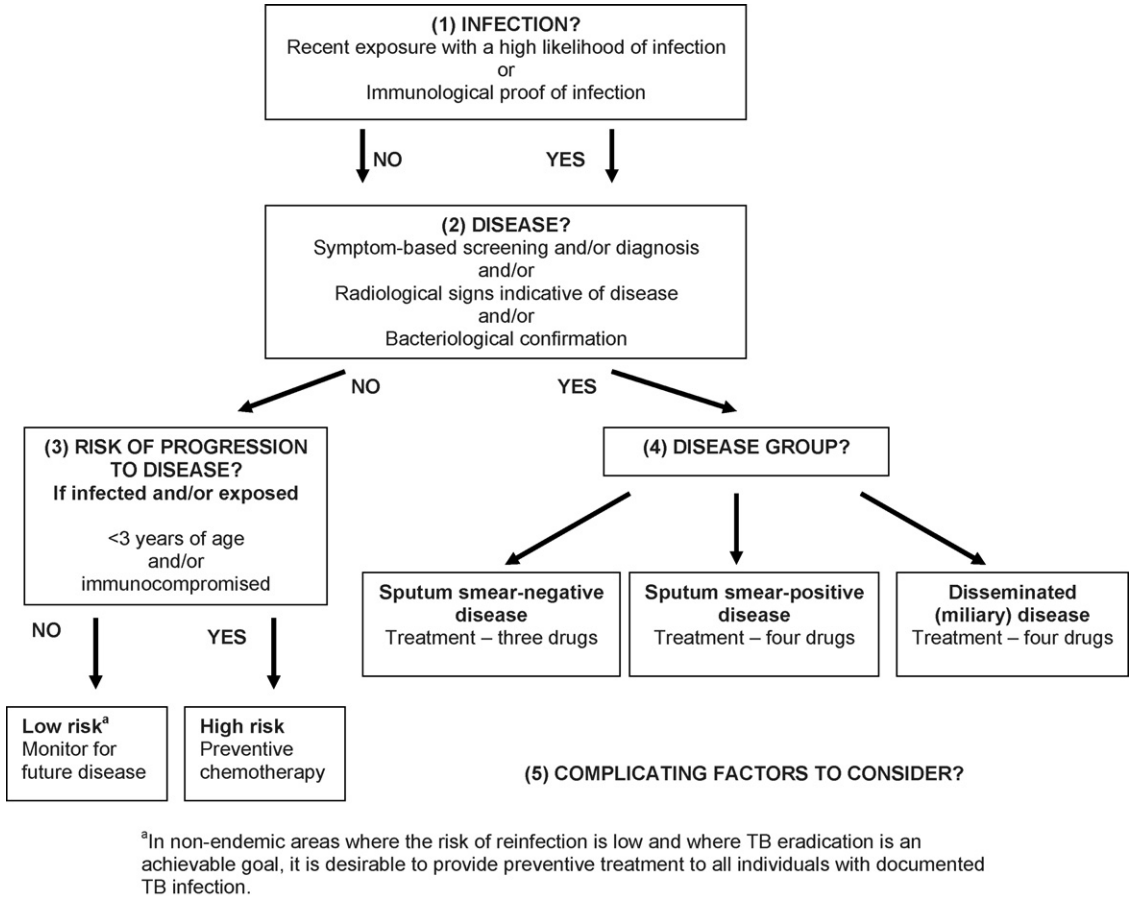


Figure 3 Flow diagram to guide the diagnosis and appropriate management of children with suspected pulmonary tuberculosis (TB). (From reference 24 with kind permission.).

4. If the child has active TB, what is the appropriate treatment regimen?
5. Are there any special circumstances to consider such as HIV infection or exposure to a drug-resistant source case?

What, then, are the research priorities for TB diagnosis among children? Based on the literature reviewed, we propose the following key research priorities:

- developing an accurate and practical case definition of active TB in childhood;
- defining the diagnostic contribution of new T-cell assays in endemic and non-endemic areas, with a specific focus on their ability to predict active disease among those with LTBI;
- developing low-cost, robust tools to accurately identify LTBI in high-risk groups, especially in HIV-infected children;
- developing new tests to accurately differentiate LTBI from active TB;
- more rigorous field-testing (which includes children) for promising new diagnostic tests.

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