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Consensus Document

Consensus Document on the Diagnosis, Treatment and Prevention of Tuberculosis ☆,☆☆

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ABSTRACT

Pulmonary tuberculosis must be suspected in patients with respiratory symptoms longer than 2–3 weeks. Immunosuppression may modify the clinical and radiological presentation. The chest X-ray is highly suggestive of tuberculosis (TB), but is occasionally atypical. The complex radiological tests (CT scan, MRI) are more useful in extrapulmonary TB.

At least 3 consecutive representative samples from the clinical location are used for diagnosis, whenever possible. Bacilloscopy and liquid medium cultures are indicated in all cases. Genetic amplification techniques are coadjuvant in moderate or high suspicion of TB.

In new cases of TB, administration of isoniazid, rifampin, ethambutol, and pyrazinamide (HREZ) for 2 months and isoniazid plus rifampin for 4 months is recommended. For meningitis cases, treatment should continue for up to 12 months, and up to 9 months in spinal TB with neurological affectation and silicosis. Appropriate adjustments with antiretroviral treatment must be made in HIV patients. Combined therapy is recommended to prevent development of resistance. An antibiogram for first line drugs should be performed in all initial extractions from new patients. Treatment control is one of the most important activities in TB management.

The Tuberculin Skin Test (TST) is positive in TB infection when ≥ 5mm, and Interferon-Gamma Release Assays (IGRA) are recommended in combination with TST. The standard treatment schedule for infection is 6 months with isoniazid. In pulmonary TB, respiratory isolation is applied for 3 weeks or until 3 negative bacilloscopy samples are obtained.

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Documento de consenso sobre diagnóstico, tratamiento y prevención de la tuberculosis

Palabras clave:
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RESUMEN

Debe sospecharse tuberculosis pulmonar en pacientes con síntomas respiratorios durante más de 2–3 semanas. La inmunodepresión puede modificar la presentación clínica y radiológica. La radiografía de tórax presenta manifestaciones muy sugerentes de tuberculosis, aunque en ocasiones atípicas. Las pruebas radiológicas complejas (tomografía computarizada, resonancia magnética) son más útiles en la tuberculosis extrapulmonar.

En el diagnóstico, siempre que sea posible, se utilizarán al menos 3 muestras seriadas representativas de la localización clínica. La baciloscopia y el cultivo con medios líquidos deben realizarse en todos los casos. Las técnicas de amplificación genética son coadyuvantes en la sospecha moderada o alta de tuberculosis.

En los casos nuevos de tuberculosis, se recomienda administrar isoniacida, rifampicina, etambutol y piracinamida (HREZ) durante 2 meses e isoniacida más rifampicina durante 4 meses más, con las excepciones de los casos de meningitis, en que se alargará hasta 12 meses, y de la tuberculosis espinal con afección neurológica y la silicosis, hasta 9 meses. Se recomiendan las formulaciones combinadas. En pacientes con infección por el VIH deben realizarse los ajustes necesarios con el tratamiento antirretroviral. Debe realizarse antibiograma para fármacos de primera línea a todos los aislamientos iniciales de pacientes nuevos. El control del tratamiento es una de las actividades más importantes en el abordaje de la tuberculosis.

En la infección tuberculosa la prueba de tuberculina (PT) es positiva cuando es ≥ 5 mm y los métodos de detección de producción de interferón gamma (IGRA) se recomiendan en combinación con la prueba de la tuberculina. La pauta estándar de tratamiento de la infección es de 6 meses con isoniacida. En la tuberculosis pulmonar se aplicará aislamiento respiratorio durante 3 semanas o hasta obtener 3 muestras con baciloscopia negativa.

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Introduction

Tuberculosis (TB) remains one of the most infectious diseases worldwide and the cause of mortality in poor countries where there is a high incidence.[1] For some years, Spain has been one of the countries in western Europe with the greatest number of cases.[2] Since the second half of the 1990s with the introduction of highly efficient antiretroviral treatment, there has been a progressive decrease in TB cases with HIV co-infection. During the last decade, demographic changes in the population help to explain why up to 35-40% of patients were diagnosed with TB in communities with a high proportion of immigrants. This brings new challenges in addressing and monitoring these patients, among them the need for early diagnosis, monitoring resistance,[3,4] introduction of appropriate treatment regimes, as well as monitoring compliance with treatment. This consensus document from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and the Spanish Society of Pneumology and Thoracic Surgery (SEPAR) aims to review the most important current aspects of diagnosis, treatment and prevention of infection with tuberculosis (TB), as well as pulmonary and extrapulmonary TB. At the end of each of the sections of the paper, a table of recommendations based on scientific observation and according to the classification of the Infectious Diseases Society of America has been included.[5] The following categories are given for the relative importance of each of the recommendations:

A: Important: supports the recommendation.
B: Moderately important: supports the recommendation.
C: Not important: neither supports nor rejects the recommendation.

And regarding the scientific quality of the recommendation:

- Grade I: Based on at least one well-designed, controlled and randomised clinical trial.
- Grade II: Based on at least one well-designed clinical trial, but not randomised, in cohort studies, multiple series or very apparent results from uncontrolled trials.
- Grade III: Based on expert opinion, descriptive studies or clinical experience.

Epidemiological Introduction

TB is one of the most contagious diseases causing morbidity and mortality. Its incidence and prevalence are directly linked with poverty levels and have declined in the developed world while steadily increasing in many poor countries or developing countries. Its eradication requires prevention, early diagnosis, effective treatment of the infection and disease and institutional support. Some circumstances, such as HIV co-infection, increasing drug resistance, geographic mobility related to current economic migration and overcrowding of poorer people in big cities, hamper the efforts of international agencies to control it.

In its report of 2009,[1] the World Health Organization (WHO) estimated the incidence of TB in 2007 at just over 9 million new cases (139/10^5 inhabitants), of which 44% were smear-positive, 15% HIV-infected patients and 500,000 resistant to a number of drugs (MDR-TB, simultaneous resistance to at least isoniazid, H, and rifampicin, R). Overall mortality was 1.3 million patients not infected with HIV and 456,000 co-infected with HIV.

During 2006 in Europe, there were 48 cases/10^5 population reported, with great variability among nations[2] (table 1). In the most industrialised countries, the mean prevalence was 10/10^5 population, with an increase among immigrant populations of 40% in many cases. Meanwhile, in eastern continental countries of the, the prevalence was 110 cases/10^5 of population.

Table 1
Annual incidence (n/105 population) of tuberculosis (TB) in Europe and other World Health Organisation (WHO) regions in 2006[1,2]

EU (notification)				Rest of Europe and North America (notification)			
EU in 2004 (15 countries)		New EU countries		Eastern Europe		Other countries and the Balkans	
Countries	Rate	Countries	Rate	Countries	Rate	Countries	Rate
Germany	7	Bulgaria	42	Armenia	72	Norway	6
Austria	10	Cyprus	4	Azerbaijan	89	Switzerland	7
Belgium	11	Slovakia	14	Byelorussia	62	Israel	6
Denmark	7	Slovenia	11	Russia	106	Canada	4
Spain	18	Estonia	34	Georgia	142	USA	5
Finland	6	Hungary	19	Kazakhstan	282	Mexico	17
France	8	Latvia	58	Kurdistan	127	*Balkan countries:*	
Greece	6	Lithuania	75	Moldova	160	Albania	16
Netherlands	6	Malta	7	Tajikistan	100	Bosnia and Herzegovina	46
Ireland	11	Romania	127	Turkmenistan	69	Croatia	25
Italy	7	Poland	23	Ukraine	89	Macedonia	31
Luxembourg	6	Czech Republic	10	Uzbekistan	94	Montenegro	28
Portugal	32	*All 12 new countries*	*46*			Serbia	29
United Kingdom	14					Turkey	28
Sweden	5					Total Balkan countries	28
Total EU 2004	*10*	*Total EU current (27 countries)*	*18*	*Total eastern Europe*	*110*	*Total Europe*	*48*
Central and South America (estimate)		*North Africa and eastern Mediterranean (estimate)*		*Sub-Saharan Africa (estimate)*		*Asia and Oceania (estimate)*	
Countries	*Rate*	*Countries*	*Rate*	*Countries*	*Rate*	*Countries*	*Rate*
Guatemala	79	Morocco	93	Senegal	270	India	168
Honduras	76	Algeria	56	Mali	280	Indonesia	234
Nicaragua	58	Tunisia	25	Guinea	265	Bangladesh	225
Costa Rica	14	Mauritania	316	Ivory Coast	420	Korea	178
El Salvador	50	Egypt	24	Ghana	203	Thailand	142
Haiti	299	Iran	22	Cameroon	192	Timor	556
Ecuador	128	Iraq	56	Namibia	767	Cambodia	500
Colombia	45	Saudi Arabia	44	Central African Republic	345	Vietnam	173
Venezuela	41	Kuwait	24	Mozambique	443	Laos	152
Peru	162	Sudan	242	Ethiopia	378	China	99
Bolivia	198	Somalia	218	Kenya	384	Japan	22
Brazil	50	Djibouti	809	Congo	403	Mongolia	188
Uruguay	27	Afghanistan	161	Zambia	553	New Guinea	250
Argentina	39	Pakistan	181	South Africa	940	Philippines	287
Chile	15			Swaziland	1.155	Total south east Asia	180
Central and South America	*37*	*North Africa and Mediterranean*	*105*	*Total Sub-Saharan Africa*	*363*	*Total western Pacific*	*109*

EU: European Union.

During this same period in Spain, there were 8,029 cases reported (18.3/10^5 of population).[2] Although, according to WHO estimates,[1] this would correspond to an incidence of 30/10^5 people, which may mean 39% of cases went unreported. Four point four percent of the reported cases were HIV-infected patients and 19.3% were foreign patients. Eighty-two point two percent of the cases were of pulmonary location.[2]

The data regarding resistance to drugs in Spain are not complete and are from various sources. Thus, EuroTB cases reported in 2006 included 3.8% of MDR-TB cases. A study conducted in Spain,[3] over 120 laboratories from 16 regions with 1,083 of TB cases analysed during one month in 2006, revealed a primary resistance rate of 8.3% (4.9% to H) and MDR-TB of 1.3%. Thirty-three point six percent of resistant cases were foreigners.

Another source of information of resistant cases in Spain was reported to the WHO,[4] which provided partial data showing 0.1% of primary MDR and 4.3% of secondary MDR cases in 2006. These are lower rates than those calculated for European Union countries (0.8 and 8.2% respectively) and very different from the primary MDR-TB between 6-23% in east European countries.[4]

In 2006 the WHO[6] reported a new type of TB, extremely resistant tuberculosis (XDR-TB), defined as MDR-TB which is also resistant to at least one second-line drug administered by injection (amikacin, kanamycin, or capreomycin) and all fluoroquinolones. Until June 2008, the WHO received reports of XDR-TB from 49 different countries, including Spain. Although only found in individual patients, it is possible that not all have been discovered, since the study of sensitivity to second-line drugs is not systematic in all resistant cases.

Diagnosis of Tuberculosis Infection

Risk Factors for Tuberculosis Infection

Except in cases of accidental puncture and infection with mycobacteria, transmission of TB in our environment is almost always aerobic. In general, intense and prolonged contact with bacilliferous patients is required for infection. Various factors are involved in the contagion, especially the following: a) the ability of the patient to be infected, which is related to the number of bacilli in the sputum, the intensity and frequency of coughing and the existence of cavitation in the chest radiograph; and b) the degree of intimacy and duration of exposure. Therefore, those living with bacilliferous patients have an increased risk of becoming infected compared with those whose relationship is sporadic or casual.[7-9]

Tuberculin Test

The standard test for diagnosis of tuberculosis infection is the tuberculin skin test (TST), which uses a sterilised and concentrated

extract from a tubercle bacilli culture filtrate. Currently the type of antigen used in the tuberculin is PPD (purified protein derivative). In Spain the variant RT-23 is used with Tween 80 as an anti-absorbent.[8,9] The most common technique for TST is intradermal, known as the Mantoux technique. It consists of the intradermal injection on the ventral forearm of 0.1mL of PPD, a dose of 2UT. To prevent errors and ensure it is administered intradermally, it is important that a mark appears which fades soon after injection. The reading is performed after 48-72h, but may be valid within 7 days. The transverse diameter, with respect to the longitudinal axis of the forearm, should be measured exclusively after induration.

PPD contains proteins that are common to *Mycobacterium tuberculosis*, the BCG vaccine bacillus and some environmental mycobacteria, which diminishes the specificity of the TST. To consider a TST as positive and indicative of tuberculosis infection, the probability of the individual to be infected by the tubercle bacillus and their risk of developing the disease should be taken into account. The BCG vaccination scar should be looked at before properly interpreting the TST.

In Spain, the test is considered positive when the induration is ≥ 5 mm in people who have not been vaccinated with BCG. For those previously vaccinated with BCG, the interpretation of the TST is complicated by the interference of the vaccination in the TST and the difficulty in distinguishing between the effect of the vaccine and tuberculosis infection. In clinical practice, it is accepted that the vaccination history is not taken into account for groups with a high risk of the disease after infection. With this criterion, a TST of ≥ 5 mm in those vaccinated is considered positive for people with frequent or close contact with bacilliferous TB patients, as well as people with residual lesions compatible with TB on the chest x-ray, with negative bacteriology and evolutionary control ruling out the disease. For those vaccinated with BCG, not included in the previous characteristics, it is not possible to be certain about the effect of the vaccine, although it is estimated that the greater the diameter of induration, the greater the possibility that the response is due to tuberculosis infection.[10,11]

In persons with significant immunosuppression (patients with HIV infection, a transplant, biological treatment or with corticosteroids), any induration of the TST is accepted as positive.[8,9]

The TST does not sensitise even if performed more than once. However, it can act as a stimulus in people previously infected by *M. tuberculosis*, although the ability to respond to this over time weakens, without actually disappearing. This phenomenon is known as a booster effect and can lead to an error in interpreting conversion to the tuberculin, which in reality corresponds to the induction or restoration of responsiveness. It is important to take this reaction into account, especially in groups at risk of tuberculosis infection in those where annual TSTs are performed.[8,9]

The booster effect is observed in people over 55 years and those vaccinated with BCG. In these cases, if the TST is negative, it is best to repeat the test after 7-10 days and to take this result as the final one. This practice can discriminate between a false negative result and prevent future false conversion diagnoses when the TST is repeated. A tuberculin conversion is defined as a change from a negative to a positive result in less than 2 years.

It should be remembered that, after infection with *M. tuberculosis,* between 2-12 weeks must elapse to prevent sensitised T cells from entering the bloodstream, which can be recognised as tuberculin being deposited under the skin. During this time, even if infection occurs, the TST may not detect a response. Therefore, with a negative TST and high risk of infection, as in the case of persons living with bacilliferous TB, a re-test should be performed 8-12 weeks afterwards, to ensure there has been no infection.[8,9]

Table 2
Causes of false negative tuberculin skin tests (TST)

Infections
Viral: HIV, measles, chicken pox, mumps
Bacterial: tuberculosis TB (serious and a proportion of serous location), typhoid fever, brucellosis, whooping cough, leprosy
Vaccination with live viruses: measles, mumps, polio
Chronic renal failure
Severe malnutrition
Lymphoid organ disease: lymphoma, leukaemia, sarcoidosis
Prolonged corticosteroid therapy (? 15mg of prednisone over 1 month)
Chemotherapy and any immunosuppressive medication
Children under 6 months and the elderly
Technical and reading errors
Exposure of tuberculin to light or heat or denaturing due to being out of date
Positive TST window

It is not necessary to repeat the TST if there is a previous confirmed positive test result. In general, a positive TST is not seen until 6 months of life. Moreover, the TST cannot distinguish between infection and disease, as it is usually positive in both cases.

False TST negatives may be due to technical defects, reading errors or diseases and conditions that cause immunosuppression, including severe TB itself (Table 2).

Gamma Interferon Tests

TST is still the method of choice for diagnosis of infection, although there are some limitations, such as low sensitivity in immunocompromised individuals, leading to false negative results; difficulties in their use in young children; administration errors; subjectivity in interpreting the results; the need for a second visit for the test reading; and its lack of privacy.

To try to overcome these problems, different laboratory techniques for diagnosing the tuberculosis infection have been recently developed. The techniques are based on the detection of gamma interferon in the blood (interferon gamma release assay, or IGRA), a key cytokine in the control of tuberculosis infection, which is released in response to in vitro stimulation of sensitised T cells with specific *M. tuberculosis* antigens. Currently, antigens in the genetic region RD1 are used to stimulate the T cells: early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), and the antigen in the gene region RD11: RV2654, present in the complex *M. tuberculosis* but absent in both the BCG vaccine and in most other mycobacteria (except for *Mycobacterium kansasii*, *Mycobacterium marinum* and *Mycobacterium szulgai*).

IGRA techniques allow discrimination between individuals infected with *M. tuberculosis* after BCG vaccination and those infected by other mycobacteria, excluding those mentioned.[12-14] They also incorporate controls to detect anergy and thus exclude false negatives. In addition, they can be repeated immediately without the risk of stimulating immunity, thereby preventing the booster effect.

IGRAs have additional advantages regarding TSTs.[12-14] As they are objective, the determination can be repeated if necessary; there is no need for a reading visit, thereby preventing the loss of individuals who do not attend; they are easy to standardise and implement in the lab; they allow the inclusion of positive controls to detect anergic

patients; and, if performed in the laboratory, they ensure the privacy of the individual. The main drawback of IGRAs is that they are more expensive than TSTs.

Further studies are needed in the different risk groups to determine their level of efficiency. However, it is accepted that in countries with a high TB prevalence, their use is the most cost-effective.

At present, two tests are available commercially: QuantiFERON-TB Gold In-Tube, using ELISA, and T-SPOT-TB, based on the ELISPOT technique. Both tests have operational advantages with respect to tuberculin and are significantly more specific in the vaccinated population.[12-14] In children, it has been found that they also test positive for infection by species of mycobacterium other than *M. tuberculosis.*[14] The agreement between the two is very high, although it seems that T-SPOT-TB is more sensitive than QuantiFERON-TB Gold. Its use in clinical practice is still in the early stages. However, some pneumology societies, such as the British, Italian and Spanish ones, among others, have already introduced them in their guides. The Centres for Disease Control and Prevention (CDC) recommend either the use of tuberculin or QuantiFERON-TB Gold for the diagnosis of a tuberculosis infection, while other companies recommend the use of new technology in combination with tuberculin.

Protocol of Action and Indications

As a rule, neither TST nor a gamma interferon test should be done if no further action is going to be taken. The indications for performing a TST are shown in Table 3.

Table 3
Tuberculin skin test (TST) indications[14]

Cohabitants and close contacts of tuberculosis patients
Persons whose chest radiograph shows images compatible with inactive tuberculosis
People with clinical and/or radiological suspicion of having tuberculosis
People who, if infected, are at increased risk of developing TB disease
HIV infection
Intravenous drug addicts
Socially excluded persons
Immigrants from countries with a high TB incidence
Immunosuppressive diseases: leukosis, lymphomas, malignancies and other
Prolonged immunosuppressive therapy, anti-TNF-α and transplant candidates
People who constitute a social and epidemiological risk if they develop active TB
Childcare workers
Teaching staff
Health personnel
Prison staff
TB epidemiological studies and control programmes

TNF: tumour necrosis factor.

Figure 1 shows the algorithm (amended as proposed by SEPAR)[9] for the use and interpretation of TST results in combination with gamma interferon detection techniques. To summarise, from current information the recommendation is to use TST as the basic technique. If this is positive and the individual has been previously vaccinated with BCG, a gamma interferon detection technique can be used to discount the vaccine effect. If the TST is negative but the individual is immunocompromised, because it is more sensitive than TST, a gamma interferon determination can be done to exclude a false negative.[9]

Recommendations Regarding TB Infection

1. The standard test for diagnosis of tuberculosis infection is the TST. It is considered positive when ? 5mm in the following cases: for those with or without a previous BCG vaccination; for those in contact with TB-infected people; those with residual TB lesions in the chest radiograph; and in people with immunosuppression and a risk of developing TB, mainly due to HIV infection (AIII).
2. New methods for diagnosing tuberculosis infection, based on the release of gamma interferon by T cells sensitised by specific antigens of *M. tuberculosis,* are not well established. Their use is currently recommended in combination with TST in protocol studies to be able to establish their usefulness in the diagnosis of infection in the future (BII).
3. Diagnostic tests for tuberculosis infection must only be performed when further action will be taken, which will depend on the results (AIII).

Clinical Manifestations of Tuberculosis

Risk Factors for the TB Disease

People infected with M. tuberculosis with an increased risk of developing TB can be grouped into two categories: recently infected individuals and patients with clinical conditions that increase the risk of progression, in general due to immune deficiency.
The first group includes close contacts of infected TB patients; TST conversions in the two previous years, especially in the first year; recent immigrants (< 5 years) from countries with high rates of TB; children under 5 years of age, especially in the first two years of life, and to a lesser extent teens and young adults; and residents and employees in places that attract high-risk patients.

The second group includes those infected with HIV; patients with previous TB or untreated fibrotic lesions; individuals 5% or more

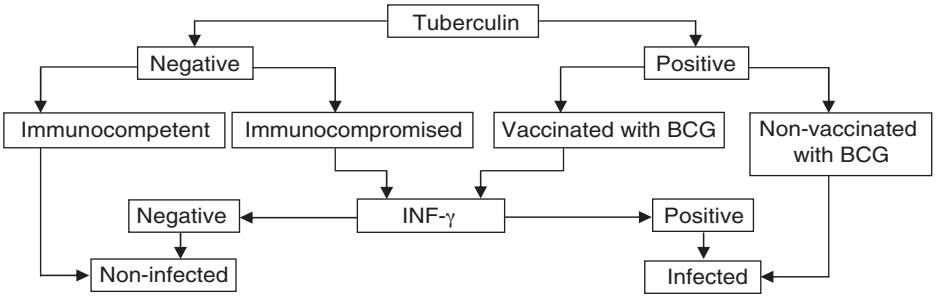


Figure 1. Algorithm for joint use of the tuberculin skin test (TST) and techniques for determining the interferon gamma (IGRA) for the diagnosis of tuberculose infection.[14] BCG: Bacillus Calmette-Guérin.

Table 4
Relative risk for the general population developing active tuberculosis[7]

Clinical condition	Relative risk
HIV/AIDS	100–500
Silicosis	30
Diabetes mellitus	2.0–4.1
Chronic renal failure, haemodialysis	10.0-25.3
Gastrectomy	2–5
Jejunoileal bypass	27–63
Solid organ transplant	
Kidney	37
Heart	20–74
Head and neck carcinoma	16

underweight; intravenous drug users; patients treated with tumour necrosis factor-alpha (TNF-α) antagonists; those under maintenance treatment with corticosteroids or other immunosuppressives; other clinical situations (Table 4).[7]

Pulmonary Tuberculosis

Pulmonary TB has non-specific clinical manifestations, and can even be detected in asymptomatic individuals in the course of contact studies or other examinations. Typically, the patient has symptoms for several weeks, consisting of coughing, expectoration, sometimes haemoptysis, chest pain and general symptoms (mild fever or fever, sweating, asthoenia, anorexia and weight loss). A chest examination may reveal little, perhaps slight rattling noises or localised or asymmetric breath sounds may be heard. It is essential to suspect the disease, and for all patients with respiratory symptoms and/or general symptoms over 2-3 weeks, to initiate diagnostic procedures, as well as in all patients with haemoptysis, regardless of the duration.

Pleural TB can occur in isolation or concomitant with pulmonary TB. Symptoms are chest pain, feeling of dyspnoea, fever or slight fever, with or without general symptoms. Its onset may be acute, over days, or weeks or months. A physical examination shows asymmetry on auscultation, palpation and chest percussion with pleural effusion semiology. The chest radiograph shows the typical image of pleural effusion which is usually unilateral and of variable size. Thoracocentesis yields a yellowish pleural fluid, usually with features of lymphocytic exudate, which is used for different diagnostic procedures. Occasionally, pleural effusion, especially if chronic, can be presented as an empyema. Very exceptionally it may cause fistulas through the chest wall.

TB can also affect the hilar and mediastinal lymph nodes, and join to other thoracic and/or cervical lymphadenites. Lymph node affectation is more common in children, where hilar affectation is a common and unique manifestation of TB. Ganglion-only forms are rarer in adults, therefore the differential diagnosis must be done with lymphoma and sarcoidosis.

Another form of TB manifestation in the chest is endobronchial affectation which, as with the previous ones, may occur alone or in combination with others. This form of TB is diagnosed during bronchoscopy performed for the study of TB or other respiratory illnesses. It manifests as localised lesions and occasionally may have an endobronchial affectation derived from a bronchial perforation due to an adenopathy. In these cases the usual bronchoscopy procedures provide diagnostic confirmation.

Another chest structure that can be affected by TB is the pericardium, which manifests as a pericardial effusion or constrictive pericarditis. This may appear alone or associated with pleurisy or another form of TB. Clinical presentation is usually insidious. Tuberculous aetiology in the pericarditis of long evolution, not responding to anti-inflammatory treatment, must be especially considered. The ECG is abnormal in almost all cases where there is a pericardial effusion, although ST-segment elevation, characteristic of acute pericarditis, occurs in only 10% of cases.[15] The presence of oedema, paradoxical pulse and increasing central venous pressure are clinical warning signs that must not be confused with pericardial tamponade. In these cases, the diagnosis must be supported by clinical and laboratory data and, if possible, by collection of pericardial fluid.

In short, the symptoms of pulmonary tuberculosis are not specific to the disease. They therefore require a high index of suspicion in patients with suggestive symptoms, to make a diagnosis as early as possible and institute appropriate treatment.

Extrapulmonary Tuberculosis

M. tuberculosis is a pathogen which lies mainly in well-oxygenated locations on the lung. However, via the blood, it can be found elsewhere in the body and multiply there to give rise to different clinical manifestations depending on the site condition. As with pulmonary TB, extrapulmonary TB may be accompanied by systemic manifestations, although less frequently observed. Weight loss is more common in disseminated TB in a gastrointestinal location. Neither leukocytosis nor biochemical alterations are usually found.[16] TB, in any of its locations, must always be considered in the differential diagnosis of fever with an unknown origin. Patients coinfected with HIV or another immunosuppression, women and non-Caucasian races are more likely to have extrapulmonary forms of TB.[17]

Once the diagnosis of extrapulmonary TB is made, association with pulmonary TB should always be investigated, as they can coexist. And, although the treatment basis is the same, the contagious nature of pulmonary TB means close associates must be tested for the disease.[18,19]

- *TB of the central nervous system (CNS)* occurs as a result of haematogenous spread from a distant source, or in the context of disseminated TB. It is rarely the result of contiguous invasion. Tuberculous meningitis is the most common presentation of TB in the CNS. Its prognosis is fatal without treatment, which must begin as soon as there is an established diagnostic suspicion, as the functional and vital prognosis depend on the precocity of treatment. The onset and progression of signs and symptoms in TB meningitis are more insidious than other forms of bacterial meningitis. The initial symptoms are non-specific (fatigue, anorexia, headache) and may last several weeks. If left untreated, there is a decreased level of consciousness and focal neurologic signs. If there is a disease of the cranial, optic or oculomotor nerve (pairs iii, iv and vi), they are most often involved. In 1947 the British Medical Council (BMC) proposed an evolutionary classification of the disease in three stages, from the absence of neurological affectation and consciousness to coma, which still has prognostic value.[20] Tuberculomas, brain abscesses, hydrocephalus with intracranial hypertension and ischaemic stroke secondary to vasculitis are other serious forms of presentation of CNS-TB.

- *Osteoarticular TB:* this is usually the result of haematogenous spread of bacilli. The vertebral location is the most common. Initial symptoms are very non-specific, of slow onset with a lack of constitutional symptoms, so diagnosis is usually late. The patient has non-specific localised pain and discomfort. If the clinical picture progresses, it leads to the extension of the lesion to adjacent vertebrae and progressive kyphosis by destruction of the anterior vertebral body. In advanced cases the infection spreads to the adjacent soft tissue, causes cold abscesses or towards the back of the vertebra and can affect the spinal cord, causing compression, which sometimes requires urgent surgery. The most frequent sites are the thoracic spine in young patients and the lumbar in the elderly. Less frequently, bone TB affects other regions, with an emphasis on the metaphyseal portion of the long bones. From this point, it may also extend to the adjacent joint space, resulting in arthritis of a tuberculous origin. A special form of joint disease is called Poncet's syndrome, where the patient has stiffness, polyarticular pain and, occasionally, inflammation and oedema. The diagnosis is by exclusion.
- *Urinary TB* caused by haematogenous spread, the symptoms are non-specific or even asymptomatic in the early stages of renal localisation. If the disease spreads through the ureter and bladder, the most common starting signs and symptoms are dysuria and haematuria. Only 20% of patients have systemic symptoms. Fibrosis can occur in the ureters and bladder, narrowing or obstructing the urinary path. Cramps may occur and eventually lead to hydronephrosis which may require bypass surgery or even nephrectomy.
- *Genital TB:* in males it can affect the prostate, epididymis and less frequently the testicles and seminal vesicles. It is often associated with urinary tract TB. Prostatic localisation manifests as local discomfort, urinary frequency, urinary urgency or haematospermia. On physical examination, the epididymitis causes local pain, scrotal swelling and irregular nodulation. If left untreated it could cause a scrotal fistula. In women, the fallopian tube is the most likely location, accounting for more than 80% of female genital TB. It is usually bilateral and also affects the endometrium. The first symptoms in patients are abdominopelvic pain without fever. Although of low prevalence in Spain, it is one of the most common causes of infertility worldwide.
- *Lymph node TB:* the most common location is in the lymph nodes of the neck and supraclavicular region. TB in this location develops after spreading by haematogenous or lymphatic route from a distant source, but may also be directly via the lymphatic system from the oropharyngeal mucosa and related structures. A cervical tumour is the most common presentation. It is not usually accompanied by constitutional symptoms or fever.[16,21] The nodes tend to grow gradually and are at first of rigid consistency and painless. With time, necrosis may occur, with fluctuating inflammation and formation of fistulas and draining of material. The disease is also known as scrofula. The nodes may remain and even increase in size in spite of effective treatment. Oriental races have a greater predisposition to TB in this location.
- *Miliary TB:* this is a haematogenous spread to multiple organs, causing small lumps to appear in them. The clinical picture is variable, with insidious symptoms such as fever or slight fever and malaise, with little appearing in a physical examination. The chest x-ray may have the typical pattern of granulomas with small pulmonary nodules, but may also be normal. Computed tomography (CT) is useful for showing lesions not visible on conventional radiology. This presentation is serious in anyone, especially in immunocompromised patients and particularly in patients with AIDS. Clinical suspicion is important to make a diagnosis and treatment as early as possible. It may be associated with tuberculous meningitis, which should therefore be confirmed or ruled out by diagnosis.
- *Laryngeal TB:* this currently occurs in less than 1% of TB patients. It is produced primarily by local extension from the bronchial tree. The most common sign for suspecting this condition is hoarseness, but any of dysphagia, cough, stridor or haemoptysis may also be present. Locally, it can produce masses, ulcers or nodules, which may be indistinguishable from a laryngeal neoplasm, requiring histological and bacteriological study. Laryngeal TB is often associated with bacilliferous pulmonary TB, and is therefore highly contagious.
- *Skin TB:* is usually a manifestation of a systemic disease, although it may be acquired by direct inoculation or extension from a contiguous focus, as is the case with scrofula. The manifestations of skin TB depend on the patient's immune status. *Lupus vulgaris* is a form of presentation in immunosuppressed patients and can lead to chronic deformities. *Tuberculosis verrucosa cutis* is usually a more indolent form in patients with mild or moderate immunosuppression. The tuberculids (Bazin's indurated erythema, scrofulous lichen and papulonecrotic tuberculids) are other forms of presentation usually found in immunocompetent patients. Although the latter are traditionally attributed to immune reactions, the presence of DNA from *M. tuberculosis* has sometimes been shown.
- *Gastrointestinal TB:* may involve any part of the digestive tract from the mouth to the anus, with ileocecal localisation the most common. An ulcerated image can be seen in an endoscopy, which sometimes suggests a carcinoma. It can also manifest as warty or fistula mages. The pancreas, bile ducts and liver may also be affected. Tuberculosis is still a common cause of granulomatous hepatitis in this country.

Clinical Manifestations in Immunosuppressed Patients

Immunosuppression associated with certain diseases (solid and haematologic malignancies treated with chemotherapy, solid organ transplantation and HIV infection, among others) can change the location; affecting any organ, the clinical and radiological presentation, as well as the gravity and evolution of the TB. The differences are particularly striking in the case of HIV infection, which has been used as an example of the effect of immunosuppression in these aspects of tuberculosis.

Clinical presentation of TB in an HIV-infected patient differs from that classically recognised in the general population. The greater virulence of *M. tuberculosis,* compared to other opportunistic pathogens that complicate HIV infection, means that most patients who develop TB have not experienced other AIDS-defining infections. The exclusive lung disease occurs in less than 50% of cases, and the rest have the extrapulmonary disease only or mixed. The state of immunosuppression of the subject is the main factor in the clinical presentation, so that patients with greater immunosuppression tend most often to have the extrapulmonary form which is, above all, scattered. Moreover, within the pulmonary disease, those presentations more similar to the classic are more common in patients with the more preserved immune status.

Symptoms and signs of TB in HIV-infected patients are often non-specific, and it is difficult to distinguish the disease from other opportunistic infections. Fever is an almost constant sign, much more frequent than in immunocompetent patients. The patient may present with an acute case of a few hours or days of evolution, similar to a classical bacterial infection, or with a period of several days or weeks, characterised by fever and systemic symptoms (anorexia, fatigue, weight loss), which may be accompanied by affectation of a focal organ. The most common location is the respiratory tract, but it also affects the lymph nodes, urinary system, central nervous system and liver very often.

Radiographic presentation of tuberculosis is highly variable, from a normal chest radiograph to extensive bilateral patterns that can lead to confusion with *Pneumocystis jiroveci* pneumonia.[22] There is a high frequency of intrathoracic lymphadenopathy seen in all series. Among the extrapulmonary organs affected are the lymph nodes, both peripheral and intrathoracic and intraabdominal is very common. In fact, in Spain the presence of significant lymphadenopathy with fever or other manifestations in HIV positive patients should always suggest the possibility of TB as a first diagnosis. The detection of lymph nodes with hypodense centres and ring contrast enhancement in the CT has been suggested as highly specific in the diagnosis of TB.

Following the introduction of highly effective antiretroviral treatments, previously unobserved clinical presentations have been recognised. Forms of TB with explosive development after starting antiretroviral treatment have been seen, including patients with diagnosis and treatment of TB prior to initiating antiretroviral therapy (immune reconstitution inflammatory syndrome, IRIS[23]). These forms can also be seen in immunosuppressed patients not infected with HIV and even sometimes in those without an underlying disease. The main risk factor appears to be the simultaneous administration of antiretroviral and tuberculosis therapy, with symptoms appearing within the first two months of treatment.[24] Clinical manifestations include high fever, exacerbation and extension of pulmonary and extrapulmonary disease, particularly nodal affectation in multiple areas. These exacerbation conditions are considered a result of the recovery of cellular immunity.[25] These manifestations of TB exacerbation do not imply a worse development or prognosis and require no specific treatment, although administration of steroids may be useful.[26]

In patients with immunosuppression caused by non-HIV infection, TB is often exclusively extrapulmonary or widespread. In organ transplant patients who develop TB, around 25-48% of the cases are extrapulmonary or widespread. In these patients, the only factor with which the presentation has been associated as a disseminated disease is the use of anti-CD3 monoclonal antibody (OKT3) to prevent rejection. These disseminated forms have also been associated with the administration of anti-TNF drugs. It should also be noted that sometimes TB cases are diagnosed in asymptomatic patients during routine monitoring for an immunosuppressive disease, and frequently necropsy is diagnosed. As opposed to patients infected with HIV, radiology shows findings closer to those of immunocompetent patients (focal or miliary infiltrates, nodules, pleural effusion and, rarely, intrathoracic lymphadenopathy).

Recommendations Regarding Clinical Manifestations

1. Pulmonary TB should be suspected, and diagnostic procedures initiated in all patients with respiratory symptoms of more than 2-3 weeks, as well as in patients with haemoptysis, regardless of length. In many cases, the clinical symptoms are non-specific, so this must always be borne in mind in the differential diagnosis of undiagnosed pulmonary diseases (AIII).
2. Extrapulmonary TB in any of its locations can have generally non-specific symptoms and those deriving from the affected organ. It is clinically important to suspect the disease when there is an inflammatory process in these organs, and fever of an unknown origin must always be considered in the differential diagnosis. CNS involvement and miliary TB are particularly serious and require diagnosis and treatment as early as possible (AIII).
3. It should be noted that immunosuppression, with HIV infection as the classical example, can modify the location, clinical and radiological presentation, as well as the severity and progression of the TB; with atypical pulmonary manifestations as well as extrapulmonary and disseminated forms being more common than in immunocompetent individuals. Fever is an almost constant symptom and lymph node affectation in various areas is frequent (AIII).
4. In immunocompromised patients, especially those co-infected with HIV, consideration should be given to explosive clinical manifestations due to immune reconstitution inflammatory syndrome, particularly within the first two months of starting antiretroviral and TB treatment (AIII).

Diagnosis of Tuberculosis

Radiology and Imaging Techniques

Pulmonary TB. The findings from chest radiography and CT, although non-specific, are a faithful reflection of structural changes in the lung and the rest of the chest area, leading to suspicion of TB. The radiological patterns correspond to pathogenesis of the disease and the continued prevalence of cellular immunity or delayed hypersensitivity.[27,28]

The main primary TB radiological findings, either alone or in combination, are: *a*) parenchymal infiltrates or opacities, corresponding to the initial pneumonic outbreak, with small and occasionally lobar segments, which in children and adolescents is accompanied by lymphadenothapy; *b*) lymphadenothapy: especially paratracheal and hilar, with the initial focus usually in the right chest area; this is the basis of diagnosis in children; *c*) segmental atelectasis: due to compression of the lymph node of the bronchial lumen or due to endobronchial TB, especially in the middle lobe, conditioning bronchiectasis; *d*) pleural effusion, usually unilateral, which is more common in the young and may be the only radiological finding, and *e*) miliary TB, which is rare; corresponding to a self-limiting initial haematogenous spread.

The main radiological findings of reactivation, secondary or post-primary TB are: *a*) bronchopneumonic type condensation, patched without air bronchograms, normally found in posterior segments of upper lobes; *b*) cavitation, single or multiple, of various sizes, with or without air-fluid level; usually located in posterior segments of upper lobes; *c*) secondary pleural effusion to bronchopleural fistula, leading to a piopneumothorax or pneumothorax if the focus is cavitated; *d*) miliary, haematogenous and diffuse dissemination of 1-3mm diameter micronodules which may be imperceptible in the beginning or coalesce and coexist with cavitated lesions in later stages; *e*) tuberculomas, which are nodules or tumour-like masses of

various sizes, with calcifications; and *f*) fibrosis: this is the attempt to heal infiltrates and cavities by calcification and fibrous retraction of the segment or lobe affected, with a distortion in its structure. It causes bronchiectasis and may lead to a destroyed lung pattern.

In immunocompetent patients, the radiological findings common in children, adolescents and young adults are often those characteristic of primary TB, while in adults and the elderly they are post-primary TB. In immunosuppressed patients, as happens in HIV infection, the findings vary depending on the degree of suppression: patients with normal CD_4^+ are similar to those described for immunocompetent individuals. However, when the immune suppression is intense, the x-ray may even be normal. CT may be useful in children and when there is some doubt.

Extrathoracic TB. The difficulty of obtaining laboratory confirmation of extrapulmonary TB often means the diagnosis must be based on other tests. Radiology and other imaging techniques can help in diagnosing extrathoracic TB. Furthermore, CT and/or magnetic resonance imaging (MRI) can also be useful for locating the affected areas accessible for a diagnostic puncture.

CNS TB, although not the most common form of extrapulmonary TB, is the most serious. So a quick diagnosis is of vital importance for patient prognosis. The manifestations of CNS TB are diverse, as reflected in both CT and MRI images. MRI is the imaging technique for earlier detection of lesions and is therefore preferred if available. In meningitis TB, the presence of a triad consisting of contrast uptake in the basal meninges, hydrocephalus and images of vascular origin infarcts are typically described. However, none of them is pathognomonic of TB and only a small percentage of meningitis TB cases present all three signs. TB that affects the brain parenchyma may present as tuberculoma, abscess or plaque tuberculomas. CT and MRI may be helpful, although there are no specific signs for differential diagnosis from other mass lesions of the CNS space.[29] Tuberculomas are usually supratentorial in location, show peripheral ring enhancement and appear with a hypo- or isointense signal on the T1 sequence and hyperintense on the T2; or, hypointense in caseating tuberculomas. They may be surrounded with varying degrees of oedema. MRI spectroscopy shows prominent lipid peaks, which are in fact quite characteristic of intracranial tuberculomas.[30] However, they may also appear in other intracranial lesions such as pyogenic abscesses or tumours.

In bone TB, the spine is the most common area, especially in the lumbar or thoracic regions. Usually, the disease affects the anterior vertebral section and may extend to the intervertebral space and paravertebral soft tissue, forming abscesses. Periarticular demineralisation is an early, but non-specific, radiological sign. As with CNS TB, MRI is more sensitive than CT in detecting lesions of tuberculous origin.

In urogenital TB, affectation of the calyces is often the first radiologically objective manifestation. The lesion may progress causing calyceal deformity, ulceration, fibrosis and stenosis, which in the end lead to caliectasis, hydronephrosis and ureteral hydronephrosis, secondary to renal parenchymal destruction. Ultrasound techniques may be of diagnostic help in tuberculous epididymitis and other forms of genital location. Genital TB shows hypoechoic foci and a low degree of blood flow in the Doppler study.[31]

For lymph node TB, the cervical or supraclavicular nodes are most commonly affected, although it can occur at any location. The peripheral enhancement with a low attenuation centre on the CT is suggestive of tuberculous aetiology, although this is not pathognomonic.

For other TB locations, such as the bowel, radiological signs of fibrosis, intestinal stenosis or retraction can be found.

Isotopic techniques, such as scintigraphy and positron emission tomography (PET), which estimate the inflammatory activity, detect the disease in its early stages, although the resulting findings are non-specific for the diagnosis of extrathoracic TB.

Imaging Technique Recommendations

1. A simple chest radiography should be used in the diagnosis of pulmonary TB (AIII).
2. A normal chest x-ray is not recommended for TB diagnosis in immunocompetent patients, although not completely ruled out (BIII).
3. Chest radiography often shows signs highly suggestive of TB, although it is sometimes atypical. Therefore, TB has to be in the differential diagnosis of unexplained radiographic abnormalities (AIII).
4. More complex radiological tests (CT, MRI) are very useful for extrapulmonary forms of TB. For pulmonary TB, they must be reserved for cases where there is clinical suspicion and results from the simple radiography and microbiology do not lead to a diagnosis (AIII).

Microbiological Diagnosis of Tuberculosis

Clinical diagnosis of TB is based on signs and symptoms. However, the differential diagnosis can sometimes be difficult. This, together with the public health and epidemiological implications of TB diagnosis, require the confirmation of diagnosis by microbiological means. However, this has to be done in accordance with the workload and experience of each laboratory, with referral to other centres when necessary.

Microscopy techniques. At present, the quickest, easiest and most accessible technique for rapid diagnosis of TB is acid-fast staining.[32] This detects all members of the *Mycobacterium* genus in clinical samples.[33,34] However, there need to be between 5,000-10,000 bacilli/mL in the sample to be detectable.[33,34] This means that a variable percentage (30-50%) of TB cases are smear-negative, so a negative result does not rule out the disease. In Spain, the detection of acid-fast bacilli in clinical specimens is often synonymous with TB, especially in respiratory samples. The most commonly used staining techniques are the Ziehl-Neelsen and auramine-rhodamine fluorescence. It has been shown that the specificity of both is similar, although the fluorescent staining has the advantage of being examined with a lesser magnification, resulting in a shorter time to evaluate each extension.[32,33] Viewing at least three acid-fast bacilli per 300 fields with a magnification of 100 must always be considered positive.[32] Counting the number of bacilli present in the sample (table 5) is also recommended, to assess compliance with treatment. Given the high number of false positives reported in several studies, the Kinyoun technique is not currently recommended in direct diagnosis.[35]

Identification of bacilliferous cases is of particular importance, as these are the most infectious patients, and their detection is a priority in all programmes for combating TB, as an essential step in breaking the disease transmission chain. Therefore, any patient suspected of

Table 5
Interpretation of bacilloscopy results

Report	Ziehl-Neelsen (× 1,000)	Fluorescent stain (×250)	Fluorescent stain (× 450)
Negative	0	0	0
Doubtful (repeat)	1–2/300 fields (3 sweeps)	1–2/30 fields (1 sweep)	1-2/70 fields (1.5 sweeps)
Positive 1 +	1–9/100 fields (1 sweep)	1–9/10 fields	2-18/50 fields (1 sweep)
Positive 2 +	1–9/10 fields	1–9/ field	4-36/10 fields
Positive 3 +	1–9/field	10–90/field	4-36/field
Positive 4 +	> 9/field	> 90/field	> 36/field

Modified Alcaide et al[32].

TB must be given a stain test. In addition, due to the fact that elimination of bacilli in respiratory secretions is irregular, at least three good quality samples obtained on different days should be considered before evaluating the patient as non-bacilliferous.[32]

For extrapulmonary clinical presentations, the performance of microscopy is related to the sample. It is high in samples obtained by biopsy or excision of tissue (> 70-80%) and particularly low in biological fluids (5-20%).[32,33]

Apart from its role in diagnosis, microscopy is essential for monitoring the effectiveness of treatment; with samples for bacilloscopy and culture taken on follow-up visits. The number of bacteria present in the stain will decrease if treatment is effective, although the conversion of staining may take some time depending on the initial bacterial load of the lesions.[34]

Culture techniques. A mycobacteria culture should be performed in all patients with suspected TB. The culture, considered the gold standard, has much greater sensitivity than staining, and can detect between 10-100 bacteria/mL of sample. In addition, the mycobacterium present in the sample can be isolated, the species identified and a study made of sensitivity to antibiotics. The main drawback of the culture test is its slowness in obtaining positive results, determined by the metabolic characteristics of the pathogen. *M. tuberculosis*, as well as the rest of the complex species, are slow-growing mycobacteria, which means that 2-6 weeks are required to provide macroscopically visible growth in solid culture media, depending on the number of bacteria present in the sample.[33] Various strategies have been used to minimise this incubation period, with the most common at present being the automatic use of liquid media. These can detect the presence of bacterial growth between 7-10 days before the solid media.[32-34] In addition, when combining the inoculum of a greater amount of sample with the characteristics of the medium, they are generally more sensitive than solid media. However, sometimes the solid medium can test positive and the liquid medium give a negative result, so it is currently recommended to use both.

A negative result after growing the culture is the main indication of cure. Therefore, it is recommended to take bimonthly sputum cultures at every follow-up visit, providing a sample can be obtained from the lower respiratory tract. Also, for bacilliferous patients, samples for growth and bacilloscopy are taken at 15 days and one month after starting treatment, when clinical criteria deem it necessary. If the issue of sputum disappears during the treatment, no saliva samples can be taken and this will therefore not be indicated.

Antibiogram. The sensitivity study of *M. tuberculosis* to antibiotics is based on detecting more than 1% resistant bacteria in a sample compared to control growth without antibiotics.[36] The existence of these resistant bacteria is due to the high rate of mutations occurring in relation to other antibiotics used in treatment.[37] At present, there are various standardised commercial systems on the market, based on the same automatic or semi- automatic liquid media used in cultivation. Antibiograms can be performed relatively easily in most microbiological laboratories.[34,38-40] However, it must be performed by experienced laboratories having a sufficiently high workload, and be subject to repeated quality controls, both internal and external. At present, it is recommended to find out the sensitivity to first-line drugs for all extractions of initial samples of new or previously untreated patients. This will provide knowledge about the response of the clinical extracts to the drugs, and treatment can be adjusted as necessary.[32,34] When resistance is detected it is important to confirm it by repeating the test or by sending the extract to a reference centre. It is also important to reject false results due to contamination by other mycobacteria or conventional bacteria. If a strain is resistant, especially to H and/or R, an antibiogram against second-line drugs will be indicated. Because there is no widely accepted standardisation,[41] it is recommended to send the extract to a reference centre with sufficient experience in performing the test.

Molecular methods in direct diagnosis. These are based on the amplification of specific gene fragments of *M. tuberculosis* directly from clinical specimens. Currently its main theoretical interest is its potential for rapid diagnosis of tuberculosis with a higher sensitivity than smear microscopy, which is considered as the quick technique of reference. However, despite the fact that over the past 15 years there have been numerous studies to establish its role in the diagnosis protocol of TB, the main obstacle in assessing its effectiveness lies in the lack of universal standardisation. This is because there is a wide methodological variety in the different levels of the technique, making it difficult to compare different studies. At the same time, there are commercial and home-made applications available for sale.[42-44]

Most studies have applied to pulmonary TB, using the polymerase chain reaction (PCR) technique with IS6110 as the genetic target. Although some extracts do not have this sequence, in general its performance is superior to other proposed targets. In the best test conditions, the sensitivity is lower than culture and is related to bacterial load. So in bacilliferous samples it is in the range 90-100%, while with a negative result it is between 60-70%. Irrespective of the bacilloscopy, the overall sensitivity of the commercial methods on the market would be lower, around 50%, compared to 80% for the homemade preparations; although they would have a higher specificity (95 vs. 80%).[42]

Of the extrapulmonary forms, the ones mainly studied are pleural[45] and meningeal, mostly with bacilloscopy negative. The average sensitivity ranges from 50-70%, with specificity close to 90-95%, so its exclusion value is little, but it confirms the disease with a high degree of certainty. For other locations, there is insufficient evidence to establish a reasonable profitability. The scarce literature may indicate that in lymph node and bone locations there is a

sensitivity of about 80%, with specificity above 90%; and urinary, peritoneal and pericardial TB would have a similar profitability to the meningeal and pleural forms.[42]

The application of amplification techniques to the diagnosis of TB requires greater standardisation as a prerequisite, taking technical and clinical factors into account. Among the technical factors are the genetic target selection, the DNA extraction method, the amplification technique and the variety selected as well as the developing system. Specificity is crucial in the choice of primers and control of cross contamination. Including more of a genetic target, using internal inhibition controls and proper assessment of extraction methods, all help to increase sensitivity. Using methods that combine automated extraction with PCR amplification in real time would offer a versatile alternative, which would be feasible under routine conditions of care. However, there are insufficient data regarding its usefulness when compared with other strategies. Regarding clinical factors, the degree of suspicion has a direct effect on the usefulness of the technique. Most studies considered bacilloscopy and culture growth as the reference methods, so that in practice the diagnostic value of the results of amplification tests should be decided after a joint analysis of clinical data and other evidence available. So when the degree of suspicion of TB is moderate to high (above 40%), a positive result will indicate TB in 80% or more of cases, whereas if the suspicion is low, it reduces the specificity to a maximum of 50%.[42]

In conclusion, the data support the use of amplification techniques as an adjunct to the diagnosis of TB, along with clinical and other examinations, but not in cases of low clinical suspicion due to its poor specificity.

Molecular methods in the identification and detection of resistance. In recent years, laboratories have seen an increase in the percentage of extracts for non-*M. tuberculosis* complex mycobacteria. Technical improvements have contributed to this increase, such as the use of semi-automated liquid culture media and new methods of identification. Also, possibly increasing the actual incidence, there are those related to new epidemiological situations, such as the greater number of immunosuppressed patients or those with chronic diseases and infections related to invasive device handling.

Mycobacteria other than the *M. tuberculosis* complex are generally ubiquitous in the environment and have different pathogenicity, lower than *M. tuberculosis*. Its isolation does not imply disease, so it is crucial in each case to elucidate its clinical significance. This is why guidelines have been developed by various scientific societies to help in their interpretation. It is therefore vital to identify the species from all clinical sample extracts. Traditionally, *M. tuberculosis* was identified via biochemical tests for niacin production and nitrate reduction. In recent years, a commercial method using chemoluminescent hybridisation probes has been used successfully for identification. Recently, laboratories have used various identification hybridisation methods with probes immobilised in nitrocellulose strips and enzymatic development (line probe assay) that can identify 10-15 different species, with close to 100% specificity.[46] Moreover, the sequencing of variable regions of genes common to the genus, such as the *16S rDNA* gene theoretically identifies virtually all mycobacterial species. It is seldom necessary to specifically identify the different members of the *M. tuberculosis* species, as they have no clinically relevant differences in Spain, with more than 95% of the extracts corresponding to *M. tuberculosis*. However, there are several methods capable of differentiating them, whether they are PCR-based, line probe assays or molecular typing techniques.

In conclusion, genetic techniques have a high efficacy in the identification of *M. tuberculosis*. Combined with biochemical and morphological characteristics, they can identify most species extracted from clinical specimens.

The therapeutic arsenal against TB is very limited and represents a major problem in patients affected by drug-resistant strains, especially in multidrug-resistant cases. The proportion antibiogram method is the gold standard for detecting in vitro resistance to first-line TB drugs of *M. tuberculosis* extracts. However, the incubation time required to obtain results (4-8 weeks after sample collection) limits the scope for designing therapeutic alternatives.

The development of molecular resistance detection methods is based on the demonstration of mutations in certain genes. Mutations resistant to various first line drugs are known, although there are a proportion of extracts where they are not observed and therefore are not detectable by this method.[47] This proportion varies depending on the drugs. Thus, over 95% of the resistances to R are due to mutations in a short fragment of 81 nucleotides of the rpoB gene. 60-65% of cases of resistance to H have been associated primarily with mutations in the katG gene and the intergenic region mabA-inhA. As for the remaining first-line drugs, there are embB mutations in 50% of the resistances to ethambutol (E), 55% of extracts resistant to streptomycin are due to the rrs and rpsL genes, and 94% of cases resistant to pyrazinamide (Z) with the gene pncA. Most screening protocols are based on the simultaneous analysis of the mutation and its corresponding wild sequence, which confers it very high specificity.

When genetic resistance screening is applied to culture extracts, the sensitivity is close to 100%, provided that the causal mutation is included in the technique design. In clinical samples it is used only when the smear is highly positive, as otherwise the sensitivity is low.

In recent years, a major breakthrough in genetic resistance screening has been developed, especially through the technology of PCR amplification and fluorimetric detection in real time,[48,49] as well as the line probe assay methods. As they are the most important drugs in the standard treatment, most strategies are applied to the detection of resistance to R and H. More recently, applications based on microarrays capable of simultaneously detecting a large number of mutations to all drugs have been developed.[50]

Detection of mutations resistant to H and R is indicated for positive culture extracts or directly in intense smear samples (3+/4+) in the following situations: treatment failure, resistant patients suspected of infection and patients from countries with a high incidence of resistance. In patients with a history of previous treatment, indications should be analysed individually, which is clear in patients suspected of not completing treatment.

However, antibiogram testing is compulsory, as neither these techniques nor the CMI detect all resistance. Their main use therefore is in providing preliminary and rapid information about the antibiogram, especially when mutations are detected.

Other Diagnostic Methods

Histology. TB has characteristic lesions that may be useful in diagnosis when biopsies or surgical specimens are available. The typical TB tissue lesion is a granulomatous inflammation with central necrosis.[51] This is considered a highly specific finding justifying the start of TB treatment. Granulomas without necrosis are also often

observed. They suggest a probable diagnosis of TB, although there are other infectious and non-infectious causes of these, such as sarcoidosis.

The observation of acid-fast bacilli in the necrotic centre confirms the diagnosis, but bacteria are only observed in about 10% of cases with necrotic granulomatosis. Therefore it is necessary to make a culture of the histological specimens, but this is not viable if the samples are preserved in formalin, as happens often. Several amplification studies on samples fixed with formalin and embedded in paraffin indicate that the sensitivity range is between 30-65% according to the method conditions. However, the most important recommendation is to obtain adequate biopsies for culture, preserved in distilled water.

Determination of adenosine deaminase (ADA) and other biochemical and cytological parameters. In the same way that the granulomatous tissue response is typical of TB, when this is found in serosas, a characteristic inflammatory response is seen. Several cytological and biochemical parameters of this reaction product are useful in the diagnosis, with determination of ADA the one that provides most information. ADA is an enzyme involved in the catabolism of purines, produced by monocytes and macrophages and is found in high concentration in the TB. It is found in three forms, with the most specific for TB being isoenzyme ADA2, produced by monocytes. However, in most techniques, total ADA is analysed and expressed in IU/L, with a cut-off point for each type of sample being considered (usually 8-10 IU/L for cerebrospinal fluid and 40 IU/L for pleural fluid).

The determination of ADA is widely used in pleural TB,[52] as it is one of the most common causes of pleural effusion, and to a lesser extent in the meningeal forms. There is less experience in peritoneal, articular and pericardial locations.

The sensitivity is generally high, between 75-98% and is the most sensitive test for all forms of TB in serosas. It is similar for the pleural forms, where the sensitivity of bacilloscopy would be less than 5%, culture would be between 25-40% and biopsy histology between 50-80%.

Specificity is directly related to the incidence of TB, so that in low incidence areas it will decrease with increasing relative proportion of false positive cases, and will have exclusion value in negative ones. In the pleural location, with false positives due to empyema, lymphoma, malignancy or rheumatoid pleuritis, it is considered to be about 90%. Also false positives have been reported in cerebrospinal fluid associated with lymphomas and *Crytococcus neoformans* meningitis, cytomegalovirus (CMV) and *Candida*. Therefore, in this location, its specificity would be lower, at about 80%.

Its maximum usefulness, with over 90% of diagnoses, is achieved when combined with favourable biochemical parameters (lymphocyte percentage over 50%, high protein and LDH and glucose lower than 25mg/dL), tuberculin positive (or one of the 2 positive gamma interferon production tests) and/or positive gene amplification. This situation could prevent a pleural biopsy to confirm diagnosis. It has been reported that second measurements performed in highly suspected cases would increase sensitivity in pleural TB.

Recommendations for Microbiological and Laboratory Diagnosis

1. At least three high quality consecutive samples of respiratory secretions obtained on different days should be studied for the diagnosis of pulmonary TB (AII).
2. In extrapulmonary TB, consecutive samples will also be obtained if not done via the invasive route. When it is necessary to use invasive methods, obtaining more than one sample must be considered if the degree of clinical suspicion is high. It is also important to ensure that the method of obtaining the sample allows it to be used as a culture (BIII).
3. All patients with suspected TB should have clinical location samples analysed by acid-fast stain (Ziehl-Neelsen, auramine) and culture (AIII). Automated liquid media should be used for the cultures, although it is recommended to also add a solid medium (AIII).
4. Gene amplification techniques are coadjuvant with bacilloscopy and culture isolation. They should only be used in cases with moderate/high suspicion of TB to avoid false positive results (BIII).
5. The genetic screening of better-known mutations resistant to H (codon 315 of katG gene) and R (rpoB gene in RRDR area) is highly specific for resistance. It is recommended to be used in specific clinical and epidemiological situations (AIII).
6. An antibiogram should be performed for first-line drugs for all initial *M. tuberculosis* extracts. This must be done by experienced laboratories and any resistance detected must be confirmed. With the emergence of resistance to H and R (multidrug resistance) a sensitivity study to second-line drugs must be performed (AIII).

Treatment of Tuberculosis

Standard Regime

The current treatment for initial or never treated TB (tables 6-8) is governed by bacteriological bases and numerous controlled therapeutic trials conducted over 30 years by various scientific societies and international health organisations. It is considered that a therapeutic regime is eligible to be used when it cures over 95% of patients and causes less than 5% serious intolerance requiring modification. The best treatment will be made up of drugs with a high bactericidal and sterilising power, with a low number of recurrences, few side effects, well accepted and tolerated and administered simultaneously in preparations containing all the drugs combined. This will facilitate compliance and prevent failures and the development of resistance.

Tuberculosis treatment should not be very costly for use even in countries with a low purchasing power. In Spain, free treatment to all patients should be offered.

Treatment regimes that meet all favourable requirements and have been universally recommended are for 6 months: two months with R, M, Z and E, followed by four months with R and H (2RHZE/4RH). When it is identified that isolation is sensitive to these drugs E may be withdrawn. In exceptional low bacillary cases, treatment with three drugs (RHZ) can be used in the initial stage.[9,53-55]

Children often have a good tolerance to drugs. Their treatment needs to be the same as in adults, with weight adjusted doses. The regime should also not be altered during pregnancy or lactation, however, the use of aminoglycosides and protionamide should be avoided.

Anovulatory patients should be advised of the induction of these drugs due to R (Table 10), and recommend they be prepared with low oestrogen doses or other methods of contraception.

Although rarely used in Spain, there are widely contrasting intermittent regimes where drugs are administered 2 or 3 times a week, with appropriately modified dosages.[53]

It is always recommended to use fixed combination drug preparations. Preparations are now available with four drugs (RHZE), 3 drugs (RHZ) and two (HR). See table 8.

Treatment in Special Situations

Certain situations require the standard treatment to be modified. Among them are the following:

1. Patients with chronic liver disease: treatment should be attempted with a standard regime, with close clinical and analytical monitoring of liver function. In cases of advanced liver disease, one of the three drugs with hepatotoxic potential (H, R, Z) will be removed. This will preferably be Z, while trying to keep R (table 6). Only in cases of acute hepatitis or terminal stages of chronic liver disease will a drug treatment with no potential liver toxicity be opted for. This will include a quinolone, an injectable (aminoglycosides or capreomycin) and E or cycloserine. The duration of treatment must conform to the regime used.
2. Chronic renal failure: there is no need to modify the standard treatment. Only for patients with creatinine clearance below 30mL/min or patients on haemodialysis is treatment recommended to be 3 times a week (always after haemodialysis), while maintaining the same daily dose regime. Generally speaking, measuring the drug plasma levels should be considered.
   If S needs to be entered in the treatment, it should be given at 12-15mg/kg, 2 or 3 times a week. The H, E and, above all, Z are all drugs that are dialysed, so for patients undergoing haemodialysis it is recommended that drug administration be done after this.
3. Extrapulmonary forms: evidence on the duration of treatment in some extrapulmonary locations is not sufficiently clear, and the recommendations of various guidelines are not unanimous. However, it is recommended to use the same regime as in the pulmonary TB and prolong the duration of treatment in some situations, especially in tuberculous meningitis and tuberculous spondylitis with neurologic involvement (table 6).
4. It is also recommended to extend the treatment of pulmonary tuberculosis for patients affected with silicosis.
5. Difficulties in oral administration due to loss of consciousness: in addition to streptomycin, there are parenteral formulations of H, R and E with doses equivalent to oral presentations. Administration via nasogastric or percutaneous gastrostomy are offered as alternatives.
6. Administration of corticosteroids: these will be indicated in the first weeks of treatment for meningeal forms (especially in stages 2 and 3 of BMC[20]) and in pericarditis. Because of its anti-inflammatory action, it can be considered for occasional use in other locations (pleural, lymph node, urinary and fallopian tube strictures, peritonitis, uveitis, miliary forms of poor outcome and very extensive and scattered forms). The recommended dose is 0.5-1mg/kg/day of methylprednisolone for 1 month, with progressive decrease until withdrawing at 2 months.
7. Administration of pyridoxine: H can cause peripheral neuropathy due to a lack of this substance in certain situations. A prophylactic

Table 6
Initial tuberculosis treatments

Situation	Treatment
Pulmonary and extrapulmonary	2HRZE/4HR*/**
Alternative initial treatments	2HRZS/4HR 2HRE/7HR
Special situations (gout, severe chronic liver disease)	2HRE/7HR
Meningitis, tuberculomas	2HRZE/10HR
Silicosis, spondylitis TB with neurological affectation	2HRZE/7HR

E: ethambutol; H: isoniazid; R: rifampin; S: Streptomycin; TB: tuberculosis; Z: pyrazinamide.
*When the antibiogram shows sensitivity to all drugs, E may be withdrawn.
**If it is not possible to use E, it can be replaced by S (2HRZS/4HR).

Table 7
First-line drug dosing guidance

Drug	Daily dose (according to weight)		
	< 40kg	40–90kg	> 90kg
Isoniazid (H)	5–7mg/kg	300mg	450mg
Rifampicin (R)	10mg/kg	600mg (maximum 600mg)	600mg
Pyrazinamide (Z)		25–30mg/kg (maximum 2,500mg)	
Ethambutol (E)		25mg/kg* (maximum 2,000mg)	
Streptomycin (S)	15mg/kg	750mg**	

*Start of the third month of treatment: 15mg/kg.
**Less than 50 years old, more than 50kg in weight: 1,000mg/day.

Table 8
Dosages in fixed combination preparations in the treatment of tuberculosis

Initial Phase: 2 months				
Weight (kg)	Rifater® (R 120+H 50+Z 300) Pack of 100 tablets	Weight (kg)	Rimcure® Pack of 100 tablets (R 150+H 75+Z 400) Pack of 100 tablets	Rimstar® (R 150+H 75+Z 400+E 275) Pack of 60 tablets
< 40	3	30-50	3	3
40–50	4	50-70	4	4
50–70	5	> 70	5	5
> 70	6			
Continuation Phase: 4 months				
Weight (kg)	Rifinah® (R 300+H 150) Pack of 60 tablets	Rimactazid® (R 300+H 150) Pack of 60 tablets	Tisobrif® (R 600+H 300) Pack of 30 sachets	
40-90	2	2	1	

E: ethambutol; H: isoniazid, R: rifampin; Z: pyrazinamide.

Table 9
Interactions between antiretroviral drugs and rifamycins

Antiretroviral	Use with rifampicin	Use with rifampicin	Comments
NARTI	Yes	Yes	
NNRTI			
Efavirenz	Yes (first choice)	Yes	Normal dose of R and efavirenz, except 800mg in > 60kg Increase RB to 450–600mg/day
Nevirapine	Yes (alternative to efavirenz)	Yes	Normal dose of RBR ↓ 37%
Etravirine	No information	Yes	No dose adjustment with RB
Rilpivirine	Contra-indicated	No information	
PI			
Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir Nelfinavir	Contra-indicated	Yes	RB 150mg, 3 times/week
Ritonavir	Yes	Yes	Normal dose of R Reduce RB to 150mg, 3 times/week
Saquinavir	Contra-indicated due to toxicity	Yes	RB 150mg, 3 times/week
Tipranavir	Contra-indicated	Yes	RB 150mg, 3 times/week
Inh fusion			
Enfuvirtide	Yes	Yes	
Inh integrase			
Raltegravir	Contra-indicated at normal dosages	Yes	RB At normal daily dose R reduces raltegravir values. No clinical studies. Pharmacokinetic studies in healthy volunteers suggest that R can be used increasing Raltegravir dose to 800mg/12 h
Inh CCR5			
Maraviroc	Contra-indicated at normal dosages	No studies	R reduces Maraviroc levels. No clinical studies. Pharmacokinetic studies in healthy volunteers suggest that R can be used increasing Maraviroc dose to 600mg/12 h

Inh CCR5: Co-receptor antagonists CCR5; Inh fusion: fusion inhibitors; Inh integrase: integrase inhibitors; PI: protease inhibitors; NARTI: nucleoside analogue reverse transcriptase inhibitors; NNRTI: non-nucleoside analogue reverse transcriptase inhibitors; RB: rifabutin; R: rifampicin.

pyridoxine supplement (10-50mg/day) is recommended in patients who are alcoholic, malnourished, pregnant, diabetic, or those with renal failure or HIV coinfection.

Treatment in HIV Patients

Patients who receive concomitant antiretroviral and tuberculosis therapy pose a special situation, due to the interaction of R with antiretroviral drugs. R is a potent inducer of cytochrome P450 and accelerates the metabolism of many antiretroviral drugs, as well as significantly decreasing their plasma levels (table 9). Therefore, R cannot be co-administered with protease inhibitors (except ritonavir).[56] Although R interacts with non-nucleoside reverse transcriptase inhibitors, plasma levels of these drugs may exceed 50% of the inhibitory concentration (IC_{50}) against the virus, despite the reduction.[57-60] There are favourable data supporting the concurrent use of R and nevirapine, but most experience has been accumulated with the use of efavirenz. In fact, administered at normal doses (600mg/day), efavirenz is the drug of choice for treating HIV-infected patients who should also receive R. There is no interaction between R and nucleoside analogues or fusion inhibitors (enfuvirtide). The pharmacokinetic interactions with new families of antiretrovirals (CCR5 coreceptor antagonists, integrase inhibitors) have been studied. According to these studies, they can be co-administered with R doubling the standard dose of both maraviroc and raltegravir. However, clinical data supporting this recommendation is not available.

It is currently recommended that treatment of TB in HIV-infected patients needing antiretroviral treatment should be done with the standard HRZE regime, provided that two nucleoside analogues are administered in combination with efavirenz, nevirapine or enfuvirtide (full-dose administration of ritonavir is not currently a practical option). If these combinations cannot be used, R can be replaced by rifabutin, whose interaction with protease inhibitors is lower and which can be co-administered with all boosted protease inhibitors, with the appropriate dose adjustment for rifabutin.[61]

Side Effects of the Medication[8,53,54]

The most common side effect is initial gastrointestinal intolerance, which may lead to abandonment or irregular compliance if no measures are taken to resolve it. Usually, it is sufficient to deliver the medication in divided doses for a few days. In other cases, metoclopramide or omeprazole should be taken, at different times from the TB medication to prevent interactions, for a short period to achieve tolerance. In more difficult cases, it is necessary to use intravenous medications, R and H, with E or S. These measures should be as brief as possible, with routine phasing over a few weeks until all medication is taken together, half an hour before breakfast.

Hepatic and skin toxicities are also frequent. Liver toxicity may be caused by H, R or Z. If cholestasis occurs, this is usually caused by R. However, cytolysis may be caused by H and/or Z. The frequency and severity of toxicity increases in the presence of hepatic risk factors, such as abuse of alcohol and other drugs, liver disease, hepatotoxic drug consumption and also with age, although severe forms may occur at any age and at any time of treatment.

Mild toxicity occurs without clinical symptoms and with increased transaminases less than 5 times or 3 times the ALP. Removing the medication is not indicated, however, the frequency of clinical and analytical controls can be increased for early detection of any worsening. The parameters usually return to normal without a change in treatment (fig. 2).

In acute toxicity, associated with clinical hepatitis, with or without jaundice, and an impairment in the analytical results, after ruling out other causes of hepatitis, all medication must be withdrawn for a week or replaced by non-hepatotoxic drugs. After clinical and analytical improvement, treatment with a standard first-line drug is gradually reintroduced. The drug responsible is identified and replaced with a non-hepatotoxic alternative.

Mild skin reactions with the characteristics of acne, rash or urticaria are common, especially during the first month. They usually require no treatment or a topical cure or antihistamines with remission after a few weeks. On rare occasions, generalised hypersensitivity reactions require temporary removal and replacement of drugs and the taking of steroids.

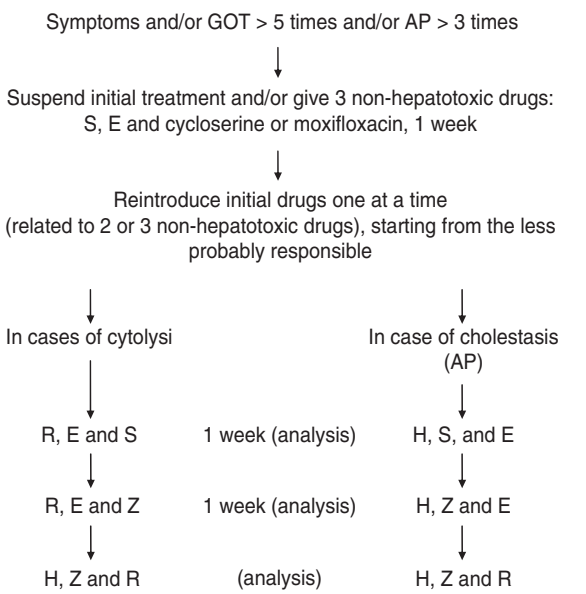
Another complication is the emergence of persistent fever due to the drugs. After excluding other causes, the causative drug must be determined and replaced. Sometimes it may be necessary to temporarily introduce a corticosteroid regime with successive dosage reductions.

Less common is polyneuritis due to H, especially in the groups mentioned above.

Other less common side effects are thrombocytopoenia, haemolysis and acute renal failure due to R, arthralgia or photosensitivity due to Z and optic neuritis due to E.

In all successive medication changes required to overcome iatrogenic effects, a 3-drug rule must be strictly followed at least in the initial treatment phase, with two drugs in the continuation phase, thereby preventing monotherapy leading to possible mutation resistance. It is advisable to have a proper antibiogram to guide drug choice. When R is reintroduced, it should be done gradually over four days, in 150, 300, 450 and 600mg doses, to prevent haemolysis and acute renal failure.

Where it is necessary to suspend or change the standard treatment, it is best to have experience in managing the disease.



E: Ethambutol; AP: alkaline phosphatase; GOT: Glutamyl oxaloacetic transaminase; H: isoniazid, R: rifampin; Z: pyrazinamide.

a) If any step reappears in the clinical symptoms and/or enzymatic elevation, definitely replace the recently used drug by S or E.

b) Rifampicin will always be reintroduced in progressive doses: 150, 300, 450 and 600mg/day (during 4 days) to avoid severe immunological reactions: haemolisis, trombocitopoenia, renal failure.

c) In patients with AIDS use moxifloxacin instead of cycloserin when using non-hepatotoxic trio.

d) If hepatotoxicity appears in the second treatment stage, use non-hepatotoxic treatment E and moxifloxacin.

Figure 2. Hepatotoxicity management algorithm.

Drug Interactions

R strongly induces different cytochrome P-450 enzymes. Other drugs metabolised by this pathway may undergo accelerated catabolism, thereby reducing their blood levels. The association of R with these drugs results in clinically significant interactions. shows the more clinically significant interactions of R. In addition, the effectiveness of R can be decreased by some azoles. H inhibits some isozymes in the P-450 system which at times can lead to significant drug interactions. It is advisable to monitor the levels of phenytoin and carbamazepine when taken together with H.

Non-standard Treatment Patterns

These are mainly indicated in patients with intolerance or resistance to one or more of the drugs. In designing treatments, the following general criteria based on experience should be taken into account: the system used must consist of at least 3-4 effective drugs; if H cannot be given, treatment duration should be at least 12 months; if R cannot be given, it must be extended to 18 months; and for Z, extended to 9 months; if it is not possible to prescribe H and R, treatment should be extended to 18-24 months; in all cases, treatment should continue for a minimum of six months after a negative culture test; before choosing a treatment plan, an antibiogram for first-line drugs and those administered to the patient in previous treatments must be performed. Moreover, it should be noted that in general there are fewer second-line drugs and they are less effective. This can pose serious problems for treatment in patients with severe intolerance or resistance to more than one drug, especially if they include H and R. The proposed regimes are often based on experience and recommendations from experts, as obviously there are insufficient randomised studies to establish the most appropriate. Those used mostly are outlined below.

Table 10
Most significant drug interactions of rifampicin (R)

Drug	Handling
Oral contraceptives	Alternative measures
Haloperidol	
Antiarrhythmics (quinidine, verapamil)	
Beta blockers	
Corticosteroids	
Enalapril, losartan	Clinical monitoring
Benzodiazepines and antidepressants	Increase dose if necessary
Digoxin	
Antiepileptics	Monitor levels
Levothyroxine	Increase dose if necessary
Immunosuppressants (cyclosporine, tacrolimus)	Monitor levels Increase dose if necessary or replace R
Hypolipaedemics	Monitor lipid levels
Sulphonylureas	Increase dose if necessary or insulin therapy
Azoles (ketoconazole, fluconazole)	Separate dose Increase dose if necessary and/or lower R dose
Oral anticoagulants	Monitor INR* Increase dose or substitute low molecular weight heparin
Methadone	Increase dose as required

*Determination of prothrombin time by international normalised ratio (INR).

- Patients with resistance or toxicity to H: this drug should be replaced by E and the treatment extended for a total of 12 months (second phase 10RE).
- Patients with resistance or toxicity to R: isolated resistance to R is rare and requires a longer treatment to be effective. In general, R can be replaced by E and treatment maintained for 18 months (second phase 16HE).[62]
- Patients with resistance or toxicity to Z: The treatment regime of 9 months (2HRE+7HR) was used widely before the introduction of Z. This achieves a similar efficacy to the standard regime of 6 months.
- Patients with multidrug-resistant TB (MDR-TB): As much as possible, drug therapy should be individualised, guided by an antibiogram and taking into account the patient's drug history. The use of an injectable agent is recommended for the first six months and includes a fluoroquinolone for the entire treatment time. If the strain is sensitive to E, this must also be maintained throughout the treatment. The system used must consist of at least four effective drugs, and must last for 18 months after consecutive negative culture results.[63]

Table 11 shows the main second-line drugs effective against *M. tuberculosis*. There are other drugs that have shown some efficacy in the treatment of TB and have been used when there are no other alternatives, such as clofazimine, amoxicillin-clavulanate, thiacetazone and, especially, linezolid.

Treatment Control

Treatment of TB is long, includes several potentially toxic drugs and, to be effective, must be done regularly and without interruption.

Table 11
Second-line drugs in the treatment of tuberculosis

Category	Drug	Dose in adults
Injectables	Amikacin Capreomycin* Kanamycin	 15mg/kg/day
Fluoroquinolones	Moxifloxacin* Levofloxacin Ofloxacin	400mg/24h 500mg/12h 400mg/12h
Others	Cycloserine PAS Protionamide	15–20mg/kg/day (in 2–3 doses) 10–15mg/kg/day (in 2 doses) 8–12g/day (in 2–3 doses)

PAS: para-amino-salicylic acid.
*Preferred drug in each group.

For these reasons, control must be strict as a number of incidents may occur that need to be evaluated and treated by experts.

The most important objectives once a correct treatment is established are: to ensure compliance; evaluate the curative efficacy; and detect and correctly manage any intolerance, interactions and side effects of the drugs.

A treatment must never be indicated without organising a system of regular monitoring by a medical expert or specialist centre in the treatment of TB. In many patients, a proper regime consists of a system of controls at 15 days, one month and 2, 4 and 6 months, with adequate information, proper incentive measures for clinical compliance, as well as monitoring, laboratory, radiological and microbiological controls when deemed necessary.

For patients at particular risk of early abandonment of the medication (the homeless, prisoners, drug addicts, etc.) and in all cases of suspected non-compliance, directly observed therapy (DOT) must be implemented. This is considered very important by international and national disease control organisations for tackling TB in developing countries. DOT consists of taking medication under observation by an appropriate health worker. It can be done in the hospital during admission of a patient, in a health centre that the patient visits daily or at home or the workplace when visited daily by the health worker. Its use must be recommended when non-compliance is predictable or when failure could have a serious impact on the community. Contagious patients who refuse treatment should be placed under a mandatory DOT in a closed centre, ordered by the health authority and ratified by a court, if necessary, taking into account the responsibilities and health and social risks of these exceptional cases.

It is important to record the final outcome of treatment according to internationally established categories in a medical record.[53,55]

Recommendations Regarding TB Treatment

1. In all new TB cases where there is no contraindication for any of the drugs, the regime is HREZ for two months and RH for four months (AI). This is the currently recommended regime in Spain (BIII).
2. The recommended therapeutic regime is the same during pregnancy (BII), breastfeeding (BII), in children (AII), in liver disease and severe chronic renal failure (BIII). It is also the same for extrapulmonary forms, with the exception of meningitis cases (AII), which has the second phase extended for up to 12 months; and spinal TB with neurological affectation (AII) and silicosis (BIII), for a period not exceeding 9 months.

3. The administration of a combination treatment using formulations that prevent single-dose monotherapy are recommended (AIII).
4. The administration of corticosteroids in meningitis (AI) and pericarditis (AI) are recommended. They may also be administered in other locations where there is significant inflammation (CIII).
5. It is recommended that for HIV-infected patients receiving antiretroviral treatment, TB treatment should be the standard HREZ scheme, provided that two nucleoside analogues are administered in combination with efavirenz, nevirapine and enfuvirtide (BIII). If these combinations cannot be used, R can be replaced by rifabutin, which can be co-administered with all the protease inhibitors with proper adjustment.
6. Liver toxicity is one of the major side effects of the standard treatment, as it includes three hepatotoxic drugs (H, R, Z). When changes in transaminases 5 times above the normal value and/or alkaline phosphatase is 3 times above, normal treatment should be discontinued for a week and replaced with treatment using non-hepatotoxic drugs, before gradually reintroducing the initial programme (BIII).
7. Before treatment is started, all drugs currently taken by the patient should be assessed for their interactions with the treatment drugs proposed (AIII).
8. The main indications for non-standard treatment are resistance and intolerance to one or more of the drugs of choice. This should be initiated and monitored by experts, according to the following basic requirements: the system employed must consist of at least 3-4 effective drugs; it must last for a minimum of six months after negative culture result tests; an antibiogram must be performed for first-line drugs and those administered to the patient in previous treatments (AIII).
9. Treatment control is one of the most important activities when tackling TB. Its objectives are to ensure compliance, assess the effectiveness of treatment and check for any side effects. This must be performed strictly and by experienced people. Patients at particular risk of early abandonment of the medication, and all cases suspected of doing so, must be subject to a system of directly observed treatment, DOT (AI).

Treatment of Infection

The treatment of tuberculosis infection (TBI) can begin once the disease has been ruled out. If the clinical situation has required culture tests to be started, it is necessary to wait until they prove negative. The possible infection study should be done with a view to initiating treatment in cases where efficacy has proved better: *a*) recent infection (infected contacts and people with a conversion from TST), *b*) HIV infection; *c*) residual lesions in the chest radiograph and without prior treatment; and *d*) infected patients who have started treatment with anti-TNF-· drugs or transplant candidates.

For close and frequent contacts with a negative TST, the test should be repeated at 8-12 weeks with TBI indicated if there is conversion of the TST. The primary chemoprophylaxis (treatment of uninfected persons) will be indicated in children and adolescent contacts and those infected with HIV as well as the immunocompromised as the most likely to develop severe and rapidly progressive TB. The TST must be repeated at 8-12 weeks[8,18] with the treatment continuing if it is positive and withdrawn if still negative, unless there is a false negative for tuberculin (HIV, severe immunosuppression).

The TBI most studied and with proven efficacy is H.[7] Its efficacy is greater if used for six months or more. Prolonging the treatment over 6 months (to 9 or 12 months) is considered to improve effectiveness, although this has not been clearly demonstrated in clinical practice.[64] Neither has the optimum time for TBI treatment with H in co-infection with HIV,[65] although the usual recommendation is to extend it to 6-9 months. An alternative to the H is four months R treatment, especially when there is resistance to H. Finally, there is the possibility of using R and H for 3 months, with an efficacy similar to H and with better compliance.[66,67]

For multi-drug resistant TB contacts, there is no recommended method with proven efficacy, so it is advisable to maintain monitoring with periodic visits.

Regarding controls to be performed during TBI, the patient must be monitored clinically to completion, noting the side medication effects. Analytical tests are not absolutely necessary, but our recommendation is to perform them before the start of treatment, then monthly, with repeated determinations at the end and whenever there are symptoms. Monitoring must be extended for the onset of symptoms in patients at risk of side effects (liver disease, alcoholism, HIV infection, 3 months postpartum, etc.).[9]

Recommendations Regarding the Treatment of Infection

1. The treatment of tuberculosis infection (TBI) will begin once the disease has been ruled out and in cases where better efficacy has been demonstrated: those recently infected, with HIV infection, with untreated residual radiological lesions, patients who have started treatment with anti-TNF-· drugs and transplant candidates (AIII).
2. The primary chemoprophylaxis is indicated mainly in children under five, teenagers and people with HIV and the immunosuppressed (AIII).
3. The TBI most studied and with proven efficacy is that with H. The standard treatment is considered to be a regime of 6 months (AI). Prolonging the treatment to 9 or 12 months is considered to increase efficiency, although this has not been clearly demonstrated in clinical practice.
4. It is recommended to prolong the treatment to 6-9 months for children, those with HIV and those with residual radiological TB lesions (AII).
5. When H cannot be used, R must be used as an alternative, administered for 4 months (BII).
6. For MDR-TB contacts, there is no standard recommended treatment of proven efficacy, with monitoring considered to be preferable (CIII).

Isolation and Prevention Measures

Measures in Tuberculosis Patients

The nature of the primarily respiratory disease means that, at present, the main measure to break the epidemiological chain of the disease is early diagnosis of patients with pulmonary or laryngeal TB and their isolation until they cease to be contagious. Regarding the latter, it is particularly important to monitor the respiratory isolation measures recommended. These measures are based primarily on

keeping patients in individual isolation rooms, either in hospital or at home. Personal protective equipment (masks, biologically safe respirators) must also be used to prevent the mycobacteria suspension in the patient's respiratory secretions from reaching those of people exposed.[68]

If the patient requires hospitalisation, the importance of a single room is widely recognised. Ideally, there should also be an antechamber with a system of locks to prevent the mycobacteria from the patient's respiratory secretions from escaping to the outside; with the doors permanently closed. It is recommended that these rooms have negative pressure with an air exchange rate of at least six volumes/hr. The extracted air must be filtered through HEPA filters before being pumped to the outside. If this cannot be done, the room should be in a sunny area which is well ventilated. Visitors should be restricted to a minimum, and the need to maintain isolation rules as closely as possible will be explained.

The use of filter systems for the air breathed is a fundamental measure in maintaining respiratory isolation for these patients. Guidelines from various organisations recommend the use by personnel and visitors of N95 biosafe respirators, with a filtering capacity of over 95%. These systems have proved effective in preventing particles of 5–10 μ^3 from entering the lower respiratory tract. Surgical masks are worn only if these devices are not available. When the admitted patients need to leave the isolation area, they must wear a surgical mask while out of the room. It is therefore very important to explain to patients the nature of their illness and the reasons for their isolation, so they collaborate as much as possible with these measures.[68]

All other respiratory isolation measures recommended (using a gown and other universal precautions) are also mandatory, although they are less important than those described above. Of particular importance is material considered to have been in contact with infectious respiratory secretions, as there is the possibility of contracting the disease through contact with contaminated materials.

These measures will apply to every patient admitted to the hospital with suspected TB until the diagnosis is ruled out, especially bacilliferous patients. Very recently[9] it was recommended that isolation be maintained for at least three weeks, with this time being extended as necessary until three negative sputum smear tests are obtained on different days, provided there is also a clinical response to the treatment. The same minimum isolation period is applied if the sputum had disappeared or if the initial sputum smears were negative. As TB no longer persists when a TB patient stops being infectious, it seems prudent not to set deadlines for lifting the isolation, with the ideal situation being the patient not sharing the room during the hospital stay. In any case, if the patient is stable and there is clinical improvement, the need for isolation does not prevent the discharge of such patients, if this is possible.[68]

Preventative Measures for Health Workers and Close Contacts

Health staff working in a facility where TB patients are treated have a risk of being infected of between 2-10 times higher than the general population, depending on the series.[69] It is worth noting that not all hospital units have the same risk, with it being much higher in emergency units and laboratories (in particular, pathology and microbiology), and lower in other units.[69,70] To minimise the risk, personnel must be trained in the respiratory isolation measures described above. Such training must be repeated periodically to ensure awareness of these measures by all staff.

Moreover, to control the contagion and establish measures for the treatment of latent tuberculosis infection, health workers must take yearly TSTs. This period may be shortened for those staff who are particularly exposed to infection (those in emergency departments, respiratory endoscopy, infectious disease wards and microbiology and pathology laboratories). These tests will be repeated while the result is still negative. If conversion occurs, the staff concerned will be studied to rule out the active disease and will be treated with the appropriate protocol depending on their condition (disease or infection).[70,71]

When a case of TB is diagnosed, close contacts of the patient are tested to determine any infection.[18] Of special relevance is TB among children, as most often the focus of infection is found among those living together. The study is currently done via TST and chest radiograph, and must be done systematically for all close contacts of the patient (i.e. those who spend at least 6 hours a day with the patient). This study may be extended to others on the basis of the existence of special contacts (special risk groups, etc) or if the number of conversions among the cohabitants exceeds the percentage expected.[18] If the initial TST is negative, it is repeated 8-12 weeks later to detect infections that may have existed but had not yet become evident in the first study. As a precautionary measure, tuberculin-negative contacts of a patient with TB who continue visits during the hospital stay have to follow the same respiratory isolation rules as health personnel, with special emphasis on keeping the number of visits to a minimum.

Vaccination

The only vaccine available today against TB is BCG vaccine. This vaccine is effective in preventing meningitis TB among children, but its efficacy against pulmonary TB cases is debatable, approximately 50%.[72] One of the drawbacks associated with this vaccine is TST conversion in a variable percentage of the population vaccinated. Although this positive result goes with time in most cases, it can lead to false conversions in studies of subsequent contacts. Because of this, and because the primary prevention strategy of populations with a low TB incidence is based on early detection of infection from patients with the disease, routine vaccination of the paediatric population in Spain is not recommended at this time.

Recommendations Regarding Prevention and Isolation

1. The minimum three-week respiratory isolation rules are applied to patients with pulmonary TB. Bacilliferous cases in this period may be extended until three sputum samples taken on different days are smear-negative (AIII).
2. Health personnel should have a TST at least once a year while the result is negative (BIII).
3. TST studies of close contacts of tuberculosis patients must be done in accordance with the concentric circle principle (BIII).
4. At present, mass vaccination of the paediatric population with the BCG vaccine is not recommended (AII).

Conflicts of Interest

The authors declare they have no conflict of interest.

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