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REVIEW

Recommendations for the diagnosis of pediatric tuberculosis

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Abstract Tuberculosis (TB) is still the world's second most frequent cause of death due to infectious diseases after HIV infection, and this has aroused greater interest in identifying and managing exposed subjects, whether they are simply infected or have developed one of the clinical variants of the disease. Unfortunately, not even the latest laboratory techniques are always successful in identifying affected children because they are more likely to have negative cultures and

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tuberculin skin test results, equivocal chest X-ray findings, and atypical clinical manifestations than adults. Furthermore, they are at greater risk of progressing from infection to active disease, particularly if they are very young. Consequently, pediatricians have to use different diagnostic strategies that specifically address the needs of children. This document describes the recommendations of a group of scientific societies concerning the signs and symptoms suggesting pediatric TB, and the diagnostic approach towards children with suspected disease.

Introduction

Tuberculosis (TB) is still the world's second most frequent cause of death due to infectious diseases after HIV infection [1], and this has aroused greater interest in identifying and managing exposed subjects, whether they are simply infected or have developed one of the clinical variants of the disease. Unfortunately, not even the latest laboratory techniques are always successful in identifying affected children because they are more likely to have negative cultures and tuberculin skin test (TST) results, equivocal chest X-ray findings, and atypical clinical manifestations than adults [2]. Furthermore, they are at greater risk of progressing from infection to active disease, particularly if they are very young [2]. Consequently, pediatricians have to use different diagnostic strategies that specifically address the needs of children.

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This document describes the recommendations of a group of scientific societies concerning the signs and symptoms suggesting pediatric TB, and the diagnostic approach towards children with suspected disease.

Methodology

Using the Consensus Conference method on the basis of the National Institutes of Health and the Italian National Programme Guidelines [3, 4] (Table 1), relevant publications in English were identified by means of a systematic review of MEDLINE and the Cochrane Database of Systematic Reviews from their inception until 31 December 2014. The search strategy was "children[Title/Abstract] OR pediatric[Title/Abstract] OR paediatric[Title/Abstract] AND tuberculosis[Title/Abstract] AND diagnosis[Title/Abstract] OR signs[Title/Abstract] or symptoms[Title/Abstract] or TST[Title/Abstract] or IGRA[Title/Abstract] or microbioogy[Title/Abstract] or radiography[Title/Abstract] AND English[lang])".

The Working Group agreed on a list of clinical problems related to diagnosing TB, and the evidence review procedures concentrated on patients aged 0–18 years, and included section-specific targeted searches as well as formal systematic reviews of selected aspects. The clinical recommendations made in the updated international guidelines were also reviewed and critically compared. The literature was critically appraised by trained personnel using the Scottish Intercollegiate Guidelines Network methodological checklists [5], and all of the data were entered in tables of evidence for each subject. The bibliographical material and a preliminary draft document were given to the panel members before the published evidence was presented and discussed at various meetings. The Delphi method was used to reach a consensus when the evidence did not provide consistent and unambiguous recommendations [5]. The final text was revised on the basis of these discussions and submitted by e-mail to the participants at the Consensus Conference for final approval.

The multidisciplinary panel of clinicians and experts in evidence-based medicine were identified with the help of the participating scientific societies, and included experts in the fields of general pediatrics, pediatric infectious diseases, neonatology, infectious diseases, pneumology, microbiology, radiology, pharmacology, public health and methodology. The panel was coordinated by the Italian Society of Pediatric Infectious Diseases (SITIP). No panel member declared any conflict of interest concerning the contents of the guideline topics. The panel met on three occasions, but many of the consultations involved in developing the document took place interactively by e-mail or telephone.

Table 1 Quality of evidence and strength of recommendation

Quality of evidence	
I	Evidence from more than one properly designed, randomized, controlled study and/or systematic review of randomized studies
II	Evidence from one properly designed, randomized, controlled study
III	Evidence from cohort studies or their meta-analysis
IV	Evidence from retrospective case-controlled studies or their meta-analysis
V	Evidence from case series without control group
VI	Evidence from opinions of respected authorities, based on clinical experience
Strength of recommendation	
A	The panel strongly supports a recommendation for use
B	The panel moderately supports a recommendation for use
C	The panel marginally supports a recommendation for use

When should pediatric TB be clinically suspected?

Pulmonary signs and symptoms

Most diagnoses of pediatric TB (about 65 %) are made on the basis of symptoms rather than case tracking, regardless of whether TB is endemic or not in the country in which they are made [6, 7].

The lungs are the most frequent site of pediatric TB throughout the world: a recent retrospective study of more than 2,500 children in the United States found that pulmonary forms accounted for about 70 % of cases [8], which is essentially similar to the 75.5 % found in an Italian study of more than 200 affected children [9].

Given the extreme variability of pulmonary involvement during the course of TB, the initial symptoms may also vary widely depending on age, the child's general clinical condition, the presence of underlying diseases, and the natural history of the disease; furthermore, TB symptoms may mimic some common acute respiratory diseases.

The most frequently reported symptoms in children with TB are cough lasting >4 weeks; a poor response to first-line treatment; dyspnea and asthenia; chest pain, particularly in older children and adolescents; and hemoptysis. In some case series, the presence of individual symptoms such as cough lasting >4 weeks have been highly predictive of TB (odds ratio [OR] 13.8, 95 % confidence interval [95 % CI] 2.3–83.1) [10], but such signs are often associated with a range of general symptoms such as persistent and often moderate evening fever, night sweats, general malaise and asthenia, and weight loss.

Combinations of multiple symptoms have proved to be highly predictive of TB in a number of large cohorts described in South Africa. One study of more than 1,000 children classified as being at low (aged >3 years and not infected with HIV) and high risk of TB (age <3 years or HIV-infected) tested the sensitivity, specificity and positive predictive value (PPV) of various symptoms and signs [11], and found that the combination of persistent and unremitting cough for >2 weeks, weight loss in the previous 3 months, and asthenia had a sensitivity of 82.3 %, a specificity of 90.2 %, and a PPV of 82.3 % in low-risk children, and corresponding values of 51.8, 92.% and 90.1 % in high-risk children. However, symptoms were poorly predictive of TB in the HIV-infected patients.

It has been found that the combination of cough and weight loss (OR 5.4; 95 % CI 1.7–16.9; p=0.001) or cough, weight loss and anorexia (OR 5.3: 95 % CI 1.5–18.8; p=0.004) is significantly predictive of TB in children aged <15 years; however, the presence of weight loss was required to reach statistical significance [12]. Similar findings have been reported in patients aged ≥15 years, in whom the probability of TB increased about 17-fold in the case of associated weight loss, chest/pleural pain and night sweats [13].

The pulmonary manifestations of pediatric TB are significantly different at different ages. Breastfeeding infants and school-aged children have extremely variable forms that may be clinically and radiographically similar to common respiratory infections but, as children grow, the pathognomic pulmonary characteristics of TB become gradually more similar until, by the time of adolescence, they are the same as those observed in adults [6]. In some cohorts of adolescents (aged 10–14 years) with an ascertained diagnosis of TB, the percentage of cavitary forms exceeds 80 % [12].

The radiological pictures of pediatric pulmonary TB are also extremely heterogeneous and age related. Moderate and aspecific parenchymal involvement (lobar or interstitial pneumonia) is common, but there may also be more typical pictures, such as cavitations, pleural effusion, calcifications or multi-lobar involvement (particularly the lower lobes). The presence of cavitations (OR 7.7, 95 % CI: 1.0–57.7) and the involvement of the upper segments of the inferior lobes (OR 12.6, 95 % CI 1.2–134.8) were found to be significantly associated with a high risk of TB in a small Taiwanese case series [10].

Signs and symptoms of extra-pulmonary TB (EPTB)

EPTB accounts for 20–25 % of all cases of TB [14], and is usually more difficult to diagnose than pulmonary forms not only because it is less frequent, but also because its clinical manifestations are extremely variable. The greatest diagnostic difficulties are due to its aspecific manifestations, which mimic those of other inflammatory or neoplastic diseases. It is therefore necessary to maintain a high degree of suspicion in order to make an early diagnosis, especially in a country with a low prevalence of TB such as Italy.

Furthermore, EPTB can involve relatively inaccessible sites and is characterised by a reduced bacillary load [14], and it is often necessary to consider epidemiological, clinical, immunological and imaging factors in order to make a diagnosis.

In addition to localised symptoms, EPTB may also be reflected by systemic symptoms; weight loss and a lack of appetite are characteristic of disseminated and gastrointestinal TB, whereas fever and night sweats, which are common in disseminated, cerebral and gastrointestinal forms, are less frequent in the case of lymph node, bone and genito-urinary TB [15–18].

Extra-pulmonary involvement may be associated with a pulmonary localisation, which makes diagnosis easier. In the case of clinical suspicion, after the initial investigations have excluded other possible etiologies, it is recommended to start anti-TB treatment as soon as possible, particularly in the case of severe forms such as disseminated TB and tuberculous meningitis and pericarditis.

About 50 % of the forms of EPTB involve peripheral lymph nodes, of which the most frequently involved are the laterocervical and supraclavicular lymph nodes. Lymphadenitis usually manifests itself in the form of the gradual, non-painful swelling of one or more peripheral lymph nodes. The nodes are initially hard and clearly delimited and the overlying is skin is normal but, subsequently, the skin tends to become inflamed and the nodes come together to form hard lumps that may undergo spontaneous fistulasation and release caseous material. Systemic symptoms are not frequent except in HIV-infected subjects. The presence of a painless lymphadenopathy of >2 cm that persists for >4 weeks and is unresponsive to the usual antibiotics should always raise a suspicion of TB [19, 20].

About 1 % of the cases of TB in small children may have an osteoarticular localisation, which represents about 10–15 % of the forms of EPTB [21]. This is more frequent in children than adults because the epiphyseal region of bones is more vascularised in the former. The spinal column is affected in 50 % of cases, whereas the hips, elbows and knees are less frequently involved, and involvement of the extremities is even less frequent. The spinal form usually affects the thoracic and lumbar vertebrae, usually two contiguous vertebrae but sometimes more and sometimes separately. Systemic symptoms are not frequent in the case of bone and joint TB. The most frequent symptom is pain, which usually progresses more gradually than in other bacterial forms [21], and may be accompanied by swelling and slight deformity. Kyphosis affecting the thoracic region is a late sign suggesting spinal TB [22]. Even in the early phases, there may sometimes be a paravertebral or psoas abscess, with consequent muscle spasm and movement alterations; neurological complications (Pott's disease) usually appear later.

In terms of laboratory findings, TB may be suggested by a discrepancy between white blood cell counts and the erythrocyte sedimentation rate (ESR) [21]. C-reactive protein (CRP) levels are usually normal, but there may be signs of chronic inflammation: anemia, hypoalbuminemia, thrombocytosis [23–27]. Furthermore, TB should be included in the differential diagnosis of isolated articular lesions (mainly of the hips and knees), particularly if they are not painful. Nevertheless, synovial fluid aspiration remains diagnostically decisive [26].

Tuberculous meningo-encephalitis (TBM) is the most serious form of pediatric TB, and appears in about 4–5 % of cases of TB [28]. Mortality may be as high as 10–15 %, and about half of the survivors have permanent neurological sequelae. The early symptoms are aspecific, and include anorexia, malaise, headache, and behavioural alterations. Breastfeeding infants may only manifest a lack of appetite, irritability, somnolence, behavioural alterations, and convulsions. The prodromic phase may last even several weeks, after which more specific signs such as fever, headache, vomiting and nuchal rigidity appear, the evolution of which is slower than in the case of bacterial meningitis. These signs are followed by focal neurological symptoms associated with altered consciousness, regression of neurodevelopmental cornerstones, and convulsion. Cranial nerve paralysis particularly involves the III and VI cranial nerves. Other neurological signs may develop depending on the site affected by arteritis or infarction: cerebellar signs, extra-pyramidal movements, hemiparesis.

A number of studies have defined the clinical characteristics predictive of TBM [29–33]. In addition to the symptoms mentioned above, these highlight the importance of their duration (>5 days) and the characteristics of cerebrospinal fluid (CSF): a clear appearance; leukocytes 5–750/mL, with a prevalence of lymphocytes; proteins 0.5–3 g/dL; and a CSF/blood glucose ratio of <50 %. These characteristics make it possible to differentiate TBM from other bacterial forms, but they are not decisive in the case of HIV-positive subjects insofar as they do not allow the differentiation of TBM and cryptococcal meningitis.

Pediatric abdominal TB is not very frequent [34]. It may involve any tract of the intestine and peritoneum, although the most common site is the ileocecal tract and the other parts of the colon and rectum are less frequently affected. The most frequent symptom is pain, which has a sub-acute or chronic

course in two-thirds of cases, and may mimic appendicitis or intestinal obstruction in the remaining third [35]. It may sometimes manifest itself as a palpable tumour-like mass with malabsorption or, at rectal level, as fistulae, abscesses, or anal fissuring and bleeding. A combination of fever and abdominal distension in a subject with ascites should arouse the suspicion of a tuberculous form. Diagnostic confirmation always requires invasive procedures [36].

Genito-urinary TB is one of the most frequent forms of adult EPTB, but it is rare in small children and not very frequent in adolescents because it appears at least 5 years after a first infection [37]. The symptoms are local rather than systemic, e.g. dysuria, hematuria and pollakiuria are frequent, and associated with pain in the side. The symptoms are so vague or mis-recognised that they are often only diagnosed at an advanced stage of renal damage. It is sometimes diagnosed after a routine urine test; the presence of hematuria and sterile pyuria in acidic urine should always prompt a search for urinary *M. tuberculosis* [38].

Pericarditis is a rare form of TB that is still sometimes fatal, particularly in HIV-positive subjects [39]. The initial symptoms are mainly systemic and aspecific, whereas cardiopulmonary symptoms (cough, dyspnea, orthopnea, lower limb edema, a dull retrosternal pain often influenced by position and inspiration) appear later. The progression of the disease and effusion may be accompanied by friction rubs, a sign of cardiac tamponade or constrictive pericarditis. However, in addition to images of exudative pericarditis, diagnosis always requires pericardiocentesis or even pericardiotomy, particularly in countries with a low prevalence of TB [39].

Disseminated TB is caused by the hematogenous dissemination of tubercular bacilli due to inadequate host defences, and is therefore more frequent in children aged less than 2–3 years, whose cell-mediated immune responses are still immature [40] and, in breastfeeding infants, it may develop in 10–20 % of cases of TB due to a recent primary infection. It has various clinical manifestations. The signs and symptoms at the time of onset are generally systemic and non-specific, e.g. fever, weight loss, night sweats, anorexia, and the loss of appetite. The other symptoms depend on the predominantly involved site; cough and other respiratory symptoms are associated with pulmonary TB with a characteristic miliary appearance, whereas headache and altered consciousness are associated with meningeal involvement. Choroidal tubercles, which are pathognomic of miliary TB, can be observed in 30 % of cases [40]. However, no systematic diagnostic approach model has yet been defined [41].

How should pediatric TB be diagnosed?

A detailed anamnesis aimed at investigating possible exposure to *M. tuberculosis* as a result of contact with someone with bacilliferous TB or coming from an endemic area, and factors favouring the development of the disease such as poor socio-economic conditions is fundamental for a diagnosis of TB [42]. Particular attention should be given to the presence of concomitant medical conditions favouring immunosuppression, such as malnutrition, HIV-1 infection, or treatment with corticosteroids or immunosuppressants [42].

Clinical analysis also plays a fundamental role, and all of the signs and symptoms suggesting active disease should be carefully evaluated: chronic cough, weight loss, asthenia, profuse sweating, fever, chest pain, hemoptysis and respiratory distress [43].

Tuberculin skin test (TST)

Since 1907, a TST has been the reference diagnostic means of detecting *M. tuberculosis* infection [15, 44]. The test is based on the delayed hypersensitivity reaction induced by the intradermal inoculation of a standardised dose of purified protein derivative (PPD) tuberculin: the WHO recommends the use of five units of PPD-S or two units of PPD tuberculin RT23. Tuberculin reactivity usually appears between two and 12 weeks after the initial infection (median 3–4 weeks).

PPD is intradermally injected in a lesion-free area of the volar forearm (Table 2). T lymphocytes sensitised by a previous infection are recruited at the site of the inoculation, where they release lymphokines that induce local vasodilation, edema, fibrin deposition and the recruitment of other inflammatory cells, resulting in an infiltrate that reaches its maximum size 48–72 h after the injection [44]. The diameter of the swelling should therefore be measured (in millimetres) within this period by trained medical personnel. The result of the test should be interpreted individually in relation to the presence of conditions predisposing to TB, and the timing and type of disease exposure as shown in Table 3.

A TST has some limitations, for example, it is an operator-dependent test that requires the patient to make at least two visits to a healthcare centre, and may give rise to false positive or false negative results [44–47]. The possibility of false positive results is due to the cross-reactivity of the PPD antigens with those of non-tuberculous mycobacteria and even of BCG vaccine, even though the reactions rarely exceed 15 mm in diameter [48]. The guidelines of the American Academy of Pediatrics recommend using the same interpretation of TST for children regardless of whether or not they have previously received BCG [44] because the post-vaccination intradermal reaction to tuberculin can be influenced by many factors, such as the child's immune status or age at the time of vaccination, the quality and type of the vaccine strain, and the time interval between the vaccination and the TST. Nevertheless, the same guidelines suggest considering any hardening of more than 15 mm as positive in previously vaccinated children. The specificity of the test obviously depends on the criteria used

Table 2 Tuberculin skin test (TST) procedure

Recommendation	Reason
Check medical prescription	Reduces the risk of error
Check the availability of the test and, if necessary, order a new supply	Reduces the risk of error and increases the appropriateness of clinical practice
Check the type of tuberculin available and, if the supplier has changed, carefully read the technical data sheet	Reduces the risk of error
Inform the patient	Guarantees the right of informed consent
Guarantee privacy	Guarantees patient well-being
Have the patient adopt a comfortable position	Reduces patient discomfort
Wash hands	Reduces the risk of cross-infection
Wear non-sterile gloves	Reduces the risk of cross-infection
Check the expiry date and characteristics of the drug to be administered	Reduces the risk of error
Attach the needle to the syringe (1.0 mL), and reconstitute the solution by aspirating the required dose from the vial (0.1 mL of PPD tuberculin solution or 2 mL of PPD RM 23 solution)	Guarantees procedure is executed correctly
Aspirate the drug in the needle into the syringe	Guarantees procedure is executed correctly
Replace the needle used to perforate the natural rubber membrane of the vial containing the drug	Reduces procedure-related discomfort and pain. Increases patient comfort
Eliminate any air bubbles inside the syringe, and fill the new needle	Guarantees procedure is executed correctly
Distract patient with the aid of an assistant, parent or specialised entertainer if appropriate	Increases patient comfort, and reduces the child's sensations of pain and anxiety
Identify an appropriate administration site on the volar forearm. Avoid the area near the elbow and the wrist	Guarantees procedure is executed correctly. Administration near the wrist or elbow may be associated with false negative results
Carefully disinfect the administration site using centrifugal movements for at least 20–30 s, and wait until the skin dries	Reduces the risk of infection. Guarantees procedure is executed correctly
Stretch the skin between the thumb and middle finger of the non-dominant hand	Stretching the nerve ends in the skin reduces pain
Using the other hand, insert a short length of needle angled upwards into the skin, maintaining a slope of 10–15° with respect to the plane of the skin and penetrating 2–5 mm below the epidermis. The needle should be visible through the epidermis during the insertion (Drug Technical Data Sheet: Statens Serum Institute, 2014)	Avoids the risk of subcutaneous or intramuscular administration
During the administration, it should be possible to note a swelling of the epidermis or a whitened area of about 6–10 mm (a sign that the procedure has been corrected executed); if this does not occur, repeat the administration on the other arm, or at a distance of at least 4 cm on the same arm	Guarantees the test is executed correctly. If the injection is mistakenly administered subcutaneously or intramuscularly, the papule will not develop and the TST must be repeated.
Administer the drug as indicated in the technical data sheet	Assures correct administration
Do not massage or press the administration site, but lightly dab it	Avoids discomfort
Do not recap the needle after use and arrange for its disposal in the appropriate container	Avoids the risk of accidental puncture
Highlight the injection site using an atoxic dermographic pen	Guarantees the correct localisation of the inoculation site during the test reading 48–72 h afterwards
Tell the child and his/her parents not to wash or scratch the forearm	Prevents reddening or other manifestations that may confound the test results
Dispose of waste material in accordance with current procedures	Prevents the risk of accidents and cross-infection
Wash hands	Prevents the risk of cross-infection

to define a positive result and the prevalence of non-tubercular mycobacterial infections in the population considered. Another possible cause of a false positive result is the booster effect of repeated tests [49].

False negative results are possible when making an initial evaluation of patients with active TB, especially in the case of disseminated, miliary or pleural TB [50]. The main causes underlying such findings include malnutrition and congenital or acquired immunodeficiency, such as HIV-1 co-infection [51], but other reasons may include recent TB infection, an age of <2 years, recent vaccinations with attenuated live viruses (such as an anti-measles vaccination), or ongoing viral infections such as measles, chickenpox or influenza [52]. It has been reported that approximately 10–40 % of immunocompetent

Table 3 Definition of positive tuberculin skin test (TST) results in infants, children, and adolescents

Diameter ≥5 mm	• Children in close contact with people with known or suspected TB infection
	• Children with chest radiography findings compatible with active TB
	• Children with clinical signs of active TB
	• Children receiving immunosuppressive treatment or with immunodepressive conditions (e.g. HIV infection)
	• Children aged <4 years
Diameter ≥10 mm	• Children coming from or who have stayed in a country with a high prevalence of TB disease
	• Children living in poor socio-economic conditions
	• Children with medical risk factors such as lymphoma, diabetes mellitus, chronic renal insufficiency, malnutrition
	• Children frequently exposed to adults with HIV infection, the homeless, drug addicts, people living at healthcare residences, prisoners, institutionalised subjects, migrant farm workers
Diameter ≥15 mm	• Children aged >4 years without risk factors for TB infection

Modified from American Academy of Pediatrics [44]

TB tuberculosis

children with confirmed microbiologically active TB are initially TST negative [50], and so a negative TST result does not necessarily exclude the possibility of TB infection and/or disease [52].

Immunological tests for the diagnosis of pediatric TB

Interferon-gamma (INF-γ) release assays (IGRAs) measure the INF-γ released by lymphocytes in response to in vitro stimulation with specific *M. tuberculosis* antigens. Two tests are currently marketed, namely, T-SPOT.TB (Oxford Immunotec, Oxford, UK) and QuantiFERON-TB GOLD (QFT-G, Qiagen, Venlo, The Netherlands) [53].

T-SPOT.TB uses the enzyme-linked immunospot (ELISPOT) method to quantify IFN-γ-producing lymphocytes; the result is considered positive if >7 spots are detected, negative if there are <5, borderline if there are 5–7, and indeterminate if there is no response [54]. QFT-G uses the enzyme-linked immunosorbent assay (ELISA) method, and the result is considered positive if >0.35 UI/mL of IFN-γ is produced after specific antigen stimulation, and indeterminate if there is no response in the mitogen-containing control tube [54]. The antigens used in these tests are synthetic peptides that simulate the early secreted antigenic target 6-kDa (ESAT 6) and culture filtrate protein 10-kDa (CFP-10) of *M. tuberculosis*.

As IGRAs are in vitro tests, they are not operator dependent, do not require a second visit to a healthcare facility, and can be repeated any number of times without causing a booster effect. However, there is evidence indicating that a previous TST can alter IGRA results when there is only a 3-day interval between them [55–57].

The tests have to be carried out scrupulously in accordance with the instructions of the manufacturer; unreliable results in a considerable proportion of patients have been reported to be related to technical problems during the execution of the test, including the drawing of insufficient blood from children. The findings of some studies support the hypothesis that the performance of the available IGRAs can be improved by prolonging incubation times [58] or modifying the currently used cut-off values on the basis of epidemiological or clinical risk [59]. However, the evidence is still insufficient to be able to recommend changes in the way the tests are carried out or the results are interpreted [60].

The sensitivity and specificity of in-house tests or of tests that use antigens or cytokines other than those in the marketed tests described in the literature are extremely variable, and cannot be recommended for use in clinical practice.

One of the advantages of IGRAs is that previous BCG vaccination has little or no effect on the results. The ESAT-6 and CFP-10 are coded at the level of region of difference 1 (RD1) of the genome of *M. tuberculosis* and, as these antigens are not expressed in the genome of *Mycobacterium bovis* BCG contained in the vaccine or in the genomes of the majority of non-tuberculous mycobacteria, it is often possible to distinguish subjects with TB infection from those who have been recently vaccinated and those with non-tubercular mycobacterial infections [61]. However, as the same antigens are also expressed by some non-tubercular mycobacteria, such as *Mycobacterium avium, Mycobacterium kansaii,* and *Mycobacterium szuigai*, false positive results are still possible.

The performance of IGRAs has been documented in many studies. One study of 336 children carried out in Italy in 2009 by Bianchi et al. suggested that they are more specific than a TST in children with suspected TB infection [62], and a German study of 73 children with latero-cervical lymphoadenopathy comparing the TST with two IGRA methods found that the specificity of QFT-G In-Tube (IT) and T-SPOT was respectively 100 % (95 % CI 91–100 %)

and 98 % (95 % CI 87–100 %), whereas that of the TST was only 58 % (95 % CI 42–73 %) [63].

A number of systematic reviews have revealed differences in sensitivity and specificity in different populations [64]. However, these meta-analyses need to be interpreted critically because the studies involved often used different diagnostic criteria to define active TB, only a few were based on head-to-head comparisons of T-SPOT.TB and QTF-G-IT, and various cut-off values were used to define TST positivity.

It is also necessary to remember that the TST and IGRAs cannot differentiate active TB and latent infection, and that their performance in children aged <5 years and immunodepressed subjects seems to be sub-optimal. The reported sensitivity of T-SPOT.TB and QFT-G- IT in children, respectively, range from 62 to 89 % and from 66 to 83 %. In 2010, Bamford et al. [65] studied 333 children in the United Kingdom and found that the sensitivity of the TST, QFT-G IT and T-SPOT.TB was 82, 78 and 66 %, respectively, whereas the combined use of TST and QFT-G IT had a sensitivity of 96 %, and that of TST and T-SPOT.TB was 91 %. It is possible that the TST is more sensitive because it is capable of exploring multiple TH1- and TH2-mediated immunological mechanisms, whereas IGRAs exclusively explore TH1-mediated responses, which may be immature in children in a manner that is inversely proportional to their chronological age [64]. Furthermore, antigen-presenting cells in children are less capable of synthesising IL-12, a fundamental mediator in the initial phase of TH1 polarisation [64].

Pediatric studies have also highlighted a larger proportion of indeterminate results in children than in adults [64]. A recent systematic review found that this proportion was 6.5 % in the case of QFT-G IT and 3.5 % in the case of T-SPOT.TB [66], but studies involving children aged <5 years have shown that it may range from 0 to 40 % [67]. Some authors have reported a larger proportion of indeterminate results not only in children aged <5 years [68], but also in the case of helminth co-infection, HIV-related immunosuppression, and immunosuppressive treatment [66]. A recent study has found that concomitant bacterial pneumonia may also be a risk factor in an indeterminate result, particularly in children aged <5 years [68]. In these situations, the increased risk may be due to possibly age-related immune system immaturity leading to a reduction in stimulated INF-γ production or an imbalance in TH1/TH2-mediated responses.

The guidelines of the American Academy of Pediatrics underline the fact that IGRA-positive children should be considered infected, and a negative or indeterminate result does not exclude infection [44]. Furthermore, IGRAs are not recommended in children aged <5 years because of the lack of unequivocal data or in immunocompromised children. The 2011 revision of the British NICE guidelines suggest a two-step approach: a TST followed by the IGRA confirmation of the TST-positive cases [69]. However, they also indicate situations in which it is advisable to use an IGRA alone, such as in the case of subjects who for any reason cannot be reached a second time 48–72 h after a TST, or if it is necessary to screen large numbers of children (including subjects aged ≥5 years), or when selected cases previously vaccinated with BCG need to be investigated as a result of coming into contact with subjects with contagious TB [69].

With reference to children aged <5 years, Detjen et al. evaluated the diagnostic accuracy of TST, T-SPOT.TB and QFT-G-IT in 73 children with a mean age of 39 months—28 with bateriologically confirmed TB, 23 with lymphadenitis due to non-tubercular nycobacteria, and 22 controls [63]. The specificity of QFT-G-IT for TB was 100 % (95 % CI 91–100 %) and that of T-SPOT.TB was 98 % (95 % CI 87–100 %, but the specificity of the TST was much less (58 %, 95 % CI 42–73 %); the sensitivity of both IGRAs was 0.93 (95 % CI 0.77–0.99) and that of the TST was 1.00 (95 % CI 0.88–1.00; k=0.91). In another study Okada et al. compared QFT-G-IT and TST results in 195 Cambodian children living with people with active TB, and found that the TST was more specific in those who had previously undergone BCG vaccination (k=0.63) [70]. Debord et al. retrospectively assessed the performance of QFT-G-IT in 19 immunocompetent French children with active TB aged <5 years (mean age 1.52 years) [71]. There were no indeterminate results and the number of positive cases was 6/10 children aged <2 years and 9/9 children aged 2–5 years, thus suggesting that QFT-G-IT may be a useful means of improving the diagnosis of TB in association with TST in children aged <5 years [71]. The studies of 397 South African children aged <3 years showed good concordance between QFT-G-IT and TST results (k= 0.79); however, the sensitivity of both tests in detecting active TB disease seemed to be poor (respectively, 38 and 35 %) [72, 73]. On the contrary, Pavic et al. reported considerable discordance between QFT-G-IT and TST results in 142 Croatian children aged <5 years (k=0.59), and concluded that both could be used in high-risk children aged <5 years provided that positivity to either was considered a sign of infection [74].

The findings of a large-scale study of more than 1,000 adults suggest that IGRA (particularly T.SPOT.TB) can be used to screen immunodeficient contacts of patients with TB [75], but there are extremely few pediatric data. Bruzzese et al. studied 80 HIV-negative immune-compromised children in follow-up for liver transplantation or rheumatologic diseases on biological treatment after coming into contact with TB using both QFT-IT and T-SPOT; QFT-IT was positive in one case (1.2 %), whereas a significantly larger proportion of cases (9.4 %) were positive to T-SPOT.TB (p=0.02) [76]. The authors concluded that the high proportion of discordant or indeterminate results meant that IGRAs were of little help in diagnosing TB infection or disease in immunocompromised children living in a country with a low prevalence of TB. The other available studies suggest that IGRA may play a role in

diagnosing TB infection and disease in the immunodepressed [77–81], but their sample sizes were small.

IGRAs have little positive predictive value in indicating the progression of active TB. Studies of adults and children have clearly shown that the serial repetition of the tests during anti-TB treatment does not aid the monitoring of therapeutic responses [82, 83].

What type of microbiological diagnosis should be used for children with suspected TB?

The microbiological diagnosis of pediatric pulmonary TB is still largely unsatisfactory, and its limited sensitivity is even more marked in the case of immunocompromised subjects. There are two main reasons for this: (1) the disease is typically paucibacillary in children and (2) children are generally incapable of providing sputum samples of acceptable quality [84]. It is therefore necessary to use alternative sampling methods, all of which are sub-optimal [85].

Gastric aspiration is the most widely used method of collecting swallowed respiratory secretions from children. It is normally recommended that these samples should be neutralised with sodium bicarbonate, but it has been recently reported that this can significantly reduce culture yields in liquid medium [86].

An alternative method is to induce sputum using a hypertonic aerosol solution. This technique is not invasive but it does require patient collaboration, which is not easy to obtain, especially in the case of small children. A number of studies have compared the diagnostic yield of gastric lavage and induced sputum, sometimes including a comparison with nasopharyngeal aspirates. The results are not always concordant, but the majority of studies have found that gastric lavage is the most appropriate [87–94].

Broncholavage has the advantage of being aimed at the lesioned site, but is rather invasive [95].

Another possibility is the string test. The patient swallows a gelatin capsule containing a length of thread. The capsule dissolves in the stomach, thus allowing the thread to become impregnated with gastric secretions. Four hours later, the thread is recovered and washed with 1–2 mL of saline solution, which is then used for the search [96].

A still inadequately evaluated approach is to search stool for possible swallowed mycobacteria [97, 98].

It goes without saying that sputum is the sample of election in the case of adolescents and children capable of producing it, whereas gastric aspirate is the sample of election from the youngest patients [94].

All of the samples should be collected in disposable, sterile containers with screw caps [94] and, if they cannot be sent to the laboratory immediately, should be stored in a refrigerator. In order to increase sensitivity, microbiological investigations for pulmonary TB are usually carried out on three samples collected on consecutive days [99]; a single sample can be used only in the case of broncholavage.

The sensitivity of a microbiological diagnosis of both adult and pediatric TBM is limited by the availability of a sufficient amount of CSF in which to seek Koch's bacillus [94].

Diagnosing lymph node TB is less of a problem when a bioptic sample is available [94].

A biopsy is also the sample of election in the case of pleural TB; alternatively, searching an induced sputum sample is much more useful than searching a pleural fluid sample [100].

Pediatric TB is quite rare in other body districts, and its diagnosis raises similar problems to those associated with diagnosing their adult counterparts [94].

Table 4 shows the specifications of the various types of biological samples, which may be examined microscopically, cultured, or tested by means of gene amplification.

Although not very sensitive, a positive microscopic examination of a sample previously stained in such a way as to reveal acid-fast bacilli allows prompt confirmation of a mycobacterial etiology [101]. The two most widely used stains are Ziehl Neelsen and the more sensitive fluorescence staining, but neither are capable of determining whether the mycobacterial infection is tubercular or not.

A culture examination is the gold standard of mycobacteriological diagnostics, but it is less sensitive when using respiratory samples taken from children than those taken from adults [102]. With few exceptions (particularly CSF), most biological samples are contaminated and therefore have to be treated in order to eliminate the accompanying and rapidly multiplying bacterial flora that would otherwise irremediably prevent the growth of possible mycobacteria. A mycobacterial culture requires the inoculation of a solid (generally Löwenstein-Jensen) and liquid medium (the most widely used is mycobacterial growth indicator tube [MGIT]), and prolonged incubation at a temperature of 35–37 °C for up to six (liquid media) or 8 weeks (solid media) before it can be considered negative.

In the case of a positive culture, it is essential to identify the species, or at least differentiate non-tubercular mycobacteria from those belonging to the *M. tuberculosis* complex, because both can grow in the most frequently used media. The isolation of *M. tuberculosis* complex is diagnostic for TB, but it is often difficult to interpret the significance of finding a non-tubercular mycobacterium [103].

Although gene amplification is less sensitive than a culture, positive findings greatly reduce the time of diagnosis [104]. Laboratories equipped for mycobacterial diagnostics generally also use amplification tests specific for the *M. tuberculosis* complex whose sensitivity in the presence of a positive microscopic examination is practically 100 %, thus allowing a rapid diagnosis; however, in the case of microscopically negative samples, their sensitivity is much less and also varies

Table 4 Specifications for collecting biological samples for diagnosing tuberculosis (TB)

Material	Requirements	Instructions	Unsuitable samples
Gastric aspirate	At least 5 mL collected in the morning after at least an 8-h fast on 3 consecutive days	Neutralisation with 100 mg of sodium bicarbonate	
Sputum	At least 5 mL of deep sputum collected in the morning on 3 consecutive days	Rinse the oral cavity with water before collecting the sample	Saliva, pooled samples
Induced sputum	At least 5 mL collected in the morning on 3 consecutive days	Delivery of hypertonic saline by nebulizer	
Broncholavage	At least 3 mL	Disinfection and sterilization of the bronchoscope	
Cavitary fluids	10–15 mL in a test tube with trisodium citrate		
Cerebrospinal fluid	At least 2 mL		
Bioptic samples	At least 1 g of tissue		Samples in formalin (although a molecular search is possible)
Pus	The maximum possible amount collected by means of a syringe and transferred into a sterile test tube		Swabs with transport medium
Urine	At least 40 mL of the first morning urine		24-h urine
Feces	At least 1 g	Not useful for a diagnosis of intestinal TB	

depending on the type of sample. If a sample is gene amplification positive but microscopically negative, the albeit rare possibility of contamination can be confirmed or ruled out by repeating the test, preferably on a new sample [104]. It is recommended to use gene amplification systems that include the monitoring of inhibitors because, in the absence of these, a negative result has no value. Despite its limitations, it is now generally accepted that an amplification test combined with microscopy and culture increases the sensitivity of a microbiological diagnosis of TB. In the case of a grounded clinical suspicion [105], the addition of gene amplification to conventional tests provides an added value that should not be underestimated in the largely deficient field of diagnosing pediatric TB.

The commercial GeneXpert amplification test not only reveals TB bacillus DNA, but also the presence of mutations responsible for resistance to rifampicin [106, 107]. This can be useful when there is a strong suspicion of infection due to multidrug-resistant (MDR) strains, i.e. in the case of patients coming from countries with a high incidence of MDR-TB who have come into contact with people with MDR-TB or who have been previously treated with anti-TB drugs. Alternatively, a line probe assay (LPA) can be used to test genotype resistance to the two principal first-line drugs, namely, rifampicin and isoniazid [107].

Many of the commercial *M. tuberculosis* complex gene amplification systems have only been validated for use on respiratory samples, but there are now many published studies showing that they are also useful for diagnosing EP-TB [108–110].

In patients with a microbiological diagnosis of TB undergoing anti-TB treatment, responses can be periodically monitored by means of microscopy and cultures alone, while gene amplification is of no use [104].

Many published studies have clinically validated the various commercial *M. tuberculosis* gene amplification systems, some studies of which have included pediatric samples. There are only small differences in performance between them, and the only factor that has a significant impact on their sensitivity is the volume of the processed clinical sample [111]. The GeneXpert system, which has been satisfactorily validated in a particularly large number of recent studies, is characterised by the fact that it requires very little effort on the part of the operator and has a high inoculation capacity; it is possible to load up to 0.5 mL of sample concentrated by means of centrifugation [112, 113].

The first strain isolated from every patient should undergo a phenotypic assay of drug susceptibility, particularly its sensitivity to isoniazid, rifampicin, ethambutol and pyrazinamide [114]. In the case of MDR strains resistant to at least rifampicin and isoniazid, the assay should be extended to second-line drugs and carried out at a reference centre. Phenotypic assays are currently considered the gold standard for studying drug resistance, but there are now also gentotypic tests available based on the use of probes for detecting the mutations responsible for resistance to rifampicin, isoniazid, ethambutol, quinolones and aminoglycosides [115–117], which guarantee a rapid response especially when they can be used directly on a clinical sample. However, their sensitivity in detecting resistance varies from 50 to 90 % depending on the drug, and so the results must always be confirmed by a phenotypic test. Their use is recommended whenever infection due to an MDR strain is suspected.

What radiological methods should be used in the case of suspected pediatric TB?

Radiology for pulmonary TB

Pulmonary TB is associated with a variable spectrum of lesions which, if described as distinct pathologic entities, would include pulmonary consolidations; lymph node disease, with or without airway obstruction; a miliary pattern; cavitary lesions; and pleural effusion [118].

The radiological examination is based on antero-posterior and lateral chest X-rays. The lateral projection improves visualisation of posterior lymphadenomegaly and lymphadenomegalies inferior to the intermediate bronchus [118]. Furthermore, the usefulness of the lateral projection and low radiation dose required lead to an advantageous cost/benefit ratio [119].

Although the indications have not been standardised, the investigation can be completed using computed tomography (CT) with contrast medium. CT is clinically useful above all in the case of children aged <5 years, particularly those aged <2 years in whom conventional chest radiography is often insufficient [118] because of the physiological protrusion of the thymus. CT is also better than conventional radiology in detecting calcifications, which are rare in the first years of life, and the contrast medium makes it possible to assess intrathoracic lymphadenomegalies [120], which are generally hilar and mediastinic, and usually prevalent on one side. Normal lymph node size in childhood is up to 7 mm (short axis). Although aspecific, another suggestive pattern is that of the "rim sign" (low density in the centre of the lymph node surrounded by a peripheral ring enhancement). Differential diagnosis includes lymphoma, other infections such as HIV infection or minor acute or chronic inflammation, and sarcoidosis (extremely rare).

In brief, even in the absence of clinical symptoms, the main signs of active TB are consolidation and non-calcified hilar lymphadenomegaly [121].

CT with contrast medium is the technique of choice for defining lymphobronchial disease, a complication of pediatric TB due to bronchial compression by an infected lymph node, erosion, ulceration, infiltration, the intrabronchial flow of caseous material, and the formation of granulated tissue [122], thus leading to air trapping, atelectasis, consolidation, necrosis and cavitation. It is also better for evaluating tuberculous consolidation and cavitation, identifying granulomas/tuberculomas, and investigating complications (lymphobronchial disease, pericarditis, outcomes). The use of CT for follow-up purposes is strictly related to the clinical/radiological evolution of TB [122]. Chest ultrasonography can play a role in evaluating pleural and pericardial effusion [118], but there are no pediatric data concerning the role of positron emission tomography (PET).

The anatomo-radiological classification of Marais et al. makes it possible to distinguish parenchymal and lymph node disease and their evolutionary stages [123]. A parenchymal Ghon focus may not be self-limiting, but cavitate and lead to intrabronchial spreading and subsequent consolidation; this expression is typical in adults but, although rare, is also possible in early infancy. However, what is typical in early infancy is lymph node (lymphobronchial) disease with a progressive cascade of the complications described above, which can be best identified with CT. The disease may spread into the pleural space, the pericardium or hematogenously (miliary TB), with possible outcomes being calcification, parenchymal destruction with fibrosis, and bronchiectasis. Andronikou et al. have in fact integrated the classification of Marais et al. with the findings of tomographic techniques [118].

Radiology for EPTB

Children with suspected EPTB should always undergo standard chest radiography [124], which is positive in 40–87 % of the patients with the disease [125].

Cervical, submandibular, supraclavicular and pre-auricular sites are the most typical locations of peripheral tuberculous lymphadenitis. Imaging only plays a relatively important role [126]; the examination of choice is ultrasonography, but the only ultrasonographic sign suggesting tuberculous lymphadenitis is calcification, which usually appears late.

Osteo-articular TB often presents with symptoms that are difficult to interpret, and so imaging represents a first approach [126]. Standard radiography reveals juxta-articular osteoporosis, peripheral bone erosion, gradual joint space narrowing and, sometimes, a 'cystic' pattern with sclerotic fissuring. Tuberculous dactylitis deserves particular mention; it is characterised by the painless involvement of the short tubular bones of the hands and feet, and radiographically revealed fusiform tumefaction of the soft tissues with or without periostitis. Subsequent alterations are a coarse trabecular pattern with acro-osteolysis, reactive sclerosis, and joint involvement [126].

Magnetic resonance imaging (MRI) is the technique of choice for obtaining a complete osteo-articular anatomical picture of all body regions, including an evaluation of possible synovitis, soft tissue involvement, and cartilage destruction [126].

Tuberculous spondylitis accounts for 30–50 % of cases of tuberculous osteomyelitis [127], with the frequently affected site in childhood being the dorso-lumbar junction. Standard radiography may show disc space narrowing and various degrees of bone destruction, with possible kyphosis. Diagnosis may be elusive or difficult, particularly in the lumbo-sacral region. CT provides a complete picture of all bone segments and is also capable of identifying collections of pus, but MRI is the investigation of choice for a sinal evaluation (vertebral

bodies, discs, the spinal canal, and paraspinal tissues). Bone scintigraphy provides a whole-body evaluation of skeletal involvement (an alternative is whole-body MRI mainly based on STIR sequences).

The follow-up is based on radiography, with MRI being used more frequently in cases of spinal locations, which require a careful evaluation not only of the bone [126, 127]. The use of CT is largely limited to conditions that contraindicate the use of MRI [126, 127].

In the case of central nervous system (CNS) TB, the typical alterations are granulomatous lesions in the leptomeninges, to the junction between the grey and white matter of the brain, and in spinal cord [126]. The presence of tuberculomas can be characteristic, whereas another manifestation is arteritis, which may be responsible for severe ischemic events [126].

In the absence of other known manifestations, the diagnosis of CNS TB can be difficult because the different neuroradiological pictures simulate other pathological conditions, particularly neoplasias. However, a combination of meningeal and parenchymal lesions should always arouse a suspicion of TB [128].

In an early phase, a cerebral CT scan without contrast medium may be negative (10–15 % of cases), or show the presence of an iso-hyperdense exudate in the CSF-filled subarachnoid spaces (basal cisterns and sulci) or iso-hyperdense, roundish or oval masses (tuberculomas) that rarely contain calcifications and are delimited by more or less extensive edema [128]. The administration of contrast medium leads to intense enhancement of the basal cisterns, and marginal or diffuse impregnation of the tuberculomas.

MRI provides much more information about CNS TB than CT, the use of which is currently limited to emergency situations or the monitoring of hydroencephalus secondary to tuberculous meningo-encephalitis [129]. An MRI protocol for studying suspected CNS TB should always include T1-weighted (with and without contrast medium), turbo spin-echo (TSE) and T2-weighted FLAIR images, diffusion-weighted (DW) images, and possibly angio-MR arterial images (MRA). DW images are very useful for better characterising tuberculoma signals and evaluating complications (ischemia, cerebritis) [129]. T1-weighted images with contrast medium can reveal meningeal involvement as intense enhancement and nodularity (an aspecific but very important sign that is highlighted better by MRI than CT); vasculitis of perforating vessels, which appears as puntate/linear enhancement at the level of the basal ganglia; ventriculitis; plexitis (rare); and pachymengitis (infrequent). Pienaar et al. found that MRI showed basal meningeal enhancement in 97 % of their patients with tuberculous meningo-encephalitis (as against 70 % in the case of CT), infarctions in 83 % (as against 70 %), and granulomas in 40 % (as against 16 %) [129]. Angio-RMI can reveal vascular involvement such as alterations in arterial profiles (stenoses, irregularities) or signs of occlusion [129].

In the case of CNS TB, MRI with contrast medium should also be extended to the whole spinal column in order to exclude spinal meningitis, which is characterised by irregular intradural enhancement, often with the formation of perimedullary pseudo-cysts, medullary granulomas, and misinterpreted vertebral alterations more or less associated with CNS lesions [126].

Although challenging, imaging is also used to diagnose abdominal TB [128–131]. Standard radiography can reveal signs of occlusion but ultrasonography and/or CT are necessary as they can reveal porta hepatis, in para-aortic sites, and mesenteric lymphadenomegalies (the last being common in children). The presence of calcifications and/or rim signs can suggest TB, whereas the signs of peritonitis (thickening, stranding) are aspecific. Gastrointestinal involvement manifests itself as thickening and parietal contrast enhancement (particularly at ileo-cecal level).

Micro- (0.5–2.0 mm) and macronodular hepatosplenic lesions are typical of disseminated disease [129–131]. The lesions are hypoechogenic/hypodense, and can suggest TB when/if they calcify.

Renal TB is characterised by hydrocalycosis (papillary necrosis) and lobar calcifications [129–131].

Conclusions

After analysing the published evidence and on the basis of their own clinical experience, the group of experts reached the following conclusions:

- Pediatric pulmonary TB should be suspected in the presence of the following symptoms and signs of pulmonary involvement:

  - Persistent, unremitting cough; cough lasting >4 weeks; persistent chest pain; or hemoptysis **[III-A]**.
  - Cough or other respiratory signs and symptoms associated with long-lasting weight loss, asthenia/malaise and an evening temperature of ≥37.5 °C **[III-A]**.
  - Forms of pneumonia poorly responding to first-line antibiotics or remitting/relapsing in nature, especially in subjects at risk **[V-B]**.
  - Radiological pictures of pneumonia with signs typical of TB: calcifications, voluminous hilar and mediastinic lymphadenomegalies prevalently on one side, with or without airway obstruction (<5 years), a miliary pattern, pulmonary consolidations/cavitations (typical sites: the apical and posterior segments of the upper lobe, and the apical segment of the lower lobe), pleural effusion **[III-A]**.

- Tuberculous lymphadenopathy should be suspected in the presence of:

– Persistent cervical lymphadenopathy (>4 weeks) with a diameter of >2 cm, in the absence of a response to antibiotics or other local cause **[III-B]**.

- Vertebral TB should be suspected in the presence of:

– Progressively increasing spinal pain; a discrepancy between leukocyte counts and the ESR; laboratory signs of chronic inflammation (anemia, thrombocytosis, hypoalbuminemia) **[IV-B]**.

- Tuberculous meningitis (TBM) should be suspected in the presence of:

– Headache, vomiting, irritability, lethargy, convulsions, coma, nuchal rigidity, paralysis of the cranial nerves (particular III and VI) lasting >5 days. CSF with leukocytes $<1.000\times10^3$/mL, of which >50 % lymphocytes; protein >100 mg/dL; CSF/plasma glucose ratio <50 %. CT/MRI findings of basal meningeal enhancement, hydrocephalus, cerebral infarction **[IV-A]**.

- The differential diagnosis of less frequent cerebral tuberculomas and other space-occupying forms is based more on imaging than clinical criteria (convulsions, varying focal neurological signs depending on site, endocranial hypertension) **[V-B]**.
- Abdominal TB should be suspected in the presence of distension and chronic abdominal pain, ascites, and is often associated with general symptoms such as fever and weight loss **[V-B]**.
- Genito-urinary TB should be suspected in the presence of symptoms of recurrent urinary pathway infections or hematuria associated with sterile pyuria **[V-B]**.
- In addition to images of exudative pericarditis, the diagnosis of tuberculous pericarditis always requires pericardiocentesis or pericardiotomy, particularly in countries with a low prevalence of TB **[V-B]**.
- Disseminated TB should be suspected in the presence of clinical manifestations that vary depending on the predominantly involved site and are associated with initially aspecific systemic signs and symptoms **[V-B]**. The only pathognomonic sign is the ophthalmoscopic presence of choroidal tubercles in about one-third of cases **[V-A]**.
- The use of a TST and/or IGRA is recommended in order to diagnose TB in children aged ≥5 years **[III-A]**. The choice of one or the other method should be based on individual considerations, including an evaluation of the likelihood that the child will or will not return to the centre for the interpretation of TST results, whether or not he or she has received BCG vaccination (a confirmatory IGRA is any case recommended if the TST result is <15 mm), and the availability of a TST and/or IGRA at the centre **[III-A]**.
- The use of an IGRA alone is not recommended for diagnosing TB in children aged <5 years; a TST is the only test that should be used **[V-C]**. However, the combined use of a TST and IGRA is possible in selected cases, especially if the child has previously received BCG vaccination **[V-C]**, although it must be remembered that there are still doubts as to how to interpret discordant results **[V-C]**.
- Regardless of age, a negative or indeterminate IGRA result should never be considered as excluding a diagnosis of tubercular infection **[III-A]**.
- The performance of TSTs and IGRAs is suboptimal in the case of children with T lymphocyte immunodepression **[III-A]**. The risk of tuberculous infection or disease in such patients can be evaluated by combining the results of both tests with the findings of clinical, radiological and microbiological investigations **[V-B]**. Nevertheless, the interpretation of conflicting test results remains doubtful, and so immunodepressed children should prudently be considered infected even if only one of the tests is positive **[V-B]**.
- The use of serial IGRAs is not recommended because it is not helpful in monitoring the response to anti-TB therapy, and does not predict the risk of latent infection evolving into TB disease in children **[III-A]**.
- A microbiological diagnosis must be based on samples originating from the involved site and preferably collected before the start of anti-TB treatment **[III-A]**. In order to increase sensitivity, it is recommended to test for pulmonary TB using gastric aspirate or induced sputum samples collected on three consecutive days **[III-A]**. A single sample can be used only in the case of broncho-lavage **[III-A]**. Induced sputum samples should always be indicated as such because their salivary appearance may otherwise lead to them being considered “unsuitable” by the laboratory **[III-A]**.
- Minimum microbiological diagnostics must include microscopy and cultures **[III-A]**. A microscopic examination is only moderately sensitive in the case of pediatric TB; a gene amplification test is more likely to be diagnostic **[IV-B]**. Negative microscopic results do not exclude a diagnosis of TB even if obtained on multiple samples **[III-A]**. In addition to microscopy and culture tests, a gene amplification test should be requested until a diagnosis has been established **[IV-B]**. If the laboratory is so equipped, it may be useful to reserve one of the available clinical samples for testing on the GeneXpert platform **[V-B]**. If MDR TB is suspected in a patient with a microbiological diagnosis of TB, it is advisable to carry out a genotypic resistance assay (e.g. GeneXpert or LPA) before starting treatment **[IV-B]**.
- Chest radiography remains the most suitable imaging technique for evaluating pediatric pulmonary TB **[IV-A]**.

The presence of asymmetric/unilateral mediastinic and hilar lymphadenomegalies should prompt a suspicion of TB, especially in children aged <5 years **[V-B]**. Cavitations are also suggestive **[V-B]**. The lateral projection may be useful in doubtful cases **[IV-B]**.

- After traditional radiology, the investigation can be completed using CT with contrast medium, which is particularly useful in children aged <5 years (especially those aged <2 years) in whom conventional chest radiography is often insufficient because of the physiological protrusion of the thymus **[IV-B]**.
- The Marais anatomo-radiological classification can distinguish parenchymal and lymph node disease and their respective evolutions **[V-B]**.
- All patients with extra-pulmonary TB should undergo chest radiography **[IV-A]**, which can be integrated with CT in the cases described above **[IV-B]**.
- Ultrasonography is the investigation of first choice in the case of peripheral tuberculous lymphadenitis **[IV-B]**.
- If osteo-articular TB is suspected, the first diagnostic and follow-up approach is conventional radiology **[IV-A]**. MRI with contrast medium generally allows a better evaluation of the extent of bone and joint involvement, although it does not usually increase diagnostic specificity **[V-B]**. MRI with contrast medium is fundamental for the volumetric evaluation of ankylosis and the assessment of osteopenia in patients with spondylitis/spondylodiscitis, as well as in order to obtain a complete spinal picture during follow-up **[IV-B]**.
- MRI provides much more information about CNS TB than CT, the use of which is currently limited to emergency situations or the monitoring of hydroencephalus secondary to tuberculous meningo-encephalitis **[IV-A]**.
- Imaging is also often used to diagnose abdominal TB; standard radiography can reveal signs of occlusion but ultrasonography and/or CT are the recommended imaging techniques **[IV-B]**.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interests.

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