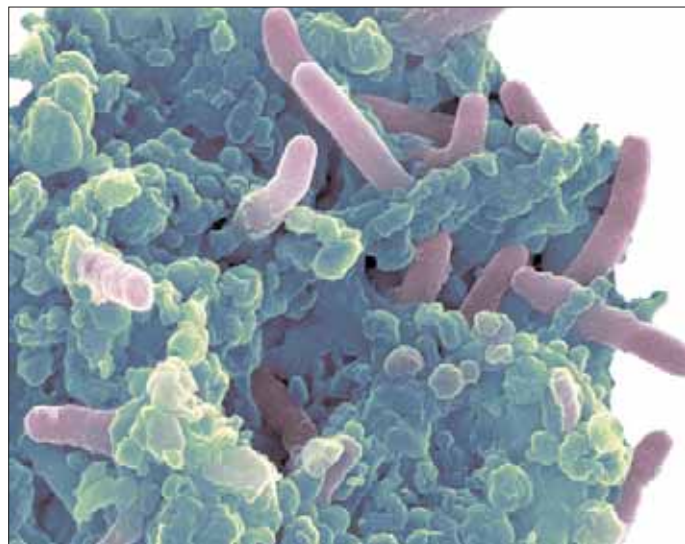


Tuberculosis

— diagnosis and investigation

By Ila Aggarwal, MB ChB, MRCP

Thirty per cent of the world's population is thought to be infected with tuberculosis, and cases of the disease in the UK are increasing. This article describes the disease and its diagnosis, and the measures taken to contain its spread in hospitals and in the community



Mycobacterium tuberculosis infecting a macrophage

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Tuberculosis (TB) is a disease that has been present in the human race for thousands of years. Evidence of spinal tuberculosis has been found in human remains from the Neolithic period and in mummies from Egypt and Peru. Hippocrates described “phthisis” (consumption — a historic term for TB) as the most widespread disease of his time, and observed that it was usually fatal. In the 17th and 18th centuries, the disease was responsible for a quarter of all adult deaths in Europe.

In the mid-19th century, TB was believed to be a hereditary illness due to malfunctioning host cells. Then, in 1882, German physician Robert Koch demonstrated the presence of a bacillus in tubercular tissue after developing a new staining technique. He cultivated the bacteria and succeeded in infecting animals with TB using isolated cultures. He thereby definitively established the infectious nature of tuberculosis.

— Bacteriology

Tuberculosis is caused by bacteria of the *Mycobacterium tuberculosis* complex. The predominant pathogen in humans is

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Mycobacterium tuberculosis, the genome of which has recently been sequenced. *Mycobacterium bovis* is the pathogen responsible for bovine tuberculosis, a cause of human TB that was common before milk was pasteurised, but is now rare. *Mycobacterium africanum* is a rare pathogen causing TB in Africa.

Mycobacteria are aerobic rod-shaped bacteria with a large proportion of high molecular weight lipids in their cell walls. The structure of the cell wall is responsible for specific staining characteristics, such as lack of decolourisation with acid-alcohol. This has given rise to the term “acid-alcohol fast bacilli” (AAFB or AFB). Specimens are termed “smear-positive” if AAFB are demonstrated by staining techniques.

— Epidemiology

Tuberculosis poses a significant threat to global health, causing the second highest mortality rates from an infectious disease worldwide, after human immunodeficiency virus. Currently 30 per cent of the world's population is estimated to be infected with the disease. In addition, one third of the 40 million people living with HIV are co-infected with TB (see p77).

In 2003, 8.8 million new cases of TB were reported worldwide, and 1.7 million deaths. Eight per cent of these new cases

and 13.5 per cent of deaths were among individuals with HIV.¹

The infection rates and risk of TB differ markedly between affluent and poorer areas of the world. Asia has the largest burden of disease, with India and China alone responsible for 35 per cent of all cases worldwide. The highest incidence rates are seen in sub-Saharan Africa due to the high prevalence of HIV infection. Despite recent data indicating that the TB incidence rate is decreasing or stable in all regions except Africa, the annual global incidence rate is continuing to increase by 1 per cent each year.¹

In the US and the UK tuberculosis rates steadily declined until the mid-1980s, when they began to rise again. Since 1987, the number of new cases reported in the UK has risen by almost 30 per cent.² Reasons for this resurgence include increased immigration and deteriorating TB control in other parts of the world. In the US, a combination of complacency, failure to implement adequate public health measures and the onset of the HIV epidemic are thought to be major contributory factors to the resurgence of the disease.³ HIV has had less of an impact on tuberculosis rates in the UK. In 1993, 2.3 per cent of notifications in England and Wales were HIV-related.⁴ Approximately 350 people die from TB in the UK every year.²

The emergence of multi-drug resistant strains of tuberculosis (MDR-TB) poses a

Table 1: Site of TB cases⁸

Disease site	Number of cases	% of total
Pulmonary	3,907	59.4
Extra-thoracic lymph nodes	1,066	16.2
Pleural	484	7.4
Intra-thoracic lymph nodes	475	7.2
Bone/joint	310	4.7
Gastrointestinal	227	3.5
Genitourinary	115	1.7
Miliary	106	1.6
Meninges	99	1.5
CNS other	52	0.8
Cryptic	49	0.7
Laryngeal	12	0.2
Other	452	6.9

further threat to TB control. The prevalence of MDR-TB is as high as 10 per cent in parts of Eastern Europe and China. Eastern Europe is an area of particular concern since 50 per cent of MDR-TB strains found there are resistant to four first-line drugs.¹ Other countries have reported an MDR-TB incidence of five to six per cent among new cases. The incidence of MDR-TB in the UK is currently 1.3 per cent.²

In the UK, tuberculosis has changed from being a disease that affects the whole population to one occurring predominantly in specific groups of people. Seventy per cent of cases now occur among people who were born abroad. The highest rates are seen in black Africans, although the largest numbers of cases occur in people of Indian, Bangladeshi and Pakistani origin. Inner city areas have a higher incidence of TB, particularly Greater London, which accounts for 45 per cent of all UK cases.² Other groups at risk of exposure include the homeless, intravenous drug users and alcohol misusers.

The prognosis of untreated TB is extremely poor, with a 50 per cent mortality rate within two years of diagnosis. With appropriate chemotherapy successful treatment should be possible in most cases. However, TB affecting the central nervous system continues to have a poor prognosis, with a mortality rate of 20 per cent in children under five years, and 60 per cent in adults over 50 years of age.⁶

Pathogenesis

Tuberculosis is transmitted by inhalation of aerosolised droplets (1–5µm in diameter) from an infected person coughing, sneezing or talking. Droplets are deposited in the alveoli, where the bacteria are ingested by alveolar macrophages, resulting in a series of host-pathogen interactions. Thirty per cent

of exposed individuals become infected. In 90 per cent of infected people, the infection is contained by host responses and becomes latent. The remaining 10 per cent develop progressive primary tuberculosis.

Mycobacteria are intracellular pathogens that can survive and multiply within macrophages. During primary infection, infected macrophages are carried by the lymphatic system to regional lymph nodes, but may disseminate throughout the body via the bloodstream. This may result in seeding to extra-pulmonary sites, where the infection can lie dormant until it is reactivated, or disseminated active infection. Five per cent of individuals with latent TB will develop active disease within two years, and another five per cent will develop it at some point in their lives.⁵

Immunity

Macrophages and T-lymphocytes are fundamentally important to the immune response to TB. Alveolar macrophages have special receptors which can recognise foreign material, such as mycobacterial lipoproteins. The macrophages ingest the bacteria and produce cytokines, in particular interleukin-12 and interleukin-18 which promote the growth of CD4+ T-lymphocytes and stimulate them to release interferon-gamma.

Interferon-γ is important in the activation of microbicidal mechanisms within the macrophage, and stimulates the macrophage to release tumour necrosis factor-alpha, which is required for granuloma formation. In addition, the macrophage processes mycobacterial antigens, and presents them to CD4+ T-lymphocytes (helper T-cells) and CD8+ T-lymphocytes (cytotoxic T-cells). This results in clonal expansion of specific T-lymphocytes. The response is a Th1 type, with CD4 cells, interferon-γ and IL-2 playing an important part. The cell-mediated immune response to mycobacteria usually develops three to eight weeks after infection. At the same time, tissue hypersensitivity develops as demonstrated by a positive skin test to mycobacterial protein (tuberculin).^{5,6}

Tissue hypersensitivity results in the formation of granulomas, which limit further replication and spread of mycobacteria. Caseating granulomas are the classical pathological lesion of TB. In severely immunocompromised individuals, tissue hypersensitivity may be minimal. This can result in a non-specific inflammatory response with few polymorphonuclear leucocytes and monocytes and high numbers of bacilli, but no granulomas.⁶

Diagnosis

The diagnosis of tuberculosis is based on clinical signs and symptoms, radiographic appearances and laboratory investigations. In adults, primary infection usually presents as a

mild self-limiting pneumonic illness which generally goes undiagnosed. The initial site of infection is usually a single lesion in the mid-lung zone, known as the Ghon focus. Disease tends to be more severe in children and the primary focus may progress to pneumonia.

In young children and immunocompromised adults extensive dissemination via the lymph or bloodstream can result in miliary tuberculosis (see p77), often followed by tuberculosis meningitis. Most symptomatic TB is believed to be due to reactivation of latent infection. Reactivation can be triggered by HIV infection or other immunosuppressive conditions, such as poorly controlled diabetes mellitus, renal failure, underlying malignant disease, chemotherapy, extensive corticosteroid therapy, malnutrition or deficiency of vitamins A or D.⁷ Symptoms may be general, such as fever, night sweats, weight loss, anorexia and malaise, or organ-specific. Pulmonary tuberculosis is the most common clinical manifestation, but other sites may be involved, including the pericardium, gastrointestinal tract and genitourinary tract. The frequency of the disease occurring at other sites of the body is outlined in Table 1 (based on Health Protection Agency surveillance data for England, Wales and Northern Ireland).

Pulmonary tuberculosis Symptoms of pulmonary tuberculosis include a persistent productive cough (for more than two weeks), pleuritic chest pain, shortness of breath and haemoptysis. Typically, an X-ray will show apical or upper lobe consolidation, with cavity formation. The pulmonary cavity favours bacterial multiplication to very high levels and patients with pulmonary tuberculosis are usually sputum smear-positive and highly infectious. Cavitation is much less common in children, coinciding with lower infectivity. Secretions from cavities may distribute widely throughout the lung, resulting in multiple foci of infection with extensive destruction and fibrosis. Pulmonary cavities may become super-infected with other organisms, such as *Aspergillus* spp or non-tuberculous mycobacteria. Pleural disease may present as a unilateral pleural effusion.

Central nervous system tuberculosis

Central nervous system tuberculosis is the most serious clinical manifestation of TB, resulting in meningitis or space-occupying lesions (tuberculomas) of the brain. Meningitis usually presents with headache, neck stiffness and fever, but symptoms are less acute than with other forms of bacterial meningitis, and progress gradually over several weeks. Alteration in mental status is common, and cranial nerve palsies may occur. A severe form of sudden onset meningitis may occur, with rapid progression to coma. Tuberculomas can cause

seizures, or motor, sensory or cerebellar defects depending on their location. Treatment includes anti-tuberculous drugs with good CNS penetration and corticosteroids.

Bone and joint involvement Tuberculosis can affect any bone or joint, but most commonly affects the spine (Pott's disease). Paraspinal cold abscesses may develop from spread of the infection, and weakness or paralysis of the lower extremities may occur.

Lymphadenitis (scrofula) Lymphadenitis usually affects the cervical and supraclavicular areas; involvement of other nodes suggests more widespread disease. Lymphadenitis typically presents as a painless, red lump. Enlargement with pain, suppuration or sinus formation may occur during treatment. These symptoms usually settle spontaneously or respond to a short course of corticosteroid therapy.

Disseminated disease Miliary tuberculosis may occur during primary infection or reactivation, and is due to extensive spread of *M tuberculosis* via the bloodstream. Pathologically, a granular pattern is produced which is said to resemble millet seeds (hence miliary). Pleural effusion, peritonitis and meningitis occur in two-thirds of cases. Symptoms can be non-specific, resulting in delays in diagnosis, especially if the lungs are not involved.

— Co-infection with HIV

The primary immune deficiency in HIV infection is a reduction in the number and impaired function of CD4+ T-lymphocytes. Following exposure to TB, individuals with HIV are at a greater risk of active disease with a more rapid progression.⁹ The risk of reactivation is much higher in patients with HIV, at 10 per cent per year.

The clinical picture is determined by the degree of immune compromise — patients with higher CD4 counts have typical pulmonary tuberculosis with cavitating lesions. In advanced HIV infection, extra-pulmonary disease is common, and can involve the liver, spleen, pancreas, bone marrow, mediastinum, peritoneum and other sites. Widespread enlargement of the lymph nodes is seen, and CNS involvement is often fatal. Diagnosis of disseminated disease can be difficult because symptoms can be non-specific, classical pulmonary changes may be lacking and sputum may be smear-negative.

Immune reconstitution inflammatory syndrome may occur in individuals with advanced HIV infection and active or latent TB on starting anti-retroviral therapy. This is characterised by a paradoxical worsening of TB, due to the development of hypersensitivity and an improved cell-mediated immune response. It usually responds to corticosteroid

treatment, but in severe cases anti-retrovirals may need to be stopped until the TB has been treated. The risk of death in HIV infected people with active TB is three to seven times higher than in those without HIV infection.

— Investigations

Patients with suspected tuberculosis should have full blood counts taken and kidney and liver function tests. The patient's bone profile and level of inflammatory markers should also be assessed. In advanced disease, anaemia, low albumin, raised gammaglobulins and hypercalcaemia may be seen. Hyponatraemia can occur with disease in the CNS or adrenal glands. Miliary disease can cause pancytopenia or raised transaminases. Inflammatory markers are usually slightly raised.

Patients with pulmonary disease should have three sputum samples sent for microscopy and culture. If sputum is not expectorated, an induced sputum, bronchoalveolar lavage or gastric aspirate can be examined. Gastric aspirates are particularly useful in diagnosing children. Other specimens taken depend on the sites affected, but may include cerebrospinal fluid (CSF), blood, peritoneal and pericardial fluid, early morning urine, lymph node aspirates or tissue samples. CSF should be tested for cell count, protein and glucose because tuberculous meningitis is associated with an elevated lymphocyte count, high protein and low glucose. Invasive procedures such as liver biopsy or mediastinoscopy may occasionally be necessary to obtain tissue samples. All tissue samples should be examined histologically for caseating granulomas and the presence of AAFB.

All patients should have a chest X-ray. Other investigations depend upon symptoms and may include X-rays of the spine or other bones, CT scans of the brain, thorax, abdomen or pelvis or an MRI scan of the brain or spine.

Microscopy should be done on all specimens. Sputum smears can be screened using fluorochrome stains such as an auramine stain where mycobacteria appear as fluorescent rods against a dark background using an ultraviolet light microscope. Other specimens should be stained using the Ziehl-Neelson method — mycobacteria are seen as pink rods against a blue or green background.

Specimens should be cultured on special media containing nutrients, antibiotics, antifungals and specific growth factors. *M. tuberculosis* grows slowly and can take three to eight weeks to show visible growth on solid culture media. However, new fully automated liquid culture systems are able to detect growth within 14–21 days. Mycobacteria isolated from culture are further identified by microscopic appearance, growth characteristics and biochemical tests.

Probes targeting ribosomal RNA can also be used to identify mycobacteria as belonging to the *M tuberculosis* complex.¹²

All mycobacterial cultures should be submitted to reference laboratories for drug sensitivity testing. Sensitivity testing can also be done using the rapid culture systems. Direct nucleic acid amplification tests that target ribosomal RNA or DNA can be used for the detection of mycobacteria in primary specimens. The tests are specific, and are 95 per cent sensitive in smear-positive patients, but have low sensitivity in smear-negative patients. Molecular tests for rifampicin resistance have also been developed, which show excellent correlation with subsequent susceptibility testing.¹⁰ As rifampicin mono-resistance is uncommon, this test can be used as a marker for the early detection of MDR-TB. Other molecular methods under investigation include polymerase chain reaction on CSF and other extra-pulmonary specimens.

The tuberculin skin test (Heaf or Mantoux) is used as a screening test for tuberculous infection or disease, and to aid diagnosis by assessing the local skin reaction to an intradermal injection of mycobacterial antigens. However, the test cross reacts with the *Bacillus Calmette-Guérin* (BCG) vaccine strain and environmental mycobacteria, and has poor sensitivity in immunocompromised individuals. A blood test has recently been developed which measures interferon- γ released from T cells in response to stimulation with mycobacterial antigens. Studies using ESAT-6 and CFP-10, two antigens absent from the BCG vaccine strain, have shown promising results for the diagnosis of active and latent infection.¹¹

— Management of tuberculosis

Before chemotherapy was available for tuberculosis, removal to a sanatorium, bed rest and closure of pulmonary cavities by lung collapse was the standard treatment. With current drug regimens, surgery is now rarely necessary. In the UK, TB is usually managed by a consultant physician and specialist nurse. Patients should be reviewed at least every month, but may need to be seen three times per week if directly observed therapy is indicated (see p84).

Tuberculosis is a notifiable disease, so confirmed or suspected cases started on anti-tuberculous medication must be reported to the consultant in communicable disease control in England or Wales, or the consultant in public health in Scotland and Northern Ireland. Notification triggers contact tracing procedures, and provides surveillance data to detect outbreaks and monitor epidemiological trends.¹⁴

Contact tracing is undertaken to detect associated cases and persons with latent infection, to identify candidates for BCG vaccination and, in instances where recent

infection has occurred, to detect a source case. Up to 10 per cent of TB cases are diagnosed by contact tracing, and disease occurs in about 1 per cent of contacts.¹² The risk of infection is determined by how infectious the source case is and the duration and level of exposure. Cases with positive sputum smears are highly infectious and cases with negative sputum smears much less so.

"Close contacts" are defined as people from the same household and frequent visitors, "casual contacts" include most occupational contacts, and "significant exposure" is defined as a cumulative total of over eight hours in the same room as an infectious case. Routine screening of close contacts of smear-positive and smear-negative pulmonary tuberculosis cases is recommended, but not of the contacts of those with non-pulmonary tuberculosis.

Investigations of contacts can include inquiry about any symptoms, BCG vaccination status, tuberculin skin testing, and chest radiography. Symptomatic contacts should be referred for rapid assessment. Previously unvaccinated contacts under 16 years old with a persistently negative tuberculin test should be given BCG vaccination. Chemoprophylaxis should be given to children under 16 years old with a strongly positive tuberculin test, to children under two years old in close contact with smear-positive cases, to recent tuberculin converters, to babies born to mothers with infectious tuberculosis and to HIV-infected close contacts of smear-positive cases. Six months of isoniazid or three months of isoniazid and rifampicin is the recommended prophylaxis.¹⁴

Most people with TB are managed as outpatients, but some may require admission to hospital. Adults with non-pulmonary tuberculosis do not require isolation unless aerosol-generating procedures, such as wound irrigation, are being performed. Patients with suspected pulmonary tuberculosis should be admitted to a single room vented to the outside until their sputum status is known and risk assessments for MDR-TB are made. Children with TB and their visitors should be segregated from the rest of the ward until the visitors have been screened to exclude them as a source of infection.

If a patient on an open ward is diagnosed with infectious tuberculosis, the risk of other patients being infected is usually low. Patients in the same bay may be at risk if the index case was coughing and in the bay for more than eight hours. Patients on the ward who are immunocompromised should have a risk assessment even if they did not share the same bay.

In settings where other patients may be infected with HIV or otherwise immunocompromised, suspected or confirmed cases of pulmonary tuberculosis should be considered potentially infectious on every

admission, and should be admitted to a single room on a separate ward or a negative-pressure ventilation room on the same ward. Aerosol-generating procedures should be carried out in appropriately ventilated areas.

After two weeks of a treatment regimen including rifampicin and isoniazid, patients with smear-positive tuberculosis with no risk factors for MDR-TB are usually non-infectious, even though bacilli might still be seen in sputum smears. These patients do not require further isolation, unless other patients on the ward are immunocompromised, in which case three negative sputum smears should be obtained before stopping isolation.

Staff members attending patients routinely are not at increased risk of infection. Staff who have undertaken mouth to mouth resuscitation, prolonged care of high dependency patients or repeated chest physiotherapy before diagnosis should be managed as close contacts. The routine wearing of masks is not necessary, except for known or suspected cases of MDR-TB.

In the UK, screening of new immigrants from countries with a TB incidence greater than 40 per 100,000 population is recommended in order to identify infected people, particularly children, who might require chemoprophylaxis, or non-infected persons who might require BCG vaccination. The incidence of TB is high among homeless people, and transmission can occur in hostels or other temporary accommodation.

Vaccination

The BCG vaccine is the only vaccine currently available for the prevention of tuberculosis. It contains a live attenuated strain derived from *M bovis*, and is administered by intradermal injection. The BCG vaccine protects against severe forms of TB in childhood, such as meningitis and miliary disease, but offers variable protection against pulmonary tuberculosis, ranging from no protection to 70–80 per cent protection in UK schoolchildren.⁸ Protection has been shown to last for 10–15 years. Because it is a live vaccine, it is contraindicated in HIV-infected or other immunosuppressed patients.

BCG vaccination was previously offered to all schoolchildren in the UK aged 10–14 years. TB rates in the indigenous UK population have continued to decline, and in 2005 the schools programme was stopped. However, TB rates remain high in recent immigrants from high prevalence countries and their families. The BCG immunisation programme is now a risk-based programme which targets infants and children either living in areas of the UK where the annual incidence of TB is 40 per 100,000 or greater, or with epidemiological links to high prevalence countries. Vaccination may also be recommended for certain contacts

and those at risk of occupational exposure, although there are limited data regarding protection afforded in people over 16 years old.⁸

Conclusion

Tuberculosis has caused considerable morbidity and mortality for thousands of years, and continues to do so today. The HIV epidemic has contributed to a resurgence of disease, with a more complicated clinical picture. Although molecular tests have improved the diagnosis of the disease, microscopy and culture remain the gold standard. Sequencing of the *M tuberculosis* genome should contribute to greater understanding of pathological mechanisms, and provide possible targets for new vaccines. The implementation of an effective control programme remains of paramount importance.

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