

Chapter 2

Clinical Diagnosis

Abstract A complete medical evaluation for tuberculosis (TB) includes the following: (1) Medical history, (2) Physical examination, (3) Tests for TB Infection, (4) Chest radiograph, and (5) Diagnostic microbiology. Tuberculin skin testing (TST) is the most common method used to screen for latent *M. tuberculosis* infection (LTBI). In 2001, an interferon release assay (QuantiFERON-TB test) was approved by the Food and Drug Administration (FDA). Active tuberculosis (ATB) is considered as a possible diagnosis when findings in a chest radiograph of a patient being evaluated for respiratory symptoms are abnormal, as occurs in most patients with pulmonary tuberculosis (PTB). The radiographs may show the characteristic finding of infiltrates with cavitation in the upper and middle lobes of the lungs, including clinical suspicion and response to treatment. Traditionally, the first laboratory test used to detect ATB in a patient with an abnormal chest radiograph is examination of a sputum smear in search for acid-fast bacilli (AFB). Definitive diagnosis of TB requires the identification of *M. tuberculosis* (MTB) in the culture of a diagnostic specimen. The most frequent sample obtained from a patient with a persistent and productive cough is sputum. Sputum is obtained by bronchoscopy and bronchial washings or bronchoalveolar lavage. Newer and faster MTB diagnostic techniques include nucleic acid amplification (NAA) tests. With molecular biology methods, DNA and RNA are amplified thus facilitating rapid detection of microorganisms; these tests have been approved by the FDA. Biopsy and/or surgery are required to procure tissue samples for diagnosis of extrapulmonary TB (EPTB).

Keywords Chest radiograph • Diagnostic microbiology • Histopathology • Medical history • Physical examination • Tests for TB Infection

2.1 A Complete Medical Evaluation for Tuberculosis (TB) Includes the Following Five Components

2.1.1 Medical History

When conducting a medical history, the clinician should ask if any symptoms of TB are or have been present; if so, for how long, and whether there has been any known exposure to individuals with infectious TB. Equally important is whether or not the individual has been diagnosed in the past with latent TB infection (LTBI) or classic TB. Clinicians should determine if the patient has any underlying medical conditions, especially human immunodeficiency virus (HIV) infection or diabetes, that increase the risk of progression to active TB in those latently infected with *M. tuberculosis* (MTB) [6].

TB most commonly affects the lungs and is referred to as pulmonary TB (PTB). PTB often leads to general signs and symptoms, including cough (especially if lasting for 3 weeks or longer) with or without sputum production, coughing up blood (hemoptysis), chest pain, loss of appetite, unexplained weight loss, night sweats, fever, and fatigue.

Extrapulmonary TB (EPTB) may cause symptoms related to the compromised organ or system. For example, TB of the spine may cause back pain, TB of the kidney may be manifested as blood in the urine, or meningeal TB may lead to headache or confusion. Both PTB and EPTB symptoms can be caused by other diseases but should always prompt the clinician to consider a diagnosis of TB.

2.1.2 Physical Examination

The physical examination is an essential part in the evaluation of any patient. It cannot be used to confirm or rule out TB, but it can provide valuable information on the patient's overall condition, point to a specific diagnostic method and reveal other factors that may affect the treatment of TB, if so diagnosed. Physical findings are usually absent in mild or moderate disease. Dullness with decreased fremitus may indicate pleural thickening or effusion. In many instances, the patient appears to be healthy. Nevertheless, a systematic examination is always required in the search of possible diagnostic clues such as:

Crackling rales in the infraclavicular space or in the interscapulo-vertebral area due to exudative and cavitary lesions.

Uni- or bilateral bronchial rales (rhonchi, subcrepitations) in case of bronchogenic disease dissemination.

In case of pleural involvement, there is dullness on percussion and absence or decrease of vesicular murmur.

2.1.3 Test for TB Infection

Selection of the most suitable tests for detection of MTB infection should be based on the reasons and the context for testing, test availability, and overall cost-effectiveness. Currently, there are two available methods for the detection of MTB infection: Mantoux tuberculin skin test (TST) and Interferon-gamma (IFN γ) release assays (IGRAs), such as the enzyme-linked immunosorbent assay (QuantiFERON-TB Gold In-Tube (QFT-GIT), Cellestis Limited, Carnegie, Victoria, Australia) and the enzyme-linked immunospot assay (T-SPOT[®].TB, Oxford Immunotec, Oxford, UK) [5, 8].

TST is the standard method of determining whether a person is infected with MTB. Reliable administration and reading of the TST requires standardization of procedures, training, supervision, and practice in Chap. 5.

For the first time, an alternative to the TST has emerged in the form of a new type of in vitro T-cell-based assay: IGRA. IGRAs are based on the principle that the T-cells of individuals sensitized with TB antigens produce IFN γ when they reencounter mycobacterial antigens in Chap. 5 [2, 3].

2.1.4 Chest X-Ray

All patients with persistent cough of more than 3 weeks duration should have a chest radiograph to rule out, among other diseases, PTB. Since PTB is the most common form of the disease, a chest X-ray (CXR) is useful in its diagnosis of TB. Chest abnormalities can suggest pulmonary TB. In some instances, a computerized tomography (CT) scan may provide additional information. A CT scan provides more detailed images that cannot be easily seen on a standard chest radiograph; however, CT scans can be substantially more expensive. In PTB, radiographic abnormalities are often seen in the apical and posterior segments of the upper lobe or in the superior segments of the lower lobe. However, lesions may appear anywhere in the lungs and may differ in size, shape, density, and cavitation, especially in HIV-infected and other immunosuppressed hosts. Often, the only vestige of a primary infection is a positive TST and the Ranke complex (Fig. 2.1).

The clinical and radiological suspicion of PTB is sufficient to initiate treatment without awaiting the culture result, but sputum should be obtained before the administration of therapy.

Mixed nodular and fibrotic lesions may contain slowly multiplying tubercle bacilli with the potential for progression to full-blown TB. Individuals with radiographic lesions suggesting “old” TB and with a positive TST reaction or positive IGRA should be considered high-priority candidates for treatment of LTBI, but only after active TB (ATB) is excluded in three specimens tested for acid-fast bacilli (AFB) by smear and culture because “old” TB cannot be differentiated from ATB on the basis of radiographic findings alone.

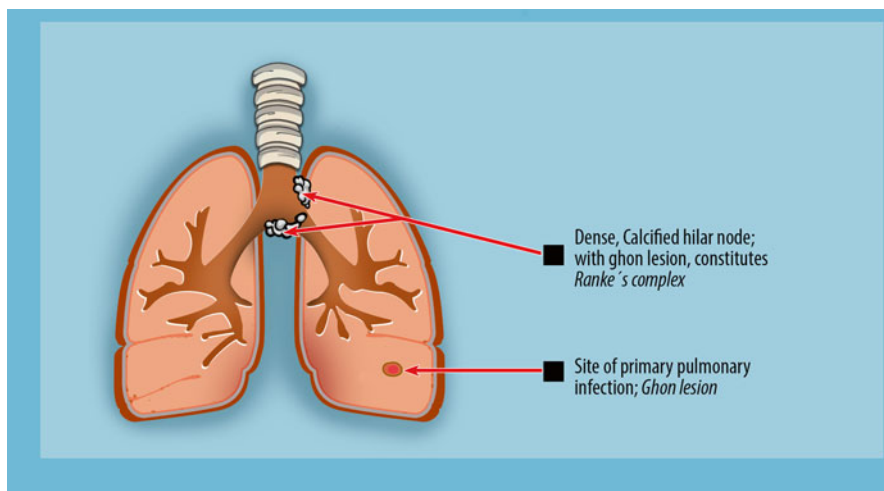


Fig. 2.1 Radiographic residuals of primary infection

In HIV-infected individuals, PTB may present with atypical findings or with no obvious lesions on the CXR. The radiographic appearance of PTB in individuals infected with HIV might be typical; however, cavitory disease is less common among such patients. Abnormalities on CXR radiographs may be suggestive of, but are never diagnostic of TB. CXR may be used to exclude PTB in an HIV-negative patient who has a positive TST reaction or IGRA and no symptoms or signs of TB. For practical purposes, a normal CXR excludes TB.

The following CXR shadows are strongly suggestive of TB:

Upper lung patchy or nodular shadows (on one or both sides).

Cavitation (particularly if there is more than one cavity). Calcified shadows may lead to diagnostic difficulties. Remember that pneumonia and lung tumors can occur in areas of previous healed and calcified TB. Some benign tumors may also be calcified.

Other shadows that may be due to TB are:

Oval or round solitary shadow (tuberculoma).

Hilar and mediastinal shadows due to enlarged lymph nodes (persisting primary complex).

Diffuse, small, nodular shadows (miliary TB).

The correct reading of a CXR requires significant experience. If you suspect TB based on the X-ray but the sputum is negative, administer a non-TB antibiotic (e.g., ampicillin, oxytetracycline) for 7–10 days and then obtain another CXR. Shadows due to acute pneumonia will have improved.

Table 2.1 Usefulness of chest radiography as a diagnostic test for TB

Radiographic finding	Sensitivity (%)	Specificity (%)
Any abnormalities consistent with TB (active or inactive)	98 (95–100)	75 (72–79)
Abnormalities suggestive of active TB	87 (79–95)	89 (87–92)
After screening positive symptoms (one study)	90 (81–96)	56 (54–58)

In individuals with a negative smear and PTB symptoms, an abnormal CXR may be very useful diagnostically. However, a diagnosis of TB can not be established only by radiography. Although the sensitivity of CXR is high, the specificity is low, as shown in Table 2.1.

2.1.5 Bacteriologic Examination of Clinical Specimens

Examination of clinical specimens (e.g., sputum, urine, or cerebrospinal fluid) is of critical diagnostic importance. The specimens should be examined and cultured in a laboratory that specializes in testing for MTB. The bacteriologic examination includes five stages: (1) Specimen collection, processing, and review; (2) AFB smear classification and results; (3) Direct detection of MTB in the clinical specimen with a nucleic acid amplification test (NAAT); (4) Specimen culture and identification; and (5) Drug susceptibility testing.

2.2 Other Tests

2.2.1 Adenosine Deaminase (ADA)

EPTB accounts for 10 % of all cases and pleural TB is the second most common manifestation, preceded only by lymphonodular TB. The diagnosis of the first condition is established by bacteriological means and identifying the bacillus in pleural fluid; unfortunately, staining when in search of MTB is usually negative and culture is positive in less than 25 % of cases. Furthermore, a pleural biopsy only shows granulomatous pleuritis in 80 % of patients with tuberculous pleural effusion (TPE) [4]; biopsy culture combined with histological examination, establishes the diagnosis in approximately 90 % of cases.

Measurement of ADA activity has proven to be sensitive (73 %) and specific (90 %) for pleural TB in special circumstances, such as in regions with a high prevalence of TB. The levels of ADA, an enzyme found in most cells, are increased in TPEs; this determination has acquired popularity as a diagnostic test in areas with a high incidence of TPE because it is noninvasive, the assay is not expensive, and it is

readily accessible. The demonstration of elevated pleural fluid ADA levels is useful in establishing the diagnosis of tuberculous effusions. ADA is an enzyme involved in purine catabolism, catalyzing the conversion of adenosine to inosine. The colorimetric test is based on the quantification of ammonium yielded as a result of the enzymatic activity. The reported cutoff value for ADA varies from 47 to 60 U/L. Specificity is increased when the lymphocyte/neutrophil ratio in pleural fluid (>0.75) is weighed in association with an ADA concentration >50 U/L. Exudative lymphocytic pleural effusions are commonly encountered in clinical practice and are often a challenging diagnostic problem. The two most common causes are malignancy and tuberculous effusions, making the test less useful in countries with a low prevalence of TB.

With the declining prevalence of PTB, the positive predictive value of pleural fluid ADA has also decreased although its negative predictive value has actually increased. Therefore, the measurement of pleural fluid ADA levels can be used to rule out a tuberculous etiology of lymphocytic pleural effusions, regardless of the prevalence of TB.

This test is mostly used to confirm the diagnosis if disease is suspected rather than to rule it out.

2.2.2 *Histopathology*

The multiplication of tubercle bacilli at any site causes a specific type of inflammation, with formation of the characteristic granuloma (Fig. 2.2). Pathology analysis involves examining the tissue for suspected TB [1]. Histopathology entails the microscopic examination of a tissue sample and it is a diagnostic aid when bacteriological techniques cannot be applied. It is especially useful for EPTB.

Types of samples: (1) Aspiration of the lymph nodes; (2) Biopsy of the serous membranes; and (3) Tissue biopsy: (a) Without surgery; (b) During surgery; and (c) Postmortem.

Methods: (1) Cytological techniques; (2) Bacteriological and histological techniques on biopsied samples; (3) Bacteriological techniques; and (4) Histology techniques.

Practical point: on biopsy, at least two fragments should be collected; one is placed in saline solution and transferred to the mycobacteriology laboratory for culture, while the other undergoes fixation for histological evaluation.

Microscopic aspects: Organ involvement by TB leads to an inflammatory reaction in the affected site. Inflammation develops in three successive stages that can overlap—acute, subacute, and chronic—and that have different histological characteristics.

Practical point: among all types of lesion, only follicular lesions with necrotizing granulomas are specific enough to confirm the diagnosis of TB, as is detection of bacilli on histological samples after appropriate staining.

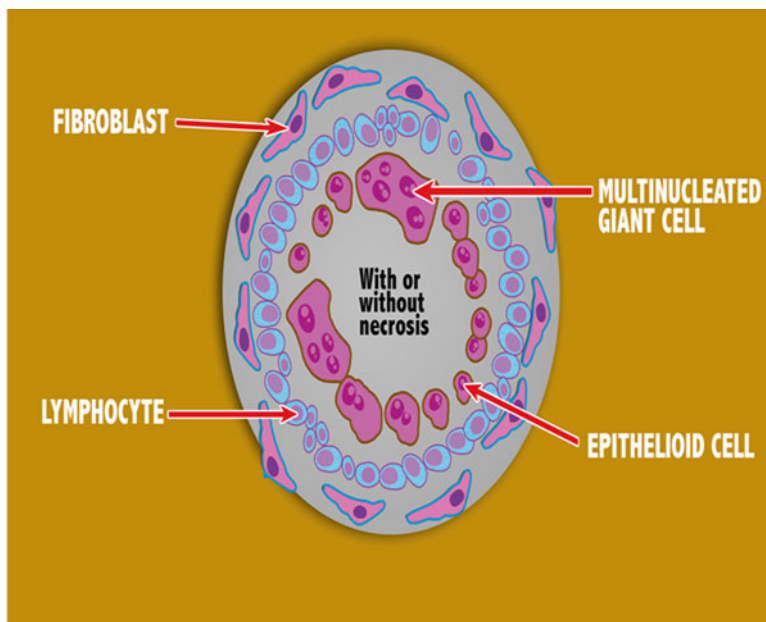


Fig. 2.2 Granuloma. Focal accumulation of macrophages (histiocytes), and/or modified (epithelioid) surrounded by a collar of lymphocytes, surrounded by a ring of fibroblasts

The histopathological examination of biopsies obtained by bronchoscopy reveals caseating granulomatous inflammation in all patients.

Renal and urinary TB diagnosis is established with certainty by identifying the Koch bacillus in special cultures and using histological examination of surgically obtained tissue. Although not in all cases, the usual pathological techniques tend to be very specific. Morphological changes are initially found in the renal cortex and granulomas subsequently develop in the medulla. The pathognomonic finding is a central area of caseating necrosis and eosinophilic nonstructured infiltrates with necrotic detritus surrounded by a row of epithelioid macrophages and a few giant cells: this characterizes the classical caseating granuloma.

The necrotic area consists of amorphous, pink, caseous material composed of the granuloma's necrotic elements as well as infectious organisms. The epithelioid macrophages are elongated with long, pale nuclei and pink cytoplasm while macrophages are grouped in what are known as giant cells. The typical giant cell in infectious granulomas is known as a Langhans giant cell, with the nuclei lined up along one edge of the cell. The necrotic area is surrounded by the inflammatory component of epithelioid cells, lymphocytes, and fibroblasts.

Although bacteriology remains the key test confirming the diagnosis of TB, histology does play an important role, particularly in the diagnostic confirmation of extrapulmonary variants. Combining histological techniques with bacteriology increases the rate of histological diagnoses. Bacteriological culture of tissue

fragments (or albeit less useful, fluid) obtained concurrently with the samples for histological analysis, increases the rate of EPTB confirmed diagnoses [7].

In cases of tuberculous cervical lymphadenitis, characteristic epithelioid cell granulomas with central caseating necrosis are seen in all the cases. Analysis by light microscopy is still a useful screening method in the diagnosis of TB cervical lymphadenopathy.

2.3 Evaluation of Diagnostic Methods in EPTB

It is recommended to obtain a sample, if possible, directly from the site of concern; if necessary, this is accomplished by needle or fine-needle aspiration and the tissue/fluid should be sufficient for histology analysis, smear, and culture. Various imaging tests are recommended, depending on the compromised organ or system, in the diagnosis of suspected EPTB. However, a CXR should always be obtained to rule out a pulmonary component. Aside from the microbiological and histological study of the sample, a rapid diagnostic technique is recommended in cases where treatment should be promptly initiated, as in tuberculous meningitis or severe disseminated TB.

2.3.1 Diagnosis of Miliary TB

The diagnosis of miliary TB can be difficult since clinical manifestations are non-specific, the chest radiographs do not always reveal classical miliary changes, and patients may present with complications that may distract the clinician. Therefore, a high index of clinical suspicion and a systematic approach to diagnostic testing is pivotal in establishing the diagnosis of miliary TB. The following criteria are useful in the diagnosis of miliary TB: (1) Clinical presentation consistent with a diagnosis of tuberculosis; (2) Classical miliary pattern on chest radiograph; (3) Bilateral diffuse reticulonodular lung lesions on a background of miliary shadows demonstrable either on plain chest radiograph or high-resolution computed tomography (HRCT); and (4) Microbiological and/or histopathological evidence of TB. Tuberculin anergy is more common in miliary TB (20–70 %) than in pulmonary and EPTB; TST conversion may occur following successful treatment.

A miliary pattern on the CXR is often the first clue suggesting miliary TB. Several other imaging modalities such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET) can further assess the extent of organ involvement and are also useful in evaluating response to treatment. A miliary pattern on the chest radiograph is the hallmark of miliary TB and it is evident in most patients. If caseous material, collagen, or both are present in the tubercles, they are visible in the CXR. A classical miliary pattern on the chest radiograph represents the summation of all densities in tubercles that

are perfectly aligned, whereas those that are imperfectly aligned result in curvilinear densities and a reticulonodular pattern.

In comparison with the pre-CT era, HRCT and thin-section multidetector CT (MDCT) have facilitated the antemortem diagnosis of miliary TB. With the availability of these imaging modalities, cryptic miliary TB that previously could have only been diagnosed at autopsy can now be diagnosed in a timely manner. The HRCT reveals a composite of both sharply and poorly defined nodules, <2 mm and that are widely disseminated throughout the lungs in association with diffuse reticulation. Importantly, the HRCT may reveal a classical miliary pattern even when the chest radiograph is apparently normal and also facilitates the identification of additional findings such as intrathoracic lymphadenopathy, calcification, pleural, and pericardial lesions [9]. CT and MRI have been useful in identifying miliary lesions in extrapulmonary sites. Abdominal CT has been useful in identifying lesions in the liver, spleen, intestine, mesentery, peritoneum, adrenals, and lymph nodes and can also detect cold abscesses. Brain and spine MRI is very useful in the evaluation of patients with miliary TB, MTB, and spinal TB. MRI is particularly helpful in identifying and delineating the extent of tuberculomas and cold abscesses as well as monitoring the response to treatment.

Although not all patients with miliary TB have a productive cough, if present, sputum must be collected for smears and mycobacterial culture. Sputum smear microscopy using the Ziehl–Neelsen stain is useful in detecting AFB. Fiberoptic bronchoscopy, bronchoalveolar lavage (BAL), bronchoscopic aspirate, brushings, washings, and transbronchial lung biopsy (TBLB) are also useful in confirming the diagnosis of miliary TB. The cumulative diagnostic yield for various bronchoscopically obtained specimens analyzed by smear and culture has been found to be 50 %. Depending on the extent of organ system involvement, appropriate tissue and body fluid samples must be obtained to confirm the diagnosis from a histopathological and microbiological perspective.

The World Health Organization (WHO) policy statement on the use of serodiagnostic tests strongly recommends that currently available commercial tests should not be used for the diagnosis of active pulmonary and EPTB including miliary TB. ADA and interferon-gamma levels in ascitic and/or pleural fluid can be helpful in the diagnosis of miliary TB. In patients with suspected miliary TB, automated molecular tests for MTB detection and drug-resistance testing may be used for early confirmation of the diagnosis, if these techniques are available. Based on current evidence and expert opinions, molecular assays to detect gene mutations signaling drug resistance have been endorsed by the WHO as being most suited for a rapid diagnosis.

Miliary TB is associated with typical interstitial lung disease abnormalities in pulmonary function tests. Diffusion impairment is the most common abnormality and may sometimes be severe. Abnormal cardiopulmonary exercise performance has been described in patients with miliary TB. Salient abnormalities include decreased maximum oxygen consumption, maximal work rate, anaerobic threshold, peak minute ventilation, breathing reserve, and a low maximal heart rate.

2.3.2 *Diagnosis of Pleural TB*

The determination of ADA in pleural fluid has a sensitivity of 73 % and a specificity of 90 %. In high prevalence countries, the probability of diagnosing pleural TB after determining ADA is 99 %.

Screening for interferon in pleural fluid has been compared in culture vs. histology, yielding a sensitivity and specificity of 89 % (CI 95 % 87–91) and 97 % (CI 95 % 96–98), respectively.

The technique of nucleic acid amplification (NAAT) has been evaluated in patients with pleural TB. Marketed tests show a combined sensitivity of 62 % and a specificity of 98 % but these results tend to be heterogeneous.

2.3.3 *Diagnosis of Meningeal TB*

NAAT tests have been evaluated in the diagnosis of MTB. The overall results revealed a relatively low sensitivity (71 %) but with a good specificity (95 %) and all with a significant variability [10].

ADA was evaluated by comparing the results obtained by culture, clinical skills, or histology. The results are very heterogeneous and sensitivity varies between 36 and 100 %, while the specificity ranged between 63 and 99 %.

The diagnostic performance of IGRA techniques is inferior in tuberculous meningitis.

2.3.4 *Diagnosis of Pericardial TB*

ADA validity for diagnosing pericardial TB has been evaluated. A cutoff value (47 U/L) was established and revealed a sensitivity of 88 % and a specificity of 83 %. The gold standard was the identification of the bacillus or the clinical outcome.

2.3.5 *Diagnosis of Lymph Node TB*

Diagnostic NAAT techniques (commercial or not) were evaluated in patients with lymph node TB. Its sensitivity ranged between 2 and 100 % and its specificity was 28–100 %. The performance of commercial tests was superior.

2.3.6 *Diagnosis of Abdominal TB*

Determining ADA in the ascitic fluid is also used in the diagnosis of abdominal TB. It has been used in patients with peritoneal TB for microbiological (smear and culture) and/or histological diagnosis. Its sensitivity was 100 % and its specificity was 97 %. Although ADA determination in ascites may prevent aggressive exploration and laparoscopy, false positive results may be due to serious diseases, such as different types of malignancies.

2.3.7 *Diagnosis of Resistance to Anti-TB Drugs*

Automated methods in liquid culture media (MGIT960, MB/BacT ALERT 3D, and Versa TREK) are currently the most commonly used due to their speed and reliability. These methods correctly detect multidrug resistance to isoniazid and rifampicin although there is some variability between laboratories in terms of the detection of resistance to other first-line drugs such as pyrazinamide, ethambutol, or streptomycin.

More recently, methods have been developed for rapid detection of resistance in clinical samples. These methods include the detection of bacteriophages with luciferase reporter phage (LRP) methods and the mycobacteriophage-based assay (MBA); both are extremely useful in the clinic.

First-line drug sensitivity in the initial isolates from all patients with TB should be tested. Susceptibility testing of second-line drugs should be conducted if microbiological resistance is established or if there is suspected clinical resistance to first-line drugs, as in case of failure in the initial response or after relapse once treatment is completed. Sensitivity studies should be carried out in laboratories with accredited quality controls.

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