A Tuberculosis Guide for Specialist Physicians

2004

José A. Caminero Luna

International Union Against Tuberculosis and Lung Disease (IUATLD) 68 boulevard Saint Michel, 75006 Paris - France

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Author: J.A. Caminero

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Dedication

To my parents, for their unconditional support, humanity, honesty, hard work, and sacrifice. Their teachings and tolerance have been the best guides in my life.

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Abbreviations

ADA	adenosine deaminase
AIDS	acquired immunodeficiency syndrome
ARI	annual rate of infection
Ak	amikacin
AUC	area under the curve
BCG	bacille Calmette-Guérin
CDC	Centers for Disease Control and Prevention
Cfz	clofazimine
C _{max}	maximum concentration
Ср	capreomycin
Cs	cycloserine
DNA	deoxyribonucleic acid
DOTS	Directly Observed Therapy, Short Course
E	ethambutol
ELISA	enzyme-linked immunosorbent assay
ETH	ethionamide
Η	isoniazid
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency syndrome
IUATLD	International Union Against Tuberculosis and Lung Disease
Kn	kanamycin
LCR	ligase chain reaction
LTBI	latent tuberculous infection
MDR	multidrug resistance
MIC	minimum inhibitory concentration
NTP	National Tuberculosis Control Programme
Ofl	ofloxacin
РАНО	Pan American Health Organization
PAS	para-aminosalicylic acid
PCR	polymerase chain reaction
PPD	purified protein derivative
PPV	positive predictive value
R	rifampicin
RNA	ribonucleic acid

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S	streptomycin
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvate transaminase
Т	thiacetazone
ТВ	tuberculosis
TLC	thin-layer chromatography
LTBI	latent tuberculous infection
T _{max}	time to maximum concentration
TU	tuberculin unit
UN	United Nations
UNICEF	United Nations Children's Fund
UV	ultraviolet
WHO	World Health Organization
Z	pyrazinamide

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Chapter 3. A brief history of tuberculosis

Chapter summary

Much has been speculated about the origin of tuberculosis (TB) in humans. TB is considered by many to be one of the oldest diseases affecting humans. However, despite this age-old relationship, there has been a considerable lack of knowledge about its pathogenesis until very recently. This chapter speculates about the possible origin of *Mycobacterium tuberculosis* and describes the efforts made throughout history to combat the disease. The only known measures for controlling this endemic disease are improving the existing socioeconomic conditions (which can lead to an annual risk reduction in infection of 4-6%) and using effective pharmacotherapeutic measures (which is associated with an additional 7-9% in risk reduction).

Speculations on the origin of Mycobacterium tuberculosis

Tuberculosis (TB) is one of the oldest diseases known to affect humans. The causal microorganism is one of the best examples of how the selection process allows the survival of a species that has been able to adapt to change and adverse conditions. Thus, although Mycobacterium tuberculosis is estimated to have existed for 15,300 to 20,400 years, based on its infrequent loss of nucleotide diversity and its mutation capacity, it is increasingly accepted that the species evolved from other more primitive microorganisms belonging to the same genus, Mycobacterium. It is reasonable to assume that if the majority of organisms belonging to this genus naturally inhabit water and soil, the genus probably originated in a similar environment. Different species have emerged in the course of history, and environmental pressures have conditioned changes in their evolution. Some species of Mycobacterium (e.g., M. ulcerans) are estimated to be 150 million years old, suggesting that the genus is far older than that of the primates, including *Homo sapiens*. It would not be unreasonable to suppose that at some time in the course of evolution, some mycobacterial species, as a result of natural selection, could have established their reservoir in animals. This may have given rise to an early precursor of *M. bovis*, which is considered by many to be the oldest of the

species forming part of the "M. tuberculosis complex", which includes M. tuberculosis, M. bovis, M. africanum, and M. microti. Based on this widely accepted theory, a next step in the evolution of Mycobacterium would be the infection of humans by M. bovis, which coincides with the domestication of cattle. It is at this point where *M. tuberculosis* may have emerged as a human pathogen. Indeed, scientific evidence has shown that in the last few thousand years the bacterium has maintained its ability to adapt to hostile environments, as did its predecessors. Thus, in the last 100 to 150 years M. tuberculosis has gradually flourished in the most vulnerable areas of the planet, where poor living conditions aid the organism's survival and transmission and limited economic resources hinder efforts to combat the disease. Indeed, at the start of the new millennium TB remains the most important infectious disease in the world, and *M. tuberculosis* is the pathogen responsible for the largest number of deaths, with rates that compete with those of human immunodeficiency virus and the agent that causes malaria. Furthermore, although TB can be cured and controlled, it has not been eliminated in many industrialised countries.

History of tuberculosis and the struggle to combat the disease

Despite the fact that TB is a disease of antiquity and it is probably one of the illnesses most dealt with in the literature, there has been surprisingly little sound knowledge of the disease through the course of history, which has not helped contemporary efforts to combat the illness. From the time of Hippocrates (c. 460-377 BC) up until the nineteenth century, the infectious nature of the disease was not even acknowledged; rather, TB was considered a hereditary disorder. However, air-a common vehicle for the transmission of live germs—was included among the interpretations of the possible origin of the disease. For this reason, the dietary regimen proposed by Hippocrates and Galen (c. 130-200 AD) remained the basis of treatment up until the Renaissance. This practice changed very little in the seventeenth century, the sole difference being the recommendation of physical exercise and the use (as with other diseases) of new medicinal substances introduced in Europe at the time, such as quinine, coffee, tea, cocoa, and even tobacco. Such lack of understanding partly explains why humankind has been unable to defend itself against this terrible illness for the most of history—the only option being to fall ill and ultimately die.

Only towards the latter half of the nineteenth century did the infectious nature of TB become apparent, as a result of the studies by Villemin (1865) and, particularly, Robert Koch (1843-1910). Koch was the first to suggest the possibility of controlling this endemic disease, with the presentation of the results of his research (in 1882) that showed that TB was a contagious disease. He not only isolated the bacterium, which was latter named after him (Koch's bacillus), from the sputum of infected patients, but also proposed that the principal measure for controlling the disease in the community would be to isolate affected patients. This suggestion paved the way for the "sanatorium" era of TB, during which prolonged confinement of patients in sanatoriums was believed to be the only effective way to cure TB and control its transmission.

Based on the above, and throughout the prolonged history of the disease, it can be seen that the human host defences were the only means to counter *M. tuberculosis*. In this confrontation between the microorganism and the immune defence system, the latter tended to prevail—as a result of which only a very small proportion of infected individuals eventually developed the disease. However, when the disease became more established, the prognosis became very bleak in most cases—with a mortality of more than 50% five years after the onset of the disease. In turn, 25% of infected patients died within 18 months. Cure was only achieved in 25% to 30% of cases; the rest remaining chronically ill while continuing to spread the disease throughout the community. This extremely poor prognosis led to the development of various treatment attempts, most of which were empirical in nature and which proved to be ineffective. For this reason, when reviewing the history of TB therapy, two major divisions should be established: treatment in the prepharmacotherapeutic era, and treatment in the period corresponding to the last 50 years during which effective cure became possible.

In the eighteenth century, treatment recommendations included moving to the countryside and partaking in moderate activities. There was still special attention to diet, with medication reserved for the initial or "inflammatory" stage of the disease. Thus, during the initial phase, treatment involved bleeding, antiemetic agents, and a light diet, whereas treatment in the "ulcerative" phase of the disease involved balsamic products, expectorants, and opium.

In the early part of the nineteenth century, the practice of bleeding became more common, after the "irritative" doctrine developed by Broussais, who introduced the use of leeches as therapy for TB in the first third of that century. Some were opposed to this practice, including Laënnec, on the grounds that bleeding neither prevented the formation of tubercles nor eliminated them once they had developed. The debate over what constituted appropriate treatment continued over the subsequent years, during which the notion of the disease being associated with "impure air" regained popularity. Thus, climate, exercise, and diet were again regarded as fundamental to TB therapy. Accordingly, patients were sent to places where they could exercise outdoors while observing a 'proper' diet and medication regimen, all under strict medical supervision.

In this way, sanatoriums for patients with TB were created and became the standard treatment in all rich countries during the second half of the nineteenth century and the first half of the twentieth century. Indeed, the level of health care in a country could be determined by the number of sanatoriums it possessed. This emphasis on sanatoriums was reinforced by physiologists at the time, who considered the disease to be a consequence of the inability of the heart to drive blood through the lungs, which supposedly favoured the growth of tubercles. For this reason, sanatoriums were constructed at high altitudes, where a reduction in atmospheric pressure was believed to increase cardiac function and, consequently, improve pulmonary circulation. These theories had epidemiological support, which suggested that communities living at high altitudes were less likely to suffer from TB.

What, however, were the actual treatment success rates in these sanatoriums? Possibly the best records are those published by Sabourin in 1913, which were based on the results of 20 years of experience with 1200 patients treated in the sanatorium in Durtol, France. The rate of complete healing was 39%, a figure very similar to that observed when TB is allowed to evolve on its own—thus questioning the efficacy of sanatoriums. However, the rate of healing reached 71% among patients in the early stages of the disease, as compared with 7.5% among the rest of patients. This difference clearly pointed to the importance of an early diagnosis and treatment, which are the same considerations in TB control today.

Another important period in the history of TB management involved the use of different surgical procedures to heal the disease. As early as the second century, Galen pointed out that the main difficulty in healing lung ulcerations in TB was the impossibility of keeping the lung at rest because of continuous breathing movements. Isolated observations indicated that when a lung collapsed spontaneously during the course of the disease, the disease proved easier to heal. Consequently, it was proposed to collapse the lung to allow it to rest and thus aid healing. This theory led to the development of surgical procedures such as chondrotomy of the first rib; thoracoplasty (removal of ribs to ensure pulmonary collapse); resection surgery; sectioning of the phrenic nerve to achieve diaphragmatic paralysis; scalenotomy (sectioning of the scalene muscles inserting in the first rib); extrapleural pneumolysis (separation of the lung and both pleural layers); filling the extrapleural space with substances such as abdominal fat, paraffin, air, polyethylene sponges, Lucite pellets, ox spleen capsules, or wax; and, especially, pneumothorax induction. The last approach marked the beginning of the surgical era in the treatment of TB and was the most widely used methodology from the late nineteenth century to well into the mid-twentieth century.

There have been no adequate studies allowing an in-depth evaluation of the impact of these invasive techniques on patient healing. What is clear is that they were associated with high morbidity and mortality. Moreover, general anaesthesia was in its infancy at the time, and most of these procedures were carried out using local anaesthesia. The healing rate probably did not exceed 40%—this being the figure recorded for thoracoplasty up until 1927, with an associated mortality of 16%. As with the non-surgical treatments, this rate of healing was only slightly higher than that associated with untreated disease.

Therapy for TB changed dramatically with the introduction of antibiotics for the management of infectious diseases. In fact, each newly introduced antibiotic was tested against TB—the main health care problem in the world at the time. Sulphanilamide was the first sulpha drug (in 1938) to be used against TB. However, this agent was found to be ineffective in humans, although it had an inhibitory effect on TB in guinea pigs. A similar lack of efficacy was observed with more complex sulpha agents such as promanide (1943) and penicillin, which Alexander Fleming began to use in clinical practice in 1941. All this changed with the introduction of streptomycin by Waksman and Schatz in 1943, which has been used against TB since 1944. Since then, other effective antituberculous agents have been developed, leading to TB finally becoming a treatable disease in the mid-1950s.

History of tuberculosis control

It should be pointed out that even before these advances in TB treatment, TB had started to come under control in the richer countries without the adoption of any specific control measures. In effect, improvements in the socioeconomic conditions in developed countries since the eighteenth century had started to have a slight effect on curbing the spread of the disease, with a reduction in the annual mortality rate of 4% to 6%.

It has not been possible to demonstrate whether patient isolation in sanatoriums had an impact on TB control or whether it contributed to the annual reduction in disease mortality attributed to improved living conditions. In reality, few patients had access to such centres and there was a lack of effective therapy, which supports the suggestion that sanatoriums did not have an important epidemiological effect on the evolution of TB. Likewise, the surgical procedures developed to treat TB most likely had little impact on the disease. Such treatments may have benefited a select few; most patients continued to spread the disease in the community for a long time. Thus, it is likely that such procedures afforded little more than what was offered by the improvement in socioeconomic conditions of the time.

The discovery of streptomycin and the beginning of the chemotherapeutic era of TB management was undoubtedly the most important advance in the struggle against the disease, not only for the individual, but also for the community. By adopting these measures alone, countries were able to lower the annual risk of infection by 7% to 9%. Furthermore, by combining chemotherapy with improved socioeconomic conditions, steady annual reductions in infection risk of 12% to 14% could be achieved. This has been the situation in the majority of developed countries in the past 30 to 40 years. In contrast, poorer regions have not seen these improvements, and consequently the gap between developed and developing countries in relation to the disease has widened.

Recommended reading

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Chapter 1. Guide rationale. The need for cooperation between tuberculosis control programmes and specialist physicians

Chapter summary

At the start of the new millennium, tuberculosis (TB) remains the most important infectious disease in the world despite efforts in the past decade to bring the problem under control. This dire situation led the World Health Organization in 1993 to declare TB a worldwide health emergency and to recommend the intensification of combined efforts against the disease, in the form of the Directly Observed Therapy, Short Course (DOTS) strategy. However, the implementation of this strategy faces an important series of limitations that, while similar in many parts of the world, differ among regions or countries. For instance, in low-income countries, where 65% of all TB cases are found, the main limitations are a deficient health care infrastructure and extreme poverty. In contrast, in middle-income countries, where 30% of TB cases are found, the problems are more of an organisational nature, such as the lack of cooperation and coordination among the different health care structures in the country, as well as the strong presence of a private health care system, which gives specialist physicians and health care groups little incentive to follow the guidelines of a National Tuberculosis Control Programme (NTP). In these countries—the majority of which have numerous universities, medical schools, and health care institutions—it is important to ensure that these specialist physicians (many of whom are in private practice) become involved with the programme, particularly since they may offer certain expertise. To this effect, it is necessary to: 1) identify and address the reasons for the lack of integration of these professionals into the NTPs; 2) define the level of participation of these professionals and the contributions they may make to the NTPs; and 3) work with this group of professionals, particularly to improve the efficacy of the programme. It is the purpose of this Guide to help achieve these aims.

Introduction

At the start of the new millennium, tuberculosis (TB) is still the most important infectious disease in the world despite efforts in the past decade to bring the problem under control. The discouraging statistics on mortality and morbidity associated with a disease for which there has been a treatment for over 40 years and that has been preventable for several decades suggest that efforts to control TB have not been optimal. In 1999, the World Health Organization (WHO) estimated that there were 8,400,000 new cases of TB worldwide—a figure very similar to that reported in 1993, when TB was declared a global health emergency and subsequent recommendations to intensify collaborative efforts against the disease led to the development of the Directly Observed Therapy, Short Course (DOTS) strategy. The use of these measures has increased steadily, and as early as 1999 it was reported that 23% of all recorded cases of positive smear microscopy had been studied and treated according to DOTS guidelines. Moreover, the DOTS strategy has begun to be implemented, albeit in various degrees, in 127 countries (60%) throughout the world, including the 22 countries with the highest prevalence of TB (an estimated 80% of all new cases).

The five basic measures of the DOTS programme involve the implementation of a series of simple but strict strategies at the most peripheral levels of health care (see Chapter 12). However, the implementation of this strategy faces a series of obstacles that, although similar in many parts of the world, differ by region and country. For example, in low-income countries, where 65% of all cases of TB are found, the main limitations for implementing the DOTS strategy are an inadequate health care infrastructure and extreme poverty. In such cases, additional resource allocations are necessary if the strategy is to be successful. On the other hand, in middle-income countries, where approximately 30% of all TB cases are found, the problems are more of an organisational nature: the lack of adequate cooperation and coordination between the different health care structures in the country, and the strong presence of a private health care system with specialist physicians and health care groups that are little inclined to follow the strict protocols of the programme. In these countries, the majority of which have numerous universities, medical schools, and health care institutions, it is important to ensure that specialist physicians (many of whom are in private practice) become involved with the programme, possibly by serving in a capacity that takes advantage of their expertise.

Thus, even though TB control is improving worldwide, there are concerns affecting certain middle-income countries, which will become a problem in many poorer countries in the near future. One priority is to ensure that physicians in private practice (mostly specialist physicians) become involved in the National Tuberculosis Control Programmes (NTPs). The present Guide will examine the reasons for the lack of integration of specialist physicians in these NTPs, and suggest ways of improving their participation.

Extent of the problem of tuberculosis in middle-income countries

In the past decade, most of the efforts to control TB have been in lowincome countries, where the prevalence of the disease has been by far the highest. However, despite the slow improvement in TB control worldwide, 30% of all cases are reported in middle-income countries. In 1997, 58 countries comprised the middle-income group-from Lesotho at the lower end to Greece at the upper end. According to WHO data published in 1999, these countries reported a total of 945,335 cases of TB in 1997, or 28.3% of the total number of new cases reported that year to the WHO. This percentage of new TB cases was similar (at around 28%) in 1998 and 1999. Of the 22 countries with the largest TB burden in 1997, seven were middle-income nations: Indonesia, the Philippines, South Africa, Russia, Brazil, Burma, and Peru. These seven countries accounted for 1,395,000 new cases in 1997, equivalent to 22% of cases from the 22 countries with the greatest TB problems in the world (6,361,000 cases in 1997) or 19% of the global total in that year (7,963,000 cases). Again, these figures were similar in 1998 and 1999. In North and South America-with the exception of the United States, Canada, Haiti, Nicaragua, and Honduras (the last three countries classified as low income)-the remaining middleincome countries reported a total of 214,342 new TB cases in 1997 (6.36% of the world's total). In most of these middle-income countries, the existing health care infrastructure is adequate, and there are economical means of implementing the basic intervention strategies. The problem often lies in the above-mentioned organisational concerns, such as the management and integration of university and specialist physician groups, which have been overlooked in the intervention actions carried out to date.

Reasons for the lack of integration of specialist physicians in tuberculosis control programmes

TB is an infectious disease. Each case not only affects a certain individual, but also places the community at risk of infection. For this reason, measures are also needed to control the transmission of the disease. The best way to arrest TB transmission is to establish a diagnosis as early as possible, and particularly to ensure complete cure. However, although treatment may be relatively easy, cure is difficult; therapy is prolonged (6-8 months), lasting for several months after the symptoms are gone. Thus, it is essential that patients strictly adhere to the prescribed treatment until cure is complete. Public health authorities in each country must assume this responsibility of monitoring patient compliance in order to ensure cure.

The implementation of a series of basic measures to control TB in the community is the objective of all NTPs. Since TB can affect all strata of society, an NTP must include all health care centres, including those at the most peripheral levels, where facilities are limited and personnel may lack specific skills. This means that the protocols must be very elementary in order to facilitate their implementation even when resources are severely limited. These basic measures-detection of respiratory symptoms, performance of serial smear microscopy in suspected cases, implementation of a single therapeutic scheme, follow-up of therapy until complete cure is achieved, and use of an elementary information/registry system-must be followed by all health care personnel, regardless of their level of training. However, it has been observed that the more specialised the staff, the more likely the lack of adherence to the NTP guidelines, and consequently the more difficult it becomes to ensure cooperation with the NTP. In short, the most qualified segment of the health care system, consisting largely of specialist physicians, has become a genuine obstacle in the course of NTPs in many low- and middle-income countries.

This lack of integration of specialist physicians can be attributed to two reasons: the characteristics of this professional group and the programme guidelines. Specialist physicians—particularly pneumologists, internists, infectious disease specialists, and paediatricians—who often possess solid training in the pathogenesis, diagnosis, and treatment of TB, are very reluctant to accept the strict and simplistic action plans, which were formulated as such to ensure that they could be carried out with the minimum of resources by individuals with limited training. These specialists must be made aware that although the knowledge of the pathogenesis, diagnosis, and treatment of the disease may be complex, the actual measures required for effective TB control are much simpler. In low- and middle-income countries, 70% of all diagnosed cases involve a positive smear microscopy, a rate that is higher in poorer countries with a worse epidemiological situation. All patients can be treated with perfectly standardised regimens that are well tolerated and easy to implement, even in the most peripheral areas. Smear microscopy is extremely simple and inexpensive to perform in any setting. For this reason, most patients with TB, including almost all of the most contagious cases, can be treated in any setting, as long as there is access to a conventional microscope and the basic antituberculous drugs are available.

The lack of specialist integration in these programmes is also partly related to the fact that NTPs have not been overly concerned about including this group of professionals in their activities. Indeed, the potential usefulness of contributions by specialists has been overlooked. Many specialist physicians are unaware of the NTP norms manual, often because the managerial body of the NTP did not contact them. Consequently, an opportunity for dissemination of the basic guidelines of the programme to this important group of physicians is being lost.

The role of specialist physicians in an NTP

Specialist physicians can play a fundamental role in many aspects of an NTP, particularly at the higher levels. All Central Units of an NTP should have a pneumologist or a specialist in a clinical area closely related to TB, an internist, an infectious disease specialist, or a paediatrician. This professional can work either full or part time for the programme, serving as its clinical authority. The work of the specialist would be fundamental to the preparation of the NTP norms manual, the training of personnel at all levels of the health care system, and the planning of health study programmes in universities and medical schools. The specialist would also serve as the chief authority for all cases involving diagnostic and treatment complications, particularly with regards to patients infected with multidrug-resistant strains. The specialist may be called upon to procure second-line drugs (these substances being costly and difficult to obtain) and to ensure that these drugs are dispensed responsibly throughout the country.

An important requirement is the establishment of a Central Unit Advisory Commission for the NTP, serving in a consulting capacity, which should meet every 1 to 3 months according to the phase and needs of the programme. The Advisory Commission should preferably include a member of each of the medical specialties related to TB, including a pneumologist, a microbiologist, an infectious disease specialist, an internist, a paediatrician, an epidemiologist, a nurse and/or auxiliary nursing staff member, and a social worker. This structure serves to ensure increased representation. Where possible, each of these individuals should be chosen by the respective scientific societies or professional colleges. This Advisory Commission will have a central role in preparing the NTP norms manual, designing the training model for health care workers in the country, planning the teaching curriculum in medical schools and universities, and preparing the action guidelines.

All regional units of the NTP should also have a clinical specialist, even if working only part time or as a consultant. This professional will be the regional specialist for the programme and will be in charge of coordinating the actions of the different specialist physicians in relation to diagnostic difficulties (e.g., performance of punctures, bronchoscopy, pleural biopsies) and treatment problems (e.g., drug intolerances, treatments in special situations, re-treatment). The regional specialist will also be involved in training personnel at the regional and peripheral levels. This specialist will preferably work in the hospital setting, where the more difficult cases are seen. This physician must therefore be completely familiar with the norms of the NTP, the performance of the different available diagnostic techniques, and the potential therapeutic options in situations of treatment difficulty. The integration of these specialist physicians in the actions of the NTP at the regional level is therefore critical.

At the peripheral level, the NTP does not require the intervention of specialist physicians, at least in the institutional sector. As has been commented above, the main functions at the peripheral level include the diagnosis of cases with positive smear microscopy and the supervision of treatment. Such activities can easily be carried out by less qualified personnel. Nevertheless, if specialist physicians are found working at the peripheral level, they must adhere to the NTP guidelines regarding the principal functions described above. The same applies to specialist physicians working in the private sector, who preferably should refer diagnosed cases to the NTP, which, in addition to providing free medication and diagnostic evaluations, can also ensure close monitoring of patients for proper adherence to the patient, he or she may conduct the required clinical controls throughout the course of therapy. Here again,

problems are frequently found among specialist physicians working at the peripheral level, owing to a lack of adherence to the NTP guidelines.

Chapter 2. Importance of the role of specialist physicians and their integration in the strategies of a tuberculosis control programme

Chapter summary

Having acknowledged the important role of specialist physicians in the activities contemplated by National Tuberculosis Control Programmes (NTPs), and particularly the obstacle such professionals may represent if they are not successfully integrated, attention should now focus on the need to implement concrete strategies for incorporating these specialist physicians in the work of an NTP. This task involves the development of specific training plans adapted to their level of expertise.

It is important to recognise that these professionals have long been excluded from the training strategies of NTPs, strategies that are key to ensuring the future and sustainability of a tuberculosis (TB) control plan. However, this group of highly qualified professionals cannot be dealt with using the same simple training terms as those designed for personnel working at the peripheral levels. Owing to the complexity of elaborating a training strategy specifically for specialist physicians, and the difficulty of finding qualified personnel to implement the strategy, NTPs have largely neglected the need to integrate and train specialist physicians.

Specialised physicians with extensive knowledge in the clinical practice associated with TB, and with sufficient operational knowledge regarding control of the disease in the community, should carry out the training of this important professional sector. In this way, specialist physicians can gain both up-to-date information on the subject and practical knowledge that can be applied to the specific problems in their respective countries. Training should be an interactive process in which specialists are free to voice their opinions. Through active dialogue, agreements can be reached regarding the activities of this professional sector, whose role is to support the endeavours of the NTP.

The above reasoning process led the International Union Against Tuberculosis and Lung Disease and the Pan American Health Organization in 1997 to identify specialist training as one of the priorities in Latin America. A series of intensive 25-hour training modules were thus designed, lasting 3 to 5 days, which have since been implemented in many countries in the region. These measures have resulted in a fundamental change in the level of participation of specialist physicians in the NTPs. The methodology of these modules is described in the present chapter.

Rationale for the operational applicability of current knowledge on tuberculosis

As with all areas of knowledge, it is important to be able to understand what information can be effectively applied in real life and what part is for reasoning or hypothesizing. In the field of tuberculosis (TB), it is important to be able to determine what information about the disease is relevant to, and useful in, the control of infection in the community.

TB is most likely the disease that has given rise to the most publications and scientific studies in the history of medicine. However, the great majority of such publications come from wealthier, industrialised countries where TB is not a major problem, and consequently their research findings often have little relevance in those parts of the world where TB is a genuine problem. Such literature, which is read mostly by specialist physicians, may lead their readers to adopt diagnostic and therapeutic attitudes that are different from those suggested by the National Tuberculosis Control Programmes (NTPs). Therefore, these physicians tend not to follow or contribute to the programme's information and registry system.

It should be noted that the main problem of controlling TB has little to do with having profound knowledge of the disease. As explained in Chapter 1, the present focus is on curing patients diagnosed by smear microscopy and detecting as many cases as possible with the least possible delay. The success of such efforts depends on the availability of drugs, the identification of most respiratory symptomatic cases with smear microscopy evaluation, and treatment that is supervised by health care personnel.

Provided sufficient medication is available, with acceptable distribution of smear microscopy laboratories—concerns of the NTP that are not a problem in most middle-income countries—the objective of effective TB control can be achieved by decentralising diagnosis and treatment routes. Only by taking diagnosis and treatment as close to the periphery of the health care system as possible can the objectives of case detection and cure rates be met. This is why the protocols must be as elementary as possible, since many patients will not be cared for by nurses or clinical auxiliary personnel.

TB control at the peripheral level requires the availability of personnel who know that any person with a cough and/or expectoration for more than 2 weeks should be subjected to serial smear microscopy, and that all individuals with a diagnosis of TB must be treated and followed for adherence to treatment until cure is complete. Any deviation from these norms will jeopardise the goals of a TB control plan.

Specialist physicians must be made aware that they should avoid underutilisation of their expertise when dealing with the diagnosis and treatment of cases with positive smear microscopy, unless these patients have complications or pose diagnostic or management difficulties. The hospitalisation of uncomplicated TB cases as a result of management by specialist physicians is a common problem in many countries, one that is associated with increased costs and losses in efficacy. Often, once the patients have been discharged, they encounter poor access to smear microscopy laboratories and difficulties in the direct supervision of their treatment. However, specialists may and should play an active role in TB control in their countries, although, as was explained in Chapter 1, such participation should take place at higher levels. Furthermore, their responsibilities must be approved by the NTP management body, which often comprises epidemiologists and health care specialists with a sound understanding of the measures required to control TB in the community.

Although the points discussed above seem reasonable, very little effort has been made to ensure that they are understood and accepted by specialist physicians. Frequently, specialist physicians do not even know what such disease control programmes are, or why the NTP defines guidelines that seem so basic to them. Much effort is therefore required to educate these physicians so as to facilitate their participation in the plans contemplated by an NTP.

The need for specific training among specialist physicians

One of the fundamental tasks of all NTPs is continuous supervision and training. This is the only way to ensure that the personnel participating in the plan are well trained and that their skills remain up-to-date. Much of the aid supplied by rich countries to nations with fewer resources is for the training of personnel. However, when the managing bodies of NTPs develop plans for the training of health care personnel, their efforts almost exclusively focus on the peripheral levels of medical care, which is a reasonable action considering that these are the areas where the main activities of the TB control plan take place. The regional or national

director of the programme may undertake training of personnel at the peripheral levels. However, the more specialised professionals have almost always been excluded from the instruction process, based on the false assumption that training in their case is much more difficult.

It is true that in the case of specialists it is not always possible to implement the standard training measures of simply teaching the basic rules of the Directly Observed Therapy, Short Course (DOTS) strategy, since such messages appear so simple to specialist physicians that they tend to ignore them. Also, the NTP management bodies often do not have the resources to train these professionals, a situation that results in a lack of consensus between specialists and the NTP about standard interventions in TB control. If this pool of professionals is small in size (as in low-income countries), they will have little influence on the results obtained by the NTP, whereas if there is a reasonable number of specialist physicians, some of whom working at the middle or peripheral levels of the health care system (as is often the case in middle-income countries), then their influence will be greater and more likely to affect the results of the programme. This group of specialist physicians therefore needs to undergo specific instructional measures to ensure that their knowledge is current and applicable to the problems in their respective countries.

Such specific training is all the more important considering that most specialists, such as pneumologists, internists, and paediatricians, from lowand middle-income countries do not undergo their postgraduate/specialist training in their own countries, but do so in developed countries where the health care and economic conditions are most likely very different. Consequently, the training of such specialists may not be applicable in their native countries where completely different approaches to addressing the problem of TB are often required.

Experience of the IUATLD in the specific training of specialist physicians

Having recognised the importance of these specialists in the actions contemplated by an NTP, and the obstacles they may inadvertently pose, the training of these professionals should be considered a priority in many low- and middle-income countries. For this reason, and because the great majority of Latin American countries meet the expressed requirements, in 1997 the International Union Against Tuberculosis and Lung Disease (IUATLD) and the Pan American Health Organization (PAHO) identified specialist training as one of the priorities in the region. It was concluded that the best way to engage this group of physicians would be to provide high-quality, specific training conducted in close cooperation with the Central Units of the NTP and the different scientific societies in each country. A series of intensive 25-hour training modules were thus designed to be conducted over the course of 3 to 5 days, with the length of the course depending on the availability of the physicians, since many physicians are in private practice and have tight schedules.

These courses or modules, titled "Importance of the actions of specialist physicians and their adequate integration in the strategies of an NTP", have been progressively adopted in the different countries throughout the region. The target audience comprises a select group of pneumologists, specialists in infectious disease, internists, paediatricians, and other specialists, as well as supervisors of the Central Units of the NTP. The course enrolment is deliberately kept small so as to facilitate interaction between instructor and trainee.

The topics covered during these training sessions are presented in Table 1. Information on each topic is updated and supported by sound scientific evidence, and sections that have practical application are detailed. There is special emphasis on the role of specialist physicians, so as to elicit their maximum participation and to ensure their best possible integration in the actions of the NTP. For each topic, 60% of the time is dedicated to providing up-to-date information on the subject, whereas another 30% is dedicated to discussing what part of the information has practical application in the field. The remaining 10% of the time is used to reach specific agreements among the participants on courses of action regarding each of the topics. As a special rule, the next topic is not dealt with until the entire group agrees on the issues presented on each subject and signs off on agreements on lines of action. These courses are
conducted by specialist physicians with extensive knowledge on the clinical practice of TB and who have been adequately instructed about the practical control of the disease in the community.

If the course takes place over 3 days, as is often the case, then the first day is dedicated to the epidemiology and pathogenesis of the disease; the second day to the diagnosis, detection, and treatment of cases, including resistance to antituberculous drugs; and the third day to TB control, NTP management, and other relevant topics, such as TB in children, extrapulmonary TB, and mycobacteriosis. In addition, each participant receives extensive literature, including the most relevant studies on the topics covered in the course. The literature is in Spanish, since the courses are given in Spanish-speaking countries. The reading package comprises at least 70 articles classified by topic, together with the most relevant TB guides published by the IUATLD and WHO/PAHO. At the end of each course, a set of collaboration agreements is drawn up to facilitate the integration of these specialist physicians in the activities of the programme. These agreements are in compliance with the NTP rules, and it is hoped that the participants will follow them.

A total of 21 courses have been conducted up until late 2002: five in Mexico, three in El Salvador, two in Honduras, two in Guatemala, two in Nicaragua, one in the Dominican Republic, two in Peru, one in Bolivia, one in Costa Rica, and two in Ecuador. In all these countries there has been a marked improvement in the integration of specialist physicians in the respective NTPs—although this improvement is somewhat difficult to quantify with objective parameters since it is based only on the observations of the national supervisors of the control programmes. Still, these supervisors have been very satisfied with the training model, and almost complete integration of these specialists in the NTP has been achieved in all the participating countries.

The activities described here should be viewed as the first specific intervention relating to TB control for this group of specialist physicians. Such interventions can be further improved in the future.

Table 1. General programme of the training courses for specialised health care professionals of the IUATLD.

- 1. Current situation of TB in the country. Implementation and results of the DOTS strategy.
- Rationale for the course. Role of the specialist in the activities of the NTP. Action levels of the specialist in the context of an NTP.
- 3. The natural history of TB.
- 4. Etiologic agent and transmission epidemiology. Factors associated with transmission.
- 5. Epidemiology of TB. Epidemic wave phenomena in TB. Principal TB evaluation indicators.
- 6. Assessment of the state of TB infection in the community. Utility of, and difficulties associated with, tuberculin evaluations.
- 7. Current situation of TB in Latin America and the rest of the world.
- 8. Future perspectives on the situation of TB in the world. Impact of poverty, migratory patterns, and HIV infection.
- 9. TB immunology. Pathogenesis of TB. Infection and disease.
- 10. Tuberculin testing. Standardisation and interpretation of results.
- 11. Clinical manifestations of TB. Symptoms and signs according to location.
- 12. Radiology and other imaging techniques in the diagnosis of TB.
- Conventional diagnostic methods. Importance of collection of samples. Smear microscopy and culture. Priorities and handling in the context of an NTP.
- 14. New diagnostic methods for TB. Applicability in low- and middle-income countries.
- Treatment of TB. Bacterial populations. Rationale of the initial treatment scheme. Special situations in TB therapy.
- 16. First- and second-line antituberculous drugs. Mechanisms of action and side effects.
- 17. Basic concepts for planning re-treatment. Re-treatment regimens.
- Side effects of antituberculous drugs. Adverse reactions to antituberculous drugs. Role of the specialist.
- 19. Drug resistance to antituberculous drugs. Detection methods. Basis and genetic markers of resistance.
- 20. Epidemiology and the current situation of drug resistance worldwide. Strategies for avoiding the development of drug resistance.
- Extrapulmonary TB. Clinical and epidemiological importance. Role of the specialist.
- 22. TB in childhood.

Differences with respect to adult TB.

- 23. Diseases caused by environmental mycobacteria. Epidemiology, pathogenesis, diagnostic methods, and therapeutic regimens.
- 24. Basic measures for TB control in the community.
- 25. Strategies for increasing cure rates.
- 26. Strategies for increasing case detection. Development of a national TB laboratory network. Quality control.
- 27. Measures for preventing TB.
- 28. Chemoprophylaxis.
- 29. BCG vaccination.
- 30. Importance of training, supervision, and evaluation.
- 31. Elaboration and management of a registry and information system within the context of an NTP.
- 32. Drug provision with adequate quality control.
- 33. Health education and community participation.
- 34. Elaboration of an infrastructure for adequate NTP functioning. Role of specialised care.
- Conclusions of the course. Collaboration agreements. Development of possible work projects integrated within the NTP.

BCG = bacille Calmette-Guérin; DOTS = Directly Observed Therapy, Short Course; HIV = human immunodeficiency virus; IUATLD = International Union Against Tuberculosis and Lung Disease; NTP = National Tuberculosis Control Programme; TB = tuberculosis.

Chapter 4. Epidemiology of tuberculosis

Chapter summary

The study of the epidemiology of tuberculosis (TB) should comprise two major parts: the epidemiological chain of transmission, and the detailed analysis of the state of the epidemic in the world and the factors influencing it. The epidemiological transmission of TB requires a causal agent (the *M. tuberculosis* complex), a reservoir or host (infected healthy and/or diseased human) capable of infecting others (diseased human), a transmission mechanism (airborne), and a susceptible host.

With regards to the state of the epidemic and its evolution, three parameters—mortality, morbidity, and infection—may be quantified by following the new cases (i.e., the incidence of the disease) reported in the population every year. The World Health Organization estimates that there were 8,417,000 new cases in 1999 (global rate 141/100,000), of which 3,724,000 corresponded to smear-positive carriers (rate 62/100,000). Eighty percent of all these cases were from 23 countries.

It is clear that a series of factors or interventions are able to influence the course of this endemic disease. Of these factors, only improvement in socioeconomic conditions (affording an annual reduction in infection risk of 4-6%) and adequately administered anti-TB therapy (offering an additional annual risk reduction of 7-9%) have been shown to halt the progression of TB. In contrast, poverty, HIV, massive immigration from highly endemic zones, the non-existence or ineffective establishment of National Tuberculosis Control Programmes, and demographic growth increase the prevalence of TB in many parts of the world.

The epidemiological chain

Section summary

M. tuberculosis is a bacillary-shaped (i.e., rod-like) microorganism and a strict aerobe. Its growth depends on the presence of oxygen and the surrounding pH level. It is highly resistant to cold, freezing, and drying, and is very sensitive to heat, sunlight, and ultraviolet radiation. Under adverse metabolic conditions, the bacterium enters a latent or dormant state, and multiplication can be postponed from days to many years. These characteristics have clearly helped the microorganism to survive in humans.

Humans (either healthy infected or diseased) are the main reservoir or host for *M. tuberculosis*, whereas cattle are the main reservoir for *M. bovis* and other animals serve this function for the *M. tuberculosis* complex. The source of infection is almost exclusively represented by the diseased human. In turn, air is the most important route of transmission, and is responsible for almost all cases of infection. In this context, pulmonary TB patients who are smear positive, along with those who cough and those who are not receiving treatment, are the most contagious. A number of conditions increase the likelihood of clinically manifesting the disease in the event of infection. These risk factors are often associated with some form of immune deficiency.

As with most infectious diseases, the tuberculosis (TB) epidemiological chain of transmission requires the existence of: 1) a causal agent capable of bringing about the disease; 2) a reservoir or source of infection where the microorganism is found; 3) a mechanism of transmission; and 4) a susceptible host.

Causal agent

Taxonomically, the causal agents for TB belong to the order *Actinomycetales* and the family *Mycobacteriaceae*. TB is caused by one of the four microorganisms comprising the so-called *M. tuberculosis* complex: *M. tuberculosis, M. bovis, M. africanum,* and *M. microti.* From a health care perspective, TB produced by *M. tuberculosis* is the most important aetiology of TB because it is by far the most common. TB caused by *M. bovis* is less frequent in industrialised countries, owing to the

existing control of TB in livestock and to the widespread pasteurisation of milk. However, *M. bovis* remains an important problem in developing countries. *M. africanum* is in turn responsible for a smaller number of TB cases in Africa, owing possibly to its lesser virulence. Finally, infection due to *M. microti* (the causal agent for TB in rodents) has recently been described in humans, mainly immunosuppressed subjects. The family *Mycobacteriaceae* includes more than 90 additional microorganisms that are found mainly in the environment. They exhibit little pathogenicity, although some can cause disease, particularly in situations of host immunodeficiency. These microorganisms are best described as environmental mycobacteria, or mycobacteria other than *M. tuberculosis*.

Although a single microorganism can cause TB from the moment it gains access to the host, it behaves as a polyvalent germ in the course of its growth. This is because the metabolism of the pathogen is dependent on the variations in oxygen partial pressure and the pH of the infected organ system.

The chemical structure of *M. tuberculosis* comprises proteins, carbohydrates, vitamins belonging to the B complex, and minerals such as phosphorus, magnesium, and calcium. The protein component is the fundamental substrate responsible for delayed hypersensitivity reactions, and rapidly induces the so-called tuberculin reaction. Although this microorganism lacks a cerulean capsule, it contains a considerable amount of mainly complex lipids. Of these, mycolic acid is the most characteristic and is considered to be responsible for the staining properties of the bacterium, which are shared by other species such as the Nocardia and can change according to the age of the microorganism. The lipid-rich wall of the bacterium is also responsible for a number of its biological characteristics, such as resistance to macrophage action and drying. The isolated lipid component is capable of inducing the same host responses as the whole microorganism, including the formation of epithelioid cells and, occasionally, caseation. M. tuberculosis is unable to produce toxins, as a result of which it lacks primary toxicity. Nevertheless, it possesses a very important and complex antigenic component that is responsible for variation in virulence and pathogenic capacity features. This topic will be described in greater detail in Chapter 5.

Another important characteristic of the bacterium is its very slow rate of division (60-fold slower than that of *Staphylococcus*), which explains the lack of specificity in the clinical presentation and the very slow development of the disease. Such slow proliferation also explains why it is not necessary to administer the medication several times a day. The growth of *M. tuberculosis* is conditioned by the presence of oxygen and the surrounding pH level. There also appears to be interdependence between the anatomical distribution of the disease and the oxygen tension available in the area. For example, in TB of the upper lung lobes, where the comparatively lesser blood flow and ventilation induces an increase in alveolar oxygen pressure, the disease tends to spread towards these regions from the post-primary seeding sites. The ideal conditions for proliferation of the bacterium comprise a pH of 7.40 and an oxygen partial pressure of 100 to 140 mm Hg. Nevertheless, even under these conditions multiplication is very slow, occurring once every 14 to 24 hours.

When *M. tuberculosis* does not encounter a favourable environment (i.e., low oxygen pressures and low pH levels), it enters a dormant state, and multiplication can be postponed from days to many years. This latent state is also responsible for the maintenance of the disease.

Lastly, *M. tuberculosis* is a bacillary-shaped (i.e., rod-like) organism. It is highly resistant to cold, freezing, and drying, and very sensitive to heat, sunlight, and ultraviolet radiation.

Source of infection

Humans (healthy and infected or diseased) are the fundamental reservoir or host for *M. tuberculosis*. Healthy infected individuals are one of the main factors that contribute to the perpetuation of TB, since they do not show any signs or symptoms of the disease. For example, a healthy infected population can act as silent carriers of the tubercle bacilli until their death, with only a number of them at some point developing tuberculous disease, especially if they have acquired some form of immune deficiency.

Although humans are the main reservoir for *M. tuberculosis*, cattle are the principal reservoir for *M. bovis*, and practically all animals (including monkeys, dogs, and cats) can also serve as reservoirs for the *M. tuberculosis* complex. However, while the great majority of these animals, particularly pets, can suffer from the disease, they are unable to transmit the illness because their size allows them to carry only relative small bacillary populations.

Only when non-diseased, infected humans develop tuberculous disease do they act as a source of infection. However, their ability to infect others depends on the location and stage of the disease. The most infectious presentations correspond to pulmonary TB, where the host capacity to spread bacteria is greatest. Among such individuals, the potential for contagion is greatest among those with the highest bacterial loads. Such patients show cavities on chest radiographs or have positive smear microscopy results.

Mechanism of transmission

The airborne route is responsible for almost all cases of TB transmission. When speaking, singing, laughing, sneezing, and especially coughing, the infected patient expels microdroplets into the air, which contain the mycobacteria. Although the largest microdroplets (>10 µm in diameter) also contain the largest number of bacteria, they tend to be deposited in the upper airways because of their greater weight, and thus possess a lower potential for infection. Aerosolised droplets measuring 5 to 10 µm reach the more proximal portions of the upper airways of the new host, where the conditions are not optimal for multiplication. However, the microdroplets that measure 1 to 5 µm in diameter and that are formed from the larger droplets as a result of condensation after losing part of their water content typically contain 1 to 5 bacilli per microdroplet. Microdroplets are highly infectious since they can be deposited within the alveolar spaces. A minimum of 10 to 200 of such microdroplets can cause infection. The ideal site of deposition for these microorganisms in the new host is the bestventilated region of the lungs, i.e., the subpleural zone of the lower lobes. It is in this distal portion of the lungs, with the high oxygen partial pressure, that conditions are ideal for multiplication. Initially, the macrophages, followed by the lymphocytes, migrate towards this region, and in the majority of cases are able to arrest microbial multiplication. However, when this initial defence mechanism is impaired, primary TB develops.

There are also other less frequent transmission mechanisms, such as the digestive route, where infection occurs via the pharyngeal or intestinal lymphatics owing to the consumption of beef infected with *M. bovis*. This route is also common in infections associated with *M. avium* in AIDS patients. Other means of transmission include the urogenital route, through urine and sexual transmission; the mucocutaneous route; inoculation; and placental transmission, particularly in cases of maternal miliary TB where the organism ultimately crosses the placental barrier (200-300 cases have been described in the literature to date). This last route of transmission gives rise to what is known as congenital TB.

The potential of a patient to infect others depends on the following factors:

- 1. The extent of disease. Patients with positive smear microscopy and those presenting radiographic evidence of pulmonary cavitations are regarded as highly infectious.
- 2. The severity and frequency of coughing. Infected patients who cough frequently are more contagious, since the microdroplets expelled are smaller.
- 3. The quality and volume of the respiratory secretions. Scantly viscous sputum often constitutes an ideal aerosol vehicle, and is therefore more contagious.
- 4. The antituberculous chemotherapy provided. Patients who receive such therapy are 50 times less infective than those who do not receive therapy. It is generally accepted that a patient is no longer infective after 2 weeks of treatment, although confirmation is not possible until smear microscopy results are negative.
- 5. The characteristics of exposure: i) the concentration of bacilli in the atmosphere, with the greatest transmission potential being found in small, closed rooms where the smear microscopy-positive TB patient spends many hours; ii) room ventilation, with fewer bacilli being found in the air of well-ventilated rooms; and iii) the duration of exposure to the infectious TB patient, where the risk of transmission increases with close and prolonged contact.

The susceptible host: risk factors

Children under the age of 5 years and elderly subjects older than 65 to 70 years are more vulnerable to TB, partly because immunity is slightly reduced at these ages. It is not known, however, why children between the ages of 6 and 14 years are less susceptible.

Worldwide, TB affects men more often than it does women (60-70%). This higher prevalence has been attributed to differences in social habits, although an increasing number of studies are suggesting a slight genetic predisposition on the part of women.

Not all individuals are at the same risk of developing TB disease once infected. Several risk factors have been identified that are known to facilitate the development of the disease. These factors imply a greater or lesser degree of immune deficiency, with up to a 1000-fold increase in the risk of developing TB as compared with normal, immune-competent individuals. These factors and the relative risk of developing active TB versus the normal population are shown in Table 2.

Table	2 .	Risk	factors	associated	with	developing	ΤВ	disease.	
Relative risk with respect to the normal population.									

Risk factor	Relative risk	
HIV infection	50-100	
Jejunocaecal shunt	27-63	
Solid tumours	1-36	
Silicosis	8-34	
Head and neck neoplasms	16	
Haemodialysis	10-15	
Haematological neoplasms	4-15	
Fibrotic lesions	2-14	
Immunosuppressive drugs	2-12	
Haemophilia	9	
Gastrectomy	5	
Low body weight	2-4	
Diabetes mellitus	2-4	
Heavy smoking	2-4	
Normal population	1	

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Analytical parameters of tuberculosis

Section summary

The magnitude and temporal course of TB in a given community can be quantified by three parameters: infection, morbidity, and mortality. Anti-TB treatment introduced on a large scale in the community rapidly reduces mortality. However, mortality is not a good parameter for assessing the magnitude and temporal course of the disease. The best way to assess the TB trend in a community is to follow the new cases detected in the population on a yearly basis, particularly among those involving positive smear microscopy findings. The most interesting parameters of infection are the annual rate of infection (ARI) and the prevalence of infected individuals. The incidence is calculated by the so-called ARI, which describes the percentage of the population that will be infected or reinfected in the course of a year, and is based on a mathematical formula requiring knowledge of the prevalence of tuberculous disease in the community at a given age. Calculation of this parameter therefore depends on the results of community-based tuberculin test surveys (primarily tuberculin skin test conversion rates), which often vary and are associated with another set of difficulties. Estimations of the incidence of the disease derived from ARI results should be interpreted with caution. A much more useful approach is to observe its trends at different time periods.

The extent and time course of TB in a given community can be quantified by three parameters: infection, morbidity, and mortality. Since the introduction of anti-TB therapy, mortality ceased being a parameter by which the evolution of this disease could be followed, since few patients in treatment tended to die of the disease. Hence, although treatment introduced on a large scale in the community rapidly reduces mortality, it does not allow a good assessment of the evolution of the disease. Other measures to ensure complete patient compliance with therapy are necessary. For this reason, when speaking of TB, treatment (which depends on the use of good therapeutic regimens) is not the same as cure (which depends on strict patient compliance). Following the introduction of anti-TB agents in the community, TB can only be accurately quantified by the corresponding morbidity parameters: incidence and prevalence. The best way to assess trends in TB (i.e., disease incidence) in the community is to follow the new cases detected in the population on a yearly basis. This parameter is dependent on accurate notification of cases, and thus on the type of information system used for case reporting by the professionals in charge of diagnosis. Since diagnosis at the peripheral levels of the health care network are based on smear microscopy (discussed in Chapters 7 and 12), the best means of following the evolution of the disease is to register the number of cases and the annual incidence of patients who have positive smear microscopy results. With TB, the incidence rates (new annual cases) and prevalence (cumulative number of cases) are expressed as the number of cases per 100,000 persons.

Despite the above considerations, it should be noted that tuberculous infection reflects the past and present TB burden in a given region and facilitates future prognosis by identifying infected individuals (reservoirs for disease). The most interesting parameters of infection are the incidence or annual rate of infection (ARI) and the prevalence of infected persons. The incidence is calculated by the ARI, which describes the percentage of the population that will be infected or reinfected in the course of a year, and is based on a mathematical formula requiring knowledge of the prevalence of tuberculous disease in the community at a given age. Despite all that has been written about ARI, many factors influence its determination and results. The information derived from this parameter must therefore be evaluated with great caution. A much more valid approach is to observe TB trends in different periods. Utilisation of ARI is not advised until a National Tuberculosis Control Programme has been established and functions effectively, and several years of reliable data on disease incidence, cure rates, and default rates, as well as information on certain basic case detection parameters (see Chapter 12), become available. Thus, estimations of the incidence of the disease and of other TB parameters based on ARI should be avoided.

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The current situation of tuberculosis in the world

Section summary

TB remains the most important infectious disease worldwide. The current global situation of TB reflects the enormous economical and social differences among countries, as can be seen from the fact that 95% of all cases of TB and 98% of all deaths caused by TB are from low-income countries. In 1999, a total of 3,689,822 new cases were reported to the World Health Organization (WHO), although the true figure is estimated to be about 8,417,000 cases (a global rate of 141/100,000), of which 3,724,000 corresponded to smear-positive microscopy cases (62/100,000). In that same year it was estimated that 80% of the TB burden in the world was confined to 23 countries, which accordingly have received priority rating from the WHO.

As has been mentioned earlier, TB remains the most important infectious disease in the world at the start of the new millennium. This ancient endemic disease, which has affected humans for thousands of years, may not only be the worst plague ever suffered by humanity, but today still escapes epidemiological control. Indeed, the global statistics on affected patients and deaths due to TB continue to rise every year. However, in the face of this bleak situation, most industrialised nations consider the disease to have been overcome, and efforts against TB have decreased as a result. In reality, the current situation of TB in the world reflects the enormous economic and social differences among countries. TB will remain a global problem until the disease disappears entirely from the planet.

In 1999, a total of 3,689,822 new cases of TB were reported to the World Health Organization (WHO), which estimated that this figure actually represented less than half of the actual cases in the world. In the great majority of countries, particularly those with low or middle incomes,

case under-detection or under-reporting exceeds 50%, a fact that led the WHO to estimate that in 1999 there was a total of 8,417,000 new cases of TB (a global rate of 141/100,000), of which 3,724,000 corresponded to smear-positive cases (62/100,000). The distribution of these TB cases varies considerably from one region to another (Figure 1). Sixty-three percent of the total case notifications in 1999 were from Asia (41% in Southeast Asia and 22% in the Western Pacific), followed by 17% in sub-Saharan Africa, despite the fact that this was the region of greatest incidence. The Americas in turn reported 6% of the cases recorded for that year, whereas Europe reported 10% and the Mediterranean 4%.

The distribution of TB by countries also differs within the same region. Thus, for a number of years it has been estimated that 80% of the global TB burden is concentrated in 23 countries (Table 3), which have been assigned priority consideration by the WHO. These are the 23 countries with the largest TB figures in absolute terms (i.e., not in cases per 100,000 inhabitants). The fact that they are highly populated also plays a role. Heading this list is India, with an estimated 1,847,000 new cases in 1999. This may be the country with the worst TB problem, and where, historically, the worst approach to antituberculous therapy—private practice—has been adopted.

It is worrisome to analyse the factors that have led to the present dire situation, especially since TB has been a treatable disease for over 40 years and the scientific rationale for its control in the community has been established for the past 30 years. Even more disheartening is the existence of enormous inequalities among different parts of the world. TB remains fully out of control in the great majority of poor countries but has been on the decline in the developed world for over a century. Such a disparate situation reflects the existence of a series of factors that contribute to the progressive spread of this disease or its sustained yearly reductions.

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Country	Population*	No. of cases	Rate per			
	_		100,000			
1. India	998	1,847,000	185			
2. China	1266.8	1,300,000	103			
3. Indonesia	209.2	590,000	282			
4. Nigeria	108.9	327,000	301			
5. Bangladesh	126.9	306,000	241			
6. Pakistan	152.3	269,000	177			
7. The Philippines	74.4	234,000	314			
8. Ethiopia	61	228,000	373			
9. South Africa	39.9	197,000	495			
10. Russia	147.1	181,000	123			
11. Dem. Rep. Congo	50.3	151,000	401			
12. Vietnam	78.7	149,000	189			
13. Kenya	29.5	123,000	417			
14. Brazil	167.9	118,000	70			
15. Tanzania	32.7	112,000	340			
16. Thailand	60.8	86,000	141			
17. Mozambique	19.2	79,000	407			
18. Burma	45	76,000	169			
19. Uganda	21.1	72,000	343			
20. Afghanistan	21.9	71,000	425			
21. Zimbabwe	11.5	65,000	562			
22. Cambodia	10.9	61,000	560			
23. Peru	25.2	58,000	228			
Total: 23 countries	3760	6,700,000	178			
World total	5975	8,417,000	141			
*Population expressed as millions of inhabitants.						

Table 3. Current global status of TB in 1999: ranking of the 23 countries with the greatest disease burden. Source: reference 6, WHO, 2001.

Causes for the increase in tuberculosis in the world

Section summary

Poverty and the growing inequalities in the distribution of wealth have always been historical allies of TB, and remain responsible for the extremely bleak situation of the disease worldwide. Moreover, over the past 20 years the HIV pandemic has affected TB (particularly in the poorer countries) by further straining the already deficient health systems in the developing world, a situation that will certainly continue to worsen in the coming decades. These three factors—poverty, HIV infection, and *M. tuberculosis*—together with uncontrolled population growth in the poorer parts of the world, operate freely in the most vulnerable parts of the planet, with extremely dire consequences for the future. In comparison, migration, albeit a problem for wealthier countries, probably has little effect on the global figures of the disease, although extensive internal migration does make TB control more difficult.

The fight against poverty is extremely complex and depends on a series of globalisation strategies. In turn, the struggle against HIV infection presently appears futile in the poorest regions of the world. This is why the only remaining possibility is to implement TB control strategies as a way of combating the disease.

Poor or no application of tuberculosis control programmes

Early detection and cure of TB cases constitute the basis for control of the disease in the community. While over the past 40 years developed nations have employed effective TB control programmes for the early detection and cure of most cases, developing countries have achieved little in the struggle against the disease and continue to have large numbers of infectious cases in the community that elude detection and treatment. This variability in exposure to a very different risk of TB infection explains why 80% of infected persons in the developed world are over the age of 50 years, whereas 75% of infected individuals in developing countries are below that age. This difference in the distribution of infected subjects is influencing the added problem posed by HIV infection. The mistakes of the past in relation to antituberculous measures are now exacting a high price in most countries, particularly with the appearance of the HIV epidemic.

The extremely serious global problem of TB led the WHO in April 1993 to declare the disease a global health emergency, and to recommend the coordination of efforts against the illness-the so-called Directly Observed Therapy, Short Course (DOTS) strategy, which comprises five components: 1) the political commitment on the part of governments to solve the problem of TB; 2) diagnosis by smear microscopy accessible to the entire population; 3) directly observed or supervised treatment (at least in the first phase); 4) the guaranteed and regular supply of drugs; and 5) the implementation of an adequate registry and information system. This strategy is relatively simple to implement, provided the political will exists to do so. This represents the only possible line of action against the factors influencing the increase in the prevalence of TB. The fight against poverty itself is unfortunately extremely complex. In turn, the struggle against HIV infection presently appears hopeless in the poorest regions of the world. However, implementation of TB control strategies is a possibility. Although the spread of such measures has been constant, in late 1999 it was acknowledged that only 23% of all reported smear-positive cases have been studied and treated following strict guidelines, and that the DOTS strategy has been initiated to some degree or other in 127 countries (60%). Figure 2 shows that many parts of the world still do not use the DOTS strategy, or do so in only a very small percentage of the population.

Poverty and the widening gap between the rich and the poor

The improvements in living conditions achieved in the past 150 years in industrialised countries remain unattainable to the great majority in the poorest parts of the world. In these areas, extreme poverty continues to be the main ally of TB. The distribution of world wealth (Figure 3), based on per capita income reported by the World Bank in 1998 and by the categories of rich, middle income, and low income, shows that the distribution of wealth coincides greatly with the global distribution of TB. The minor variations between the two maps are attributable to the fact that the range corresponding to middle-income level is very broad (US\$786-9655). However, if the group of middle-income countries is further divided into two subgroups (Figure 4)—poorest (US\$786-3125) and richest (US\$3126-9655)—the similarities between TB case distribution and wealth distribution are even more pronounced, confirming that poverty, the historical partner of TB, remains the main factor influencing the serious situation of the disease in the world today.

However, economics not only affects the poorer nations but also the lower-income groups in richer countries. Studies conducted in the United States, Canada, and Western Europe have shown increased rates of TB in families with the lowest incomes, particularly among those who live below the poverty threshold. These studies also show that there is a level of income above which no further reduction in TB rates is recorded—this level corresponds to families with fewer children and secured access to food and shelter. This relation between socioeconomic level in wealthier countries and TB reflects the extreme sensitivity of TB as a parameter of development, inequality, and poverty.



Figure 2. Worldwide distribution of the use of the Directly Observed Therapy, Short Course (DOTS) strategy. Source: WHO, 1999. DDR = percentage of patients with TB subjected to DOTS; TS = percentage treatment success.



Figure 3. Per capita income distribution in the world. Source: World Bank, 1998.





Using this association between socioeconomic level and TB, the future global distribution of TB can be estimated. In effect, the future course of the disease can be charted by examining the annual United Nations (UN) report on the distribution of wealth in the world, which emphasises the increasing gap between rich and poor countries.

The impact of HIV infection

Undoubtedly, HIV infection has greatly complicated the problem of TB control in the world. In fact, it is difficult to imagine a microorganism better suited than HIV to function as an ally of *M. tuberculosis*. The virus selectively destroys or alters the function of those immune cells that defend the host against Koch's bacillus. In short, TB, the oldest of infectious diseases, and HIV, the most recent pandemic to affect the human species, have combined their pathogenic effects to become the primary cause of death in many parts of the world. It is estimated that substantial areas of the poorest countries will lose their youngest populations in the coming decades.

Towards the end of 1999, the WHO estimated that 33.6 million people worldwide had HIV infection/AIDS and that this disease had already caused 16.3 million deaths. The number of AIDS cases continues to grow each year. In 1999, there was already an estimated 5.6 million new cases of HIV infection. The best indication of the devastating spread of this disease is the fact that in 1999 a total of 2.6 million people were estimated to have died of AIDS, causing HIV to surpass M. tuberculosis as the infectious agent responsible for the largest number of deaths in the world. In the same way as with TB, the world distribution of AIDS is very heterogeneous, with 95% of all cases being located in the poorest parts of the globe. Both pathogens have therefore gradually spread to the most vulnerable parts of the planet. At the end of 1999 it was estimated that 70% of all cases of HIV infection (23.3 million individuals) were from sub-Saharan Africa, 20% were from Southeast Asia and the Western Pacific (6.6 million cases), and 5% were from Latin America and the Caribbean (1.6 million). The number of new infections and deaths had a similar distribution, as did the location of patients with dual HIV-TB infection. Indeed, the distribution map for HIV infection can be superimposed on the distribution map for TB infection and disease, as well as poverty. Thus, poverty, HIV infection, and *M. tuberculosis* operate freely in the most vulnerable parts of the planet, with extremely dire consequences for the future. According to the predictions of the Population Division of the UN, the life expectancy of the

populations in the nine African countries with the highest prevalence of HIV infection will be reduced by an average of 16 years between 2010 and 2015—a further obstacle to development in these countries, where poverty can be expected to increase with the decimation of an economically active population.

The situation, however, is completely different in the industrialised world, where only 5% of all HIV infection/AIDS cases are found, together with 5% of the cases of HIV-TB infection. Because most developed countries had implemented appropriate antituberculous measures during the past few decades, 80% of persons with *M. tuberculosis* infection are over the age of 50 years, whereas 85% to 90% of all HIV-infected persons are under this age. In other words, the two groups with TB or HIV infection are unlikely to overlap, as a result of which the impact of HIV on TB has been (and can be expected to remain) limited. In contrast, the developing countries are characterised by large numbers of individuals from the same age group (ages 20-49 years) who are infected with both pathogens—a situation that will undoubtedly worsen in the coming decades.

Moreover, since 1996, highly active antiretroviral therapy (HAART) has been available for the treatment of HIV infection. HAART, which comprises the combination of three or more antiviral drugs, including a protease inhibitor, affords marked and sustained increments in peripheral blood CD4+ lymphocyte counts, together with reductions in the plasma HIV viral load to undetectable levels. These drugs, however, are very expensive, and their use has largely been limited to industrialised countries. Here again, the countries with the greatest HIV problem are not benefiting from these treatments.

Massive immigration from tuberculosis endemic zones

The industrialised nations that have combated TB effectively in the past 40 to 50 years made the initial mistake of believing that the struggle against the disease ended at their own frontiers, and as a result failed to help poorer countries overcome the disease. Now, because of the massive migratory movements due to extreme poverty in much of the world, and the ease of long-distance travel, industrialised nations are paying a high price for their past neglect and witnessing a substantial increase in their TB rates resulting from the arrival of infected immigrants from regions where TB is still endemic. In their countries of destination, these immigrants reproduce the same endemic situation found in their original countries.

This situation persists for two to three generations, since immigrants from developing countries tend to live in relatively closed communities, with their own people and common cultural values, under very similar living conditions. Moreover, the great majority live in marginalised communities, which have limited access to health care services and which are less likely to be tested for TB.

Immigration has been one of the main reasons why TB rates have not only failed to decrease but have even increased in the past decade in many industrialised nations. Because TB rates are higher among immigrants in many developed countries, specific antituberculous strategies have been developed, including the systematic examination of all immigrants upon entering a country. There has also been an increase in funds donated to poorer countries in the last 5 to 10 years, with the aim of solving the problem at its origin.

Another consideration is the existence of internal migration in lowand middle-income countries. Such internal migration has grown considerably in the last two decades, owing to factors such as drought, famine, insecurity, internal conflicts, and terrorism. As a result, the population characteristics in many low-income countries are changing very rapidly, from a rural to a predominantly urban population. These largescale internal migratory phenomena lead to the development of urban settlements that lack the minimum required health infrastructure, with extensive crowding and poverty that assist the spread of *M. tuberculosis*. Moreover, it may be years before health services are available in these poverty zones, making it very difficult to combat TB in these areas.

The demographic explosion

Population growth is leading to an increase in the absolute number of TB cases in the poorest parts of the world. The demographic explosion in the Third World has led to increased crowding and poverty, which in turn has facilitated the transmission of *M. tuberculosis* and led to an increase in the number of TB cases. It has been estimated that the world population will double within the next 30 years, fundamentally as a result of population growth in the developing (and poorest) countries. An analysis of the predictions by the WHO for Africa for the period 1990-2000 reveals that a percentage of the new cases of TB can be attributed to this population explosion. This fact becomes all the more important when one considers that high population and low income characterise the 23 countries that account for 80% of all TB cases in the world.

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Measures for reducing tuberculosis in the community

Section summary

Of all known measures for controlling TB, only improvement in the existing socioeconomic conditions (leading to a 4-6% reduction in infection risk) and adequate chemotherapy (affording an additional annual risk reduction of 7-9%) have been able to arrest the evolution of the disease. The rest of measures adopted to date have had little impact on TB in the community, including chemoprophylaxis and bacille Calmette-Guérin vaccination.

Improvement in socioeconomic conditions

Mortality due to TB has been steadily decreasing in the developed countries since the late eighteenth century, almost a full century before TB was identified as an infectious disease and M. tuberculosis was first discovered. Consequently, the disease had begun to come under control in richer countries without the adoption of any specific control measures. The improvements in the socioeconomic conditions in developed countries from the mid-eighteenth century had already started to have a slight effect on the disease, with a sustained decrease in associated mortality and morbidity. It is now accepted that once an optimum level of development has been achieved, the resulting reduction in crowding and poverty has an important effect on TB disease burden. By reducing crowded living conditions, each source of bacterial transmission is no longer able to generate sufficient new infected cases to ensure a new infectious smearpositive TB patient. In this sense, each TB patient would have to infect 20 people, of whom 20% (2 subjects) would ultimately develop disease with positive smear microscopy and the remaining without. This is why just the reduction in the number of people living in a home can have a significant effect on the transmission dynamics of TB. Moreover, extreme poverty results in malnutrition, which is another risk factor for developing the disease. It has been calculated that improved socioeconomic conditions ultimately lead to a sustained 4% to 6% annual drop in infection risk. Hence, if adequate living conditions could be guaranteed worldwide, TB could potentially be eradicated without the need for any medical intervention.

Adequate chemotherapy with high cure rates

After the discovery of streptomycin in 1943, other drugs such as paraaminosalicylic acid and isoniazid soon followed in its wake, along with the scientific reasoning required to define the first therapeutic regimen capable of curing TB. This progress led many to believe that definitive control of the disease in the community was at hand. Indeed, in developed nations a steadily decreasing trend in TB-related mortality could be observed from the late eighteenth century, with a marked acceleration beginning in the 1950s and coinciding with the introduction of effective treatment regimens. None of the control measures have been as effective as chemotherapy in modifying the natural decline in TB rates, which, when administered correctly and with good patient compliance, can decrease rates by 7% to 9%. In sum, chemotherapy is the only certain method for shortening the transmission chain, by quickly curing diseased individuals and preventing them from infecting others. However, as has been mentioned, successful treatment in TB requires patient compliance to guarantee cure. Thus, ensuring compliance to appropriate treatment is the main challenge in order to attain high cure rates and have an impact on tuberculosis control.

Chemoprophylaxis of infected individuals at high risk of developing tuberculosis

Although chemoprophylaxis is regarded the primary tool for eradicating TB in the United States, its impact has been very limited in countries where the measure has been implemented on a large scale. As will be analysed in detail in a later chapter, the efficacy of this measure depends on three major factors: 1) the risk group selected for administration of preventive therapy; 2) the efficacy of the prescribed regimen; and 3) patient adherence to therapy. The first and, particularly, last of these factors greatly influence the results of preventive therapy. As a result, before deciding to implement this strategy in the community, mechanisms should be established to ensure compliance. On the other hand, the results obtained cannot be compared with those afforded by standard therapy, for while the latter directly treats the sources of infection (i.e., a diseased patient), preventive chemoprophylaxis only acts on the reservoir (and most individuals do not go on to develop the disease). Chemoprophylaxis has only been able to reduce TB rates by less than 1% per year, and only when correctly implemented.

BCG vaccination

Although the best measure for ensuring the eradication of an infectious disease is mass vaccination, the lack of efficacy shown by bacille Calmette-Guérin (BCG) vaccination in many parts of the world has effected practically no change in the epidemiology of TB. Moreover, if the vaccine were able to protect effectively against TB, the beneficial effect would mainly be in children under the age of 5 years who practically do not transmit the disease (and of whom 95% show negative smear microscopy).

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Chapter 6. Diagnosis of tuberculous infection: the tuberculin test

Chapter summary

The tuberculin test demonstrates the existence of a state of host hypersensitivity to the proteins of the tuberculous bacillus, most often acquired as a result of infection with *M. tuberculosis*, although hypersensitivity can also be induced by bacille Calmette-Guérin (BCG) vaccination or infection with environmental mycobacteria. The tuberculin skin test gives rise to an inflammatory reaction with an important dermal cellular infiltrate at the location of the tuberculin inoculation. This reaction is identified as a visible and palpable induration at the site of inoculation, and can be accompanied by swelling, erythema, and, sometimes, vesiculation, necrosis, and regional lymphadenitis.

The performance of the tuberculin skin test requires the use of 2 units of tuberculin purified protein derivative (PPD)-RT23 or 5 units of PPD-CD68, both doses being bioequivalent to 5 units of PPD-S, which is considered the international standard. The result is expressed in terms of millimetres of induration, taking into account that its interpretation is very complex and dependent on many variables that can influence not only reaction size but also the appearance of false-negative and false-positive readings, particularly in the case of BCG vaccination. The tuberculin test does not affect non-infected individuals, regardless of how often it is performed.

In low- and middle-income countries, because of the difficulty of preserving tuberculin at the most peripheral levels of the health care system, and because of large-scale BCG vaccination, this test is rendered practically unusable. Its use should therefore be restricted to centres where a paediatrician is available and to referral hospitals. In these settings, the indication for the tuberculin test is limited to children with suspected tuberculosis (TB) (disease, not infection), health care personnel (in an attempt to identify recent converters), and to very select cases of severe immune deficiency. The tuberculin test cannot be used as a discriminatory test for indicating preventive treatment or chemoprophylaxis. The test should not be repeated if there is evidence that the test had already been performed in the past and shown to be positive, regardless of the diameter of the induration. The second test might amplify the reading, giving rise to erroneous conclusions.

In those subjects in whom the skin test is performed, the positivity limit is 5 mm, based on the demonstrated high positive predictive value of this size in individuals at high risk of TB. These high-risk groups constitute the sole indication for the tuberculin skin test, since they represent the only individuals in whom performance of the test can lead to intervention (treatment or preventive chemotherapy).

The only method available for diagnosing tuberculous infection is the tuberculin skin test. Even today, this test remains a widely used technique, although it is one of the topics most frequently addressed in the history of medicine and which has generated much controversy. In view of its limitations, which will be described later in this chapter, the tuberculin test should be used much less frequently, particularly in low- and middle-income countries where there is a high prevalence of *M. tuberculosis* infection and where bacille Calmette-Guérin (BCG) vaccination is frequently administered at birth. Although the tuberculin test is an old technique, it has not been surpassed to date by any other method as a means of diagnosing tuberculous infection. There have been recent attempts to develop alternative tests capable of assessing tuberculous infection with greater specificity, of which the most promising may be the detection of the ESAT-6 antigen (secreted by T lymphocytes) based on an enzyme-linked immunosorbent assay for interferon γ (ELISPOT).

The history of the tuberculin skin test goes back to Robert Koch, who used tuberculin in his tireless quest for a vaccine against tuberculosis (TB). Koch had obtained a sample ("Koch's lymph") from tuberculous bacterial cultures, with the purpose of curing the disease. Unfortunately, the potential importance of his first conclusions presented at the 10th International Congress of Medicine in Berlin in 1890—that tuberculin could be used to detect a past or present tuberculous state—was not recognised and left a negative mark on his otherwise brilliant career. In 1905, von Pirquet (who coined the word "allergy" in reference to certain immune phenomena of TB) introduced a test based on the cutaneous reaction, while in 1908 Mantoux and Mussou developed the intradermoreaction, which remains the most widely used technique today.

Pathogenic basis of the tuberculin test

Specifically sensitised T lymphocytes proliferate in the regional lymph nodes of the host penetration site of *M. tuberculosis*. After 2 to 8 weeks. these lymphocytes enter the circulation where they remain for a prolonged period of time. The tuberculin test consists of the administration of tuberculin via the intradermal route. When tuberculin penetrates the skin, it is partially removed or cleared via the lymphatic system; the rest remains localised and is phagocytosed by the macrophages. This phenomenon produces an inflammatory reaction of mild-to-moderate intensity, with the involvement of polymorphonuclear cells and some mononuclear cells. In non-sensitised individuals, this inflammatory reaction soon disappears. In persons who have been sensitised as a result of prior mycobacterial infection, the initial inflammatory response intensifies, and lymphomonocytic perivascular infiltration develops as a result of recruitment by the lymphokines secreted by the circulatory T lymphocytes specifically sensitised to the bacterial antigens, which have recognised the tuberculin injected into the dermis. This inflammatory reaction, which occurs at the site on the skin where tuberculin was administered, gives rise to a visible and palpable induration (Figure 6) that can be accompanied by oedema and erythema because of alteration of the permeability of the vessels located within the inflammatory site. More intense reactions can show vesiculation and necrosis with regional lymphadenitis and, occasionally, febrile syndrome. The host response to tuberculin begins within 5 to 6 hours, usually peaking in intensity after 48 to 72 hours and persisting for several days. This phenomenon represents a cell-mediated delayed immune reaction.

However, from the early days of the tuberculin test, several limitations were identified. Such problems became more relevant when the test began to be used on a large scale. It became necessary to standardise the technique (e.g., in terms of the tuberculin used, dosage, method of administration, and reading and interpretation of the result), and to assess the limitations posed by storage of the product and the possibility of falsepositive and false-negative results. These aspects greatly influence the use of the test and, particularly, interpretation of results.



Figure 6. Tuberculin test performed with 2 units of purified protein derivative–RT23, yielding a 22-mm induration after 72 hours.

Factors affecting the result of the test

Test standardisation

Tuberculin

Tuberculin is obtained from a sterilised and concentrated *M. tuberculosis* culture filtrate. The first tuberculins used (referred to as "old tuberculin") contained impurities from the culture medium and mycobacterial development, and their composition varied from one batch to another, which posed a problem when comparing results. In 1934, Seibert prepared a purified protein derivative (PPD). Most of the components of this substance are low molecular weight proteins, and their multiplicity explains the incomplete specificity of PPD, since some of the allergenic constituents of the product, obtained from *M. tuberculosis* cultures, are also common to the other environmental mycobacteria. In 1951, this PPD was adopted by the World Health Organization (WHO) as the international standard, and the designation PPD-S was used. Consequently, all

commercial PPD formulations must be standardised with respect to PPD-S. Such formulations are identified by a suffix following the initials "PPD", such as PPD-RT21, PPD-RT23, and PPD-CT68. The formulation most widely used in the world, and recommended by the WHO, is PPD-RT23.

Dosage

The tuberculin dose must be administered in 0.1 ml of solvent, which is the volume to be injected into the dermis. Many studies were performed to define a PPD dose that allowed the maximum possible number of true reactive individuals (i.e., infected with *M. tuberculosis*) with the fewest possible false reactors. The ideal dosage was found to be 5 tuberculin units (TU) of PPD-S. Since the biological equivalence of PPD-RT23 is more than twice that of PPD-S, only 2 TU of PPD-RT23 are required (bioequivalent to 5 TU of PPD-S).

Method of administration

Charles Mantoux introduced and developed an intracutaneous method that remains common practice today. It is recommended both in view of its precision and sensitivity to 5 TU of PPD-S (or its bioequivalent doses of other PPDs) when performing the skin test in individuals who are known to be infected with *M. tuberculosis* (bacteriologically confirmed diseased TB patients).

The tuberculin skin test is performed with a syringe graded in tenths of a millilitre (as in the case of insulin syringes), which has a short, bevelled needle (27G or 0.4/12). The syringe can be made of glass or, preferentially, disposable plastic. The injection site should be the anterior or posterior surface of the forearm, although any other skin region can also be used. It is advisable to perform the injection away from the veins, and for the skin to be free of lesions. Injection is performed beneath the skin, with the needle bevel positioned upwards (Figure 7). The confirmation that tuberculin is deposited intradermally is based on the appearance of a pale skin elevation (blister) at the injection site, which persists for some time after the inoculation.



Figure 7. Photograph showing administration of the tuberculin skin test.

Reading of results

Before the factors influencing test specificity became known, the results of the test were simply expressed as negative if no reaction was observed, or positive if a reaction was observed. Previous attempts were made to quantify the reaction based on a cross-numbering system (e.g., +, ++). However, once the tuberculin dose was standardised and the formulation could be administered with considerable precision (owing to the Mantoux technique), the size of the reaction began to be assessed, particularly when it was seen that the induration diameter adopted a normal (Gaussian) distribution. At the same time, advances were being made in understanding the cellular infiltrative nature of the response found in tuberculin-sensitive individuals.

The result of the tuberculin skin test performed with 5 TU of PPD-S or its bioequivalent in other PPDs, based on the Mantoux technique, should always be expressed in millimetres of induration, and measured along the diameter transverse to the long axis of the forearm. The reading can be obtained 48 to 72 hours after performing the test, which is when induration is most apparent, although the reaction may remain without much variation for 4 to 7 days, followed by gradual weakening. This induration is visible, palpable, and measurable (Figures 6 and 7).
The procedure by which the induration is measured using a ballpoint pen is known as the "Sokal ballpoint pen method". Its sensitivity is no better than that afforded by direct and careful palpation. In fact, the latter technique is better able to detect the point where the elevation of induration begins. Correct measurement of the diameter is important, since interpretation of the result is dependent on an accurate reading. The longitudinal diameter should not be measured, since it has not been assessed in any of the epidemiological studies on which knowledge of test interpretation is based. If there is no induration, the result should be registered as zero (0 mm). Although the term "negative" is often used when referring to such cases, it is better to use the designation "nonreactor". Induration is often accompanied by erythema, which usually exceeds the limits of the induration, although only the diameter of the actual induration should be taken into account. If only erythema without induration is noted, the reading is 0 mm (i.e., non-reactor status). When the reaction is very intense, it tends to be accompanied by vesiculation, necrosis (Figure 8), and, occasionally, lymphangitis and satellite adenopathies. These observations should be duly recorded since they are highly specific of reaction due to *M. tuberculosis* infection.

The most frequent reason for erroneous tuberculin skin test readings is the interpretation of the test as being either positive or negative, instead of simply reading the result. As will be discussed below, the same result can be interpreted as positive or negative depending on many circumstances. Thus, a test report should include each of the steps expressed in this chapter on standardisation of the test. In other words, the report should include the type of tuberculin used, the dose administered, the administration technique, and the detected induration (in mm). An example of such a report would be: "Tuberculin skin test performed with 2 TU of PPD-RT23, revealing an induration of _____ mm after 72 hours".



Figure 8. Tuberculin test performed with 2 TU of PPD-RT23, revealing a 30-mm induration after 72 hours, with vesiculation and necrosis.

Tuberculin storage

Tuberculin should be stored at 4°C to 8°C, since it loses activity over time outside this temperature range. This consideration is very important, particularly in many poor countries that have tropical climates. In such environments, a refrigerator and a continuous electrical power supply would be necessary. Moreover, tuberculin undergoes denaturalisation when exposed to sunlight. These aspects alone make it practically impossible to perform the test at the peripheral level in the great majority of low- and middle-income countries.

False-negative and false-positive readings

False-negative readings

Twenty-five percent of *M. tuberculosis*—infected individuals can yield a negative tuberculin test at the time of diagnosis. In many instances, individuals with active TB with a negative test result (anergy) represent

atypical TB, although a negative result is more frequent in cases of severe and disseminated disease. Patients with TB disease who develop a negative result heal with adequate treatment, and the tuberculin test response undergoes positive conversion in over 50% of cases. The lack of cutaneous response to tuberculin has been attributed to a quantitative or qualitative depression of circulating T lymphocytes mediated by the action or intervention of suppressor cells (monocytes or macrophages). This effect is only observed in the area where tuberculin is applied (i.e., distant from the active TB site), with no inhibitory effect on adequate response in the area of inflammation. Extensive clinical and experimental evidence supports the fact that delayed sensitivity to PPD, while linked to cellular immune function, is actually differentiated from true and effective cellular immunity.

Other potential causes of false-negative responses to the tuberculin skin test are related to the type of tuberculin administered, its storage, and the technique used to perform the test. These factors are very important, for it has been estimated that inexperienced clinical auxiliary personnel perform the technique incorrectly and/or interpret the test result incorrectly in 75% of cases. Moreover, other conditions also affect cellular immunity and can lead to false-negative results. For instance, HIV-infected or AIDS patients, depending on the degree of host immune suppression, can have a negative result over 50% of the time. The factors that may give rise to false-negative results are detailed in Table 4.

On the other hand, it is necessary to remember that after infection, 2 to 12 weeks are required for the sensitised T lymphocytes to be able to recognise tuberculin deposited within the dermis. During this latency period, and although infection is present, there is no tuberculin test response. In newborns, tuberculin test positivity cannot be detected until after 8 to 12 weeks of life in some instances, and after 6 months in others. In turn, the tuberculin response capacity does not remain invariable in the course of a lifetime; in effect, it tends to weaken over time and can become undetectable in elderly individuals who were infected in youth. In this setting, in order to detect the so-called booster effect (which will be discussed in greater detail in the following section), a new test is performed 7 to 10 days later, and the result of this second test is used to classify the subject as either a reactor or non-reactor.

Table 4. Factors that may give rise to false-negative tuberculin test results.

- 1. Factors related to the person subjected to the test:
 - High fever of any origin
 - Malnutrition
 - Viral infection: HIV, measles, parotitis, varicella
 - Bacterial infection: TB, particularly severe forms and involving pleural location, typhoid fever, brucellosis, whooping cough, leprosy
 - Blastomycosis
 - Live viral vaccination. Measles (does not suppress response in first 48 hours after vaccination), poliomyelitis, parotitis, varicella, yellow fever
 - Oral antityphoid vaccination
 - Chronic renal failure
 - Leukaemia, lymphomas, Hodgkin's disease
 - Sarcoidosis

2.

3.

4.

- Newborn infants or advanced age
- Stress, surgery, burns, mental disorders
- Immunosuppressors, corticoids
- Factors related to the tuberculin used:
 - Inappropriate storage (exposure to heat or light)
 - Inappropriate dilutions
 - Chemical denaturalisation
- Adsorption by the container (partially controlled by Tween 80 detergent)
 - Factors related to the method of administration:
 - Administration of too little antigen
 - Subcutaneous injection
 - Delay in administration after extracting the dose from the container
 - Injection too close to other antigens
- Factors related to registry of the result:
 - Reader inexperience
 - Errors

False-positive readings

False-positive tuberculin test readings can occur due to multiple reasons, although the most important is the interpretation of a tuberculous infection when in fact the infection involves other environmental mycobacteria or the patient had previously received a BCG vaccination. Occasionally, the presence of a haematoma (bruise) or small abscess at the injection site can be interpreted as an induration when it is indeed secondary to injection-related trauma or another infection.

When infection by environmental mycobacteria is suspected, the ideal approach is to resort to a specific antigen of the microorganism in

question, so as to allow dual testing. If the response to the antigen is greater than the response to PPD, the infection may be attributed to the environmental mycobacteria instead of to *M. tuberculosis*. However, this second test is very difficult to perform in practice (in view of the limited availability of specific antigens) and to interpret.

The BCG vaccination is a particularly important consideration, especially in low- and middle-income countries where BCG vaccination at birth constitutes one of the priorities of local health care policies. Adequate interpretation of the tuberculin skin test requires an evaluation of the vaccination history of the individual, with particular emphasis on the identification of the post-vaccination scar in the deltoid region or in some other skin zone. The post-vaccination scar is characteristically small and located on the same level as the surrounding skin; it has a pearly smooth appearance, and fine folds are formed on compression of the scar between the fingers. However, not all vaccinated individuals become reactors to tuberculin, and the indurations recorded tend to be smaller than in patients infected with *M. tuberculosis*. Moreover, hypersensitivity to tuberculin as a result of BCG vaccination decreases before hypersensitivity attributable to *M. tuberculosis* infection develops, although it is not possible to determine precisely when the reaction disappears, since some studies have shown that a substantial percentage of vaccinated individuals exhibit a positive tuberculin test (attributable to the vaccine) after as long as 25 years. Therefore, the tuberculin sensitivity induced by BCG vaccination may last indefinitely, and although sensitivity disappears in a substantial proportion of cases, in others it can persist and interfere with test interpretation even if the positivity limit is established at 15 mm.

For this reason, countries with BCG vaccination coverage from birth (i.e., practically all low- and middle-income countries) should not use the tuberculin skin test as a discriminatory diagnostic test for possible indication of preventive therapy. In these settings, the criteria of epidemiological antecedents and age should prevail. Accordingly, a child living with a person with TB and who exhibits smear-positive results should always receive preventive treatment, regardless of the tuberculin test result obtained.

Interpretation of results

Interpretation of results is perhaps the most complicated aspect of the tuberculin skin test. Efforts have been made to simplify this step, although often with little success.

The tuberculin test, like any other diagnostic test, has a sensitivity and specificity that varies according to where the positivity threshold or cut-off value is established. The closer the cut-off point to 5 mm, the greater the test sensitivity, and the test will be able to diagnose more cases of M. tuberculosis infection. However, this also increases the possibility of more false-positive readings—in other words, a loss of specificity. In contrast, when the cut-off is established at larger diameters, particularly 16 to 17 mm, which corresponds to the normal distribution of TB patients, sensitivity decreases and there is an increase in the number of falsenegative readings as well as an increase in specificity. For this reason, when it is important to establish a diagnosis of *M. tuberculosis* infection because the patient has a high risk of developing TB, the cut-off point should be set at 5 mm to ensure maximum sensitivity, avoiding falsenegative diagnoses that would prevent the patient from receiving treatment. It may be better to prescribe one preventive treatment too many (in the event of a false-positive case attributable to environmental mycobacteria because of use of such a low cut-off value in an individual at high risk of TB) than to do without treatment and run the risk of developing TB. In contrast, if there is no particular risk of developing TB, the cut-off value should be set at a point (e.g., 15 mm) that would be associated with the least possible number of false-positive diagnoses. This way, maximum specificity at the expense of sensitivity would be achieved, which in this setting would not have negative effects since the risk of disease would be sufficiently low to contraindicate preventive therapy.

The issue dealt with above is closely related to the positive predictive value (PPV) of the tuberculin skin test, which represents the probability that an individual with a positive test result is truly infected with *M. tuberculosis*. The PPV is what is important when interpreting a tuberculin test result, and it is directly related to the prevalence of the latent infection of *M. tuberculosis*. Consequently, in a population composed of contacts of infectious smear-positive TB patients, the prevalence of infected individuals is much greater than in the general population, since the cut-off value of 5 mm affords a PPV of 99%. The PPV of the tuberculin test has been calculated for different prevalences of true *M. tuberculosis* infection,

according to whether the test specificity is 99% (the value estimated when no cross-reactions exist with other environmental mycobacteria or with BCG vaccination) or 95% (when there is interference by environmental mycobacteria).

Thus, the cut-off point established in a certain geographical setting should take into account not only the existing situation in terms of infection by environmental mycobacteria, which may increase the possibility of false-positive results, but also in terms of the prevalence of TB in each population group studied. If the intention is to establish an exact limit for the entire population, without properly assessing the risk of TB in each group, it must be taken into account that the limit in question is different for each population (i.e., it is dependent on the prevalence of the infection), and that it changes in different communities over time because of changes in infection prevalences.

Consequently, it is practically impossible to impose a precise cut-off value for tuberculin skin testing, since it is influenced not only by the size of the reaction but also the corresponding PPV.

Tuberculin test repetition. Tuberculin conversion. "Booster effect"

Before addressing this section, it should be pointed out that the tuberculin test does not sensitise non-infected individuals, regardless of how often it is performed. In some instances, the test must be repeated due to the existence of a more-or-less sustained risk of TB infection, as with health care workers.

Tuberculin conversion is defined as a test response in an individual previously classified as a non-reactor. This diagnosis is extremely important if the time elapsed between the two tests is less than 2 years, since it implies that the individual had been infected during this period, and that infection is recent. The risk of developing the disease during this time is the highest and thus constitutes an absolute indication for preventive treatment. Tuberculin conversion consists of the detection of a tuberculin test result of over 5 mm in an individual previously unresponsive to tuberculin. This limit is not unanimously accepted; several well-respected scientific societies have instead established a conversion limit of 6 or 12 mm. The phenomenon implies the acquisition of tuberculous infection provided a booster effect has previously been rejected.

With time, the *M. tuberculosis*-infected individual weakens in his or her capacity to react to a tuberculin test, as the result of a loss of memory T lymphocyte capacity, giving rise to a negative test result. However, since the response capacity persists, the PPD used in the first tuberculin skin test can produce a stimulant or "booster effect". Thus, a second test may yield a positive result because of this recovered memory phenomenon, and the individual may be classified as a tuberculin converter, when in fact he or she had been previously infected. Whenever present, the booster effect is not detectable until 7 days after the tuberculin test result is regarded as negative, and it may persist for years. Therefore, and in order to reject the possibility that a lack of tuberculin test reaction may be due to a weakening in response and not to the absence of infection, testing should be repeated 7 to 10 days later, accepting the outcome of the second test as the definitive result. If positive, it will help to avoid the false diagnosis of recent conversion. Since TB infection is generally acquired in infancy and youth, this weakening in response capacity to the tuberculin skin test is observed more often in elderly individuals. Therefore, in the industrialised world, a second test is advised in subjects over the age of 55 years who previously had negative tuberculin skin test results. However, although less frequently, such weakening response may also occur in non-elderly individuals. Thus, a booster effect should also be ruled out in certain highrisk groups, such as health care personnel.

The sensitivity to tuberculin due to BCG vaccination decreases at a faster rate than the sensitivity acquired as a result of natural infection with *M. tuberculosis.* Consequently, vaccinated individuals of any age who have a negative result should in theory be retested after 7 to 10 days to reject or detect a possible booster effect. This imposes serious limitations to the diagnosis of latent infection of contacts of active TB cases in low-and middle-income countries where BCG vaccination is carried out extensively.

Skin testing should not be repeated when there is indication that the test had already been performed in the past and was positive (regardless of the diameter of the induration). Indeed, a second test might result in a booster effect and amplify the reading, thereby giving rise to erroneous conclusions.

Indications for tuberculin testing

The tuberculin skin test, in the same way as any diagnostic test, should only be performed in individuals in whom the test result may lead to some

therapeutic intervention. Only two intervention possibilities exist for TB: the treatment of active disease TB patients, and preventive treatment of infected individuals at high risk of developing TB disease. In helping to diagnose TB disease, the tuberculin test only offers a high PPV in children, with considerable lesser performance in patients with immune deficiencies. Regarding the possibility of preventive treatment, in the case that TB infection is detected, such therapy is only indicated in groups at high risk of developing TB disease, in whom the PPV of the test is very high. In low- and middle-income countries, these high-risk groups are limited to those subjects who live with patients with confirmed smear-positive results, and to HIV-infected persons. However, among people living with smear-positive subjects, the tuberculin test is not indicated in view of the important interference represented by BCG vaccination. Thus, possible preventive management must be decided based on epidemiological risk factors. Likewise, in HIV-infected patients, the tuberculin test loses much of its usefulness, since immune deficiency is a frequent source of falsenegative test results. Since low- and middle-income countries have a high prevalence of *M. tuberculosis* infection, preventive treatment would be indicated in these high-risk groups regardless of the tuberculin test results obtained. Obviously, this recommendation, which is accepted by the WHO, is questioned by some experts on the grounds that it is possible that a considerable number of anergic patients may not benefit from such preventive treatment, and that these patients have a significant chance of not being infected.

In view of the above, a tuberculin test would only be indicated in lowand middle-income countries in the following situations:

- 1. *Children with symptoms suggestive of TB*. Here, a tuberculin test offers a high PPV in diagnosing the disease. However, the test should not be used to diagnose infection or for managing contacts. Specialists in paediatrics should carry out the procedure.
- 2. *Severe immune deficiencies*. Use in support of the diagnosis of TB disease. These cases are to be dealt with in the reference hospital.
- 3. *Health care personnel*, particularly those beginning to work in health care. If a tuberculin test proves positive, it can be assumed that the previous infection can afford some protection against later exposure to *M. tuberculosis*, and no further actions would be required. However, in health care workers with an initial negative tuberculin test, periodic tuberculin screening is indicated (every 6-12 months, depending on the risk) to establish when a tuberculin test turns positive. At this

point, recent conversion has occurred and preventive treatment would then be indicated.

Positivity criteria and indications in low- and middle-income countries

The above considerations imply that there is more than one limit to the tuberculin test, depending on the risk of TB-which greatly complicates its application in the field, since people working at the peripheral level would then be required to make decisions based on the risk factors of each individual. In truth, the different cut-off points would only be applicable in the reference centres and to highly expert personnel, not at the peripheral level. Nevertheless, the tuberculin test should be used sensibly, taking care to avoid excessive, inappropriate use. As has been mentioned above, the test should only be used to make a clinical decision, or for epidemiological studies. The most important consideration is to define the clinical indications, since conducting tuberculin surveys is very complex and expert personnel should only design such studies. From the perspective of supporting a clinical decision, the test is only indicated in those persons in whom the result implies some intervention: treatment if TB disease is diagnosed, or preventive therapy, which is only recommended in high-risk groups. To simplify things, the indications for a tuberculin test should be restricted to groups at a high risk of developing TB. The positivity threshold for the test should be set at 5 mm, which affords a PPV of 99% in such groups. In this way, interpretation of the test is simplified and its utilisation is facilitated.

In summary, it is necessary to consider that low- and middle-income countries not only suffer from unreliable health care distribution and resources, which makes storing tuberculin in the peripheral centres almost impossible, but that these countries also practise extensive BCG vaccination, which complicates interpretation of the tuberculin test result. For these reasons, these countries should only indicate the tuberculin test in centres where children with suspected TB are seen and where there is a paediatrician, as well as in reference hospitals to help in the diagnosis of TB infection in patients with immune deficiencies. The test should be contraindicated at the peripheral level, and should not be used as a discriminatory test for indicating preventive treatment. As will be seen in the corresponding chapter, the indication for preventive treatment should be evaluated according to the risk of TB disease among the different population subgroups, without the need for a tuberculin skin test.

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Chapter 5. Pathogenesis of tuberculosis: infection and disease

Chapter summary

Alveolar macrophages are the key cells in the host immune response to tuberculosis (TB). After successfully phagocytosing the bacteria, alveolar macrophages process the antigens of *M. tuberculosis* and present them to the specific T lymphocytes. Before a cellular immune response is elicited (within 4-8 weeks), the bacteria grow unhindered, and are therefore able to enter the bloodstream and spread to other parts of the body, mainly the apical regions of the lungs, but also to any other organs.

Ninety percent of all people have the tubercle bacilli under control in a latent state throughout their lifetime, as a result of their immune defence system. Five percent develop progressive primary TB, while another 5% develop the disease in late stages of life (i.e., reactivation TB). This situation changes drastically in the case of patients with HIV infection, of whom 50% to 60% who are also infected with *M. tuberculosis* will develop active TB in the course of their lifetime.

Based on the above considerations, all TB-infected individuals are potential patients.

Tuberculosis (TB) is the example of the interaction between an exogenous agent and the host immune defence system. It may be estimated that while 1900 million people throughout the world are infected with M. *tuberculosis* (representing an enormous reservoir that contributes to perpetuation of this disease), only 8 million actually suffer from the disease each year. This situation is explained by the fact that the human defence mechanism is highly effective and can overcome the disease in most cases.

The chemical characterisation of the structural components of M. *tuberculosis* has facilitated the investigation of the biological properties of the pathogen. The protein and peptide components are known to be responsible for host cellular immune response stimulation and for delayed hypersensitivity reactions, whereas polysaccharide elements such as the arabinomanans, while able to induce a humoral defence response, possess immunosuppressive properties. In turn, certain glycopeptides (e.g., cord factor or sulpholipids) modify macrophage function. Cord factor is a potent chemoattractant that induces granuloma formation, whereas sulpholipids inhibit phagosome-lysosome fusion.

Understanding of the pathogenesis of TB is largely attributable to research in animal models. Although many models have been developed that illustrate different aspects of the disease, the contributions of Lurie should be noted, as well as those of Dannenberg and Lefford. Lurie used syngenic rabbits that were sensitive or resistant to TB infection. In his model, three phases of tuberculous infection were defined based on bacterial counts in the lungs: a first phase of bacterial destruction (3-7 days); a second "symbiotic" phase involving exponential growth of the bacterial population; and a third phase of immune control of the infection. The resistant and sensitive rabbit strains differed only in relation to the first phase. Resistant animals were able to destroy the bacteria faster and more effectively than the sensitive rabbit strains. The studies carried out by Lurie and Dannenberg highlighted the importance of the natural host defences against Koch's bacillus, and the added role played by immune response. In comparison, Lefford emphasised the importance of an acquired immune response using rat models. Lefford and colleagues infected rats with an avirulent strain of *M. tuberculosis*. After a few days, they reinfected the same animals with a virulent strain of the same microorganism. They found that the rats had acquired resistance to infection, and that this resistance was of a cellular nature, since the lymphocytes obtained by lymphatic drainage protected those animals that had not been in contact with the bacterium. Thus, these experimental studies clearly demonstrated that the antituberculous host defence involves the participation of both natural and acquired mechanisms.

Still, the results from animal models should be interpreted with caution, since immunologic characteristics differ with mammalian species. For example, Rook reported a markedly different bactericidal macrophage response in murine (mouse) and human macrophages to the administration of interferon γ and vitamin D₁₀.

Based on the above considerations, the most widely accepted pathogenic mechanism of TB infection and disease clearly distinguishes between tuberculous primary infection and the post-primary infection phenomena—each of which has its own clinical manifestations.

Primary infection

Primary infection refers to the general biological phenomena that take place when an individual comes into contact with the tubercle bacillus for the first time. During primary infection, 95% of all affected individuals remain asymptomatic or present with only minimal clinical manifestations similar to those seen with the common cold. Only 5% develop manifest disease. Tuberculin skin test conversion usually occurs in these individuals. This phenomenon typically takes place in childhood, as a result of which primary infection is often associated with childhood TB. Nevertheless, primary infection can occur at any time in life, and is the result of inhalation of bacteria-loaded particles by an individual who has not been previously exposed to the microorganism. Because of the weight of these particles, some of them tend to sediment and are therefore not infective. Other airborne particles, known as Pflüger droplets, with a diameter of 5 to 10 µm, either sediment or are cleared by the defence mechanisms of the airways. However, upon condensation of these droplet nuclei and the loss of part of their water content, smaller particles measuring 1 to 5 µm are formed, containing approximately three tubercle bacilli each; these droplets are infective. The defence mechanisms of the upper airways (i.e., cough reflex, mucociliary system) non-specifically prevent particles measuring over 5 µm from reaching the lung parenchyma. Under infective conditions, however, some particles measuring 1 to 5 µm reach the distal airways and are deposited in the alveoli. It is believed that at least 10 to 200 of such microdroplets must reach the alveoli in order for infection to take place. The preferential zone of arrival is the best-ventilated part of the lungs, corresponding to the subpleural region of the inferior lobes.

Upon arrival in the alveolar region, the bacteria encounter three types of cells that potentially oppose infection: the alveolar macrophages within the alveolar lumen, the natural killer cells, and the γ/δ T lymphocytes. In mice, exposure to aerosols containing mycobacterial antigens, or immunisation with *M. tuberculosis*, gives rise to a marked increase in the presence of this cell line in the lungs and regional lymph nodes. On the other hand, γ/δ T lymphocytes are able to recognise certain epitopes of *M. tuberculosis*. Studies involving tuberculous patients, however, have for the most part been unable to confirm this cell line increment in either the blood or granulomas of infected patients. It should be noted though that all of these studies have been conducted outside the context of primary infection, which makes extrapolation of results difficult.

In humans, alveolar macrophages are considered to be the key type of cell involved in the initial interaction with the tubercle bacillus. Some important characteristics of alveolar macrophages should be pointed out. First, these cells originate in the bone marrow and reach the alveoli after coming into contact with the systemic circulation. As a result, different systemic and local factors can influence their functional characteristics. For example, HIV is able to infect these alveolar macrophages and thereby increase host sensitivity to tuberculous infection. Second, the antigenpresenting capacity of alveolar macrophages is low in humans, as compared with in other animal species. Third, alveolar macrophages are cells that live in an oxygen-rich environment, as a result of which their free oxygen radical production potential is theoretically great. However, and probably to avoid toxicity due to these radicals, alveolar macrophages lack myeloperoxidase (although they do generate superoxide radicals). Fourth, alveolar macrophages contain abundant lysosomal enzymes.

The initial interaction between *M. tuberculosis* and alveolar macrophages involves non-specific phagocytosis of the bacilli and their inclusion within phagocytic vacuoles. Considering that these alveolar macrophages have not been primed by lymphocytic cytokines and that various mycobacterial components inhibit the bactericidal systems of these cells, it is reasonable that bacterial growth predominates in this initial stage. Practically all bactericidal macrophage systems are inhibited by products derived from the mycobacteria. Thus, glycolipids inhibit phagosome-lysosome fusion, while other less well-known components alter lysosomal acid pH, thereby complicating enzyme action. Catalase, in turn, destroys hydrogen peroxide, and different mycobacterial components inhibit superoxide production. This phase concludes with destruction of the alveolar macrophages by proliferating intracellular bacilli. Natural resistance to the infection fundamentally occurs during this phase.

Tubercle bacilli products such as cord factor and the activation of other chemokine factors exert a potent chemical effect, attracting blood monocytes that ingest the released bacilli. At this point, a symbiotic relation is established in which the bacteria and young macrophages do not destroy each other. The monocytes have not been activated, and the bacteria are not toxic, at least on an acute basis. The tubercle bacilli increase exponentially in a similar manner, killing host cells and spreading locally. In the lung, intense alveolitis takes place at the expense of the young cells of the mononuclear phagocyte system. The third essential phenomenon in this phase of the disease is mycobacterial spread systemically via the lymphatics towards the regional lymph nodes. In this region, the host immune response to tuberculous infection takes place. In some instances, this immune response is sufficient to arrest the progression of infection, although often times the bacilli escape towards the lymphatic duct and penetrate the pulmonary bloodstream, from where there is hematogenous spreading of the bacilli to the other organs. The main metastatic or target zones of such bacterial dissemination are the highly irrigated organs and tissues—the central nervous system, spongy bone, liver, kidneys, and genitals. In each of these zones, the arriving bacilli are phagocytosed by the local cells of the mononuclear phagocyte system.

In most cases, this period implies immunologic control of the infection as a result of two mechanisms: cell-mediated immunity and delayed hypersensitivity. From the bacteriological perspective, the consequence of this situation is an abrupt interruption of the bacterial growth curve in both resistant and susceptible individuals. Cellular immunity is not responsible for this growth arrest, since susceptible individuals have only a weak cellmediated immune response and resistant subjects have not yet developed an effective immune reaction. Delayed hypersensitivity is the phenomenon responsible for the destruction of macrophages that contain intracytoplasmic bacteria, thereby forming a characteristic focus of caseous necrosis. Although the bacteria may survive within this necrotic focus for years, they are unable to reproduce due to the prevalent acidosis, the lack of oxygen, and the presence of inhibitory fatty acids. The principal factors influencing delayed hypersensitivity reactions are the cytotoxic T lymphocytes, although other factors such as cytokines (tumour necrosis factor α), oxygen reactive species, and nitrous oxide may also be involved. Such initial necrosis is therefore beneficial for control of the infection. However, delayed hypersensitivity must be "reinforced" by cell-mediated immunity, since susceptible hosts with weak immune responses are not only unable to control the infection but also produce granulomas with an increased caseous presence, probably due to the intervention of mycobacterial proteins.

Resistant animals, in the same way as immunocompetent humans, avoid tubercle bacilli spread from the tuberculous focus in a second phase, owing to the development of a potent cell-mediated immune response at the expense of the helper T lymphocytes, which activate the macrophage population. From the clinical perspective, immunocompetent individuals develop a balance between themselves and the mycobacteria, which persists throughout life until some predisposing event is able to reactivate the infectious focus. An indirect approach to demonstrate this immunologic phenomenon is represented by the tuberculin skin test. In immunocompromised individuals who are unable to control the infection, TB disease develops and the subjects do not usually show a positive tuberculin test. In addition to prior immunodeficiency, one factor that clearly influences the conversion from latent infection to disease is the age at which primary infection takes place. Conversion to disease is more frequent in the very young and very old. Adolescents and early youth also show a tendency towards conversion to disease, although for reasons that remain unclear.

Tuberculous reactivation

Tuberculous reactivation is defined as the development of tuberculous disease in a patient who had already been infected with the tubercle bacillus in the past. Although there are well-documented cases of exogenous reinfection, it is currently accepted that most cases of infection are attributable to endogenous reactivation. However, this assumption may change in the coming years, in view of recent information appearing in the literature.

It has been calculated that only a minority of people infected with *M. tuberculosis* actually progress to active disease. In general terms, 90% of infected individuals can be expected to keep the tubercle bacilli in a latent state for life, owing to the intervention of the host immune defences. Five percent will develop progressive primary TB, and the remaining 5% will develop the disease in later stages of life, a condition known as reactivation or post-primary TB (Figure 5). This situation changes drastically in patients with HIV infection, of whom an estimated 50% to 60% who are also infected with *M. tuberculosis* will develop active TB in the course of their lifetime. Individuals presenting with risk factors for TB should be actively evaluated. The number of patients at risk of TB has increased in the course of history, and presently includes some populations that do not demonstrate immunodeficiency but that have other risk factors for TB (Chapter 4, Table 2).





In resistant individuals, the immune control of haematogenous seeding sites is dependent on local as well as systemic factors. Some systemic factors (e.g., HIV infection, corticotherapy, malnutrition) may account for tuberculous reactivation, although it is less clear how other local factors affect reactivation. It has been speculated that a decrease in interferon γ production and the intervention of arabinomanans may be involved in this

reduction or in the generation of a specific suppressor response. One of the known important pathogenic phenomena of reactivation is caseum liquefaction. Although not all the factors implicated in such liquefaction are known, the phenomenon has been attributed to lysosomal enzymes released by the macrophages, and to a delayed hypersensitivity reaction to mycobacterial products. The immediate consequence of caseum liquefaction is the production of an excellent growth medium for the bacteria, which begin to multiply and release products similar to tuberculin that have great toxic potential. In the case of the lungs, these released products rupture the adjacent bronchi, forming cavities and spreading the bacteria via the bronchogenic route.

Based on the above considerations, all TB-infected individuals should be considered patients, and the greatest protection against *M. tuberculosis* corresponds to a subject who has never been infected. The great problem with TB facing us today is that there is a very large number of infected individuals who are never free from the risk of the disease. These individuals, while potentially capable of developing the disease at any time in life, particularly if they develop some form of immunodeficiency, are expected to present with alerted memory lymphocytes in the event of past exposure to tubercle bacilli. Theoretically, this would confer relative protection against such possible exogenous reactivation. In this sense, there has been speculation as to whether the ideal situation is instead represented by the infected subject, who would be more protected against future exogenous reinfections. However, such reasoning is mistaken, since the development of TB due to endogenous reactivation in infected individuals is much more likely than the development of the disease due to exogenous infection in non-infected subjects.

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Chapter 7. Diagnosis of tuberculosis

Chapter summary

The diagnosis of tuberculosis (TB) should be based on a series of ancillary methods and confirmatory microbiological techniques. The accessory diagnostic methods are non-specific and include the clinical manifestations, radiological findings (highly sensitive), histopathology (more specific than the other approaches), and tuberculin testing (which contributes little in terms of diagnosis). Efforts must be made to ensure that the diagnosis can be confirmed by smear microscopy (which is accepted for the purpose of TB control programmes) and/or culture.

When faced with a possible case of tuberculous disease, it can be presumed that *M. tuberculosis* infection has already progressed to the point where a series of clinical manifestations have developed, compelling the host/patient to seek medical help. It is therefore necessary to be familiarised with the clinical picture of tuberculosis (TB) and with the necessary diagnostic techniques. It should be emphasised that among the different diagnostic techniques, the microbiological study of samples is by far the most important instrument and the only method available for establishing the disease with certainty. It is therefore reasonable to refer to microbiological study as the diagnostic method for TB, with the rest of techniques constituting ancillary diagnostic procedures. Each of these methods will be dealt with in this chapter. We begin though with the clinical manifestations of TB, since they constitute the initial medical consultation and play a role in the subsequent suspected diagnosis.

Clinical assessment

Section summary

TB lacks the symptoms, exploratory findings, and analytical/laboratory data that can clearly differentiate it from other respiratory diseases. In most instances, the onset of clinically manifest TB is insidious and not particularly alarming; as a result, months can go by before the diagnosis is established. This points to the importance of implementing complementary measures in the case of even the slightest clinical suspicion. Hence, the

physician must be perfectly familiarised with the symptoms and signs suggestive of TB, since such knowledge can lead to increased suspicion of the disease and to an earlier diagnosis. Consequently, clinical evaluation is very important in strategies designed to increase case detection.

TB can manifest as any sign or symptom in any part of the body. However, since respiratory TB is the most frequent presentation and is the form associated with the greatest potential for contagion, increased case detection and an earlier diagnosis prompt the suspicion of TB in any patient with cough and/or expectoration lasting more than 2 to 3 weeks. Such patients are referred to as symptomatic respiratory subjects. In all subjects presenting with signs and symptoms suggestive of TB, a series of pertinent tests are indicated to rule out the possibility of the disease. Serial sputum smear microscopy is the minimum but most important measure that should be included in the diagnosis of TB.

Clinical symptoms

TB lacks specific clinical manifestations that allow it to be differentiated from other respiratory diseases. In most cases, the onset of clinically manifest TB is insidious and not particularly alarming; as a result, months can go by before the diagnosis is established. Thus, it is important to implement complementary measures in cases of even the slightest clinical suspicion. The physician must therefore be perfectly familiarised with the symptoms and signs suggestive of TB, since such knowledge can lead to increased suspicion of the disease and to an earlier diagnosis. Early diagnosis affords a double benefit: the affected individual undergoes earlier treatment, which results in fewer sequelae and improved survival, and the period during which the individual is able to spread the infection in the community is shortened. Ruling out the disease is an important measure for controlling this infectious condition.

As has already been commented, *M. tuberculosis* can spread to any part of the body from its initial phase of entry into the host. In short, TB can affect any organ or tissue. The most common location is the lungs—the primary entry site of the tubercle bacilli—which is implicated in 80% to 85% of all TB cases in immunocompetent individuals. The clinical manifestations of TB depend on the location of the disease, although in all cases the manifestations are vague and non-specific. Accordingly, it is possible to include TB in the differential diagnosis of any clinical syndrome, regardless of its location and presentation. In other words, any sign or symptom, in any location, may be suggestive of TB.

Apart from pulmonary involvement, the most common extrapulmonary locations of the disease are (in decreasing order): pleural, lymphatic, urogenital, osteoarticular, and meningeal—although as has been pointed out, any organ or tissue can be affected. In immunocompetent patients, the frequency of presentation of extrapulmonary TB is no greater than 15% to 20%, with this figure increasing in situations of immune deficiency, as in the case of patients with AIDS, in whom extrapulmonary disease accounts for 50% to 60% of all TB cases.

In addition to the local manifestations, TB often produces general signs and symptoms, including febricula, intense perspiration, asthenia, anorexia, and weight loss—which are suggestive of a chronic infectious disease.

Primary infection is usually subclinical, or may involve symptoms as non-specific as cough and febricula. This is why the persistence of respiratory symptoms for more than 15 days in a child constitutes an indication for chest radiographs, particularly when accompanied by systemic or extrapulmonary manifestations such as anorexia, weight loss, and erythema nodosum.

In turn, the onset of adult or post-primary TB often includes cough, mucopurulent expectoration, nocturnal sweating, and easy fatigue. In some instances, the onset can be acute, in the form of high fever, chills, haemoptoic expectoration, or haemoptysis—a situation that tends to lead to earlier medical consultation and a comparatively lesser delay in establishing the diagnosis. A special type of onset is represented by TB pneumonia, involving a clinico-radiological syndrome similar to that seen in patients with bacterial pneumonia. Extensive pulmonary spread is associated with progressive dyspnoea and respiratory failure, which in severe cases can result in adult distress syndrome.

On the other hand, miliary TB, which always implies haematogenous spread of the disease and is therefore serious (although the patient may sometimes present with few symptoms), is mainly characterised by general signs and symptoms. In such situations, it is often necessary to establish a differential diagnosis with a fever of uncertain origin, particularly if during the initial period no miliary pattern is observed on the radiographs.

An essential consideration for optimum case detection is adequate diagnostic suspicion. It is therefore very important to define which patients constitute suspected TB cases, in order to reduce the study population and therefore increase the positive and negative predictive values of the diagnostic tests used.

Based on the above, and considering that pulmonary TB is the most frequent presentation and the form with the greatest contagion potential, efforts should focus on the detection of these cases. Since pulmonary TB is most often characterised by persistent cough and expectoration, increased case detection and an earlier diagnosis call for the suspicion of TB in any patient with cough and/or expectoration lasting more than 2 to 3 weeks. Such patients are referred to as symptomatic respiratory subjects. These symptoms, in addition to being the most frequent manifestations of pulmonary TB, are also responsible for the greatest contagion potential (infectivity increases with the degree of coughing). In symptomatic respiratory patients, and in all subjects presenting with signs and symptoms suggestive of TB, a series of pertinent tests are indicated to rule out the disease.

One topic deserving special mention is TB associated with HIV infection. If such HIV-positive individuals have not yet developed immune deficiency, the TB symptoms tend to be similar to those observed in the rest of cases. However, in the immunosuppressed AIDS patient, the initial manifestations tend to be non-specific, with a predominance of systemic symptoms (e.g., nocturnal fever, asthenia, weight loss, peripheral adenopathies), a high likelihood of tuberculin test negativity, and a high incidence of extrapulmonary involvement. All AIDS patients should therefore be subjected to active screening to identify TB disease or infection.

Physical examination

The physical examination of the patient with TB disease is likewise lacking in specificity and often contributes very little to the diagnosis. In many instances, the patient appears to be healthy. Nevertheless, a systematic examination is always required, noting possible clues such as the following:

- Crackling rales in the infraclavicular space or in the interscapular-vertebral zone, in relation to exudative and cavitary lesions.
- Uni- or bilateral bronchial rales (rhonchus, subcrepitations) in cases of bronchogenic disease dissemination.
- In cases of pleural involvement: dull percussion, absence or reduction of vesicular murmur.

- Evidence of extrathoracic locations:
 - Erythema nodosum
 - Cervical and submaxillary fistulas and adenopathies, anal fistulas, osteoarticular involvement
- If the patient presents with dysphonia, an indirect laryngoscopic exploration is advised.
- If haematogenous spread is suspected, the central nervous system and ocular fundus should be explored.

General laboratory tests

The general laboratory study likewise offers no characteristic information, although it should always be carried out for diagnostic purposes and, in some instances, for patient follow-up during treatment. While very non-specific, the following should be noted:

- Moderate anaemia and hypoproteinaemia in long-evolving cases.
- An increased erythrocyte sedimentation rate, which usually does not exceed 50 to 60 mm in the first hour.
- Altered coagulation test results.
- Acute and febrile presentations can show leucocytosis with neutrophilia, although lymphocytosis is more common in the subacute and chronic forms of the disease.
- In some instances, before starting treatment, changes in liver enzyme levels can be detected (e.g., increases in transaminase and/or gamma-glutamyl transferase levels). These changes are often not attributable to liver infiltration but to the host toxic state or alcoholism.
- Some serious disseminated cases can present with hyponatraemia or hypochloraemia due to inappropriate antidiuretic hormone secretion.
- The presence of haematuria without colic pain and pyuria with negative urine culture suggest possible renal TB.

Tests indicated for patients with suspected tuberculosis

When dealing with a patient with suspected TB at the peripheral level of the health care network, a serial study of three sputum smears is always required. If the results prove positive, the diagnosis of TB is assumed and the patient is admitted for treatment into the National Tuberculosis Control Programme (NTP). In turn, the approach indicated in the event of negative smear studies depends on the available resources and diagnostic methods. The most rational decision for low- and middle-income countries is to recommend a broad-spectrum antibiotic to assess patient response. If the clinical manifestations persist after this treatment cycle, repeat serial smear microscopy is indicated, with culture of one of the samples in solid medium. Where available, this step should also include a chest radiograph. On the other hand, a tuberculin test would be indicated in children. Other diagnostic tests are not indicated except in very rare situations.

Figures 9 and 10 show the algorithms indicated for attempting to establish a diagnosis of TB in symptomatic respiratory patients.



Figure 9. Approach indicated in symptomatic respiratory patients: first medical consultation.



Figure 10. Approach indicated in symptomatic respiratory patients: second medical consultation (patient continues to cough). *If the radiographs are suggestive of TB, action should be planned according to the available resources. At the peripheral level, if there are no further available resources, TB treatment can be started under TB control programme conditions. However, if the patient is at a reference centre that has other diagnostic possibilities, sampling involving other techniques should be assessed before prescribing treatment, such as invasive procedures (e.g., bronchoscopy, biopsy).

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Microbiological diagnosis

Section summary

Sample collection and handling influences the sensitivity of the different microbiological techniques employed. Whenever possible, samples should be collected before starting chemotherapy and in open areas or wellventilated rooms. The patient should be instructed on the correct way to collect sputum; if difficulties are encountered, induced sputum sampling can be attempted by clapping and/or a physiological saline aerosol, taking care not to discard any sample, even if saliva is involved. Bronchoscopy is not advised except in special instances. In children who fail to expectorate and who have been hospitalised, gastric lavage can be performed on 3 successive days. In the case of pulmonary TB, a serial study is required, collecting three sputum samples on 2 consecutive days. These samples should be clearly labelled and sent to the laboratory as quickly as possible, with refrigeration during the waiting period. All biopsy specimens should also be sent to the microbiology laboratory, without fixation and with a few drops of distilled water added to avoid drying. Formalin should not be used.

Despite the advances made in the past 20 years in relation to the microbiological techniques used for diagnosing TB, only a small portion of the global world population can benefit from them. The main means of diagnosis and treatment of TB in countries with low or middle incomes is smear microscopy with the Ziehl-Neelsen technique, due to its simplicity, rapidity, reproducibility, low cost, and effectiveness in detecting infectious cases. Its main inconvenience is its low sensitivity, which in turn is influenced by the severity of disease, sample quality, and time dedicated by the observer to read the smear microscopy result as negative. However, the specificity of the technique is very high (almost 100% in countries with average or high rates of endemic disease) and is only limited by false-positive readings attributable to other environmental mycobacteria.

The other basic technique in the diagnosis of TB is culture, which is the only method available that can establish a definitive diagnosis and which is appropriate for evaluating patient follow-up and ensuring cure. Culture, moreover, has higher sensitivity than smear microscopy. However, the inconvenience posed by the long waiting time to obtain a result (at least 6-8 weeks), its increased cost, and complexity in performance and maintenance prevent its use at the most peripheral level of health care. Consequently, for practical purposes and under programme conditions, the diagnostic and follow-up technique of choice is smear microscopy, despite its limitations. The above considerations imply that the indication for culture is dependent on the endemic area involved, and on the available health care infrastructure and resources. Thus, while the general use of culture is recommended in industrialised countries, use of culture is much more restricted in poorer nations, and somewhat more common in middle-income countries where bacterial culture should be included in the diagnostic algorithm of cases with negative smear microscopy findings (Figure 10). Solid media cultures should be the only ones indicated for routine use in low- and middle-income countries.

With regards to identification of the different mycobacterial species, biochemical techniques can be used, as well as chromatography and genetic probes. However, only biochemical testing is indicated on a routine basis in countries with low or middle incomes, particularly because of the lower cost, even though these methods may be complicated to perform, are slow, and lack reproducibility. It should also be noted that that the importance of species identification is very relative, in comparison with the importance of smear microscopy and culture, since in countries with medium or high rates of TB, over 99% of the cases of positive smear microscopy are attributable to *M. tuberculosis*. Indeed, cases involving environmental mycobacteria are so rare that only one laboratory with the capacity to identify mycobacteria species based on biochemical tests is needed per country.

In countries with low- or middle-income levels, the study of drug sensitivity should be carried out based on Canetti's method of proportions, using Löwenstein-Jensen medium. The time to reading is 4 to 5 weeks, and the laboratory must inform the clinician of the amount of growth occurring in the media with antituberculous drugs, in comparison with the media without medication. Again, the indication for performing such tests depends on the available resources and on the endemic disease found in each region, although sensitivity assays should always be performed in cases of treatment failure, relapse, and treatment discontinuation, as well as to periodically monitor levels of resistance. These susceptibility tests, however, have limited use in poor countries, since the information they offer is obtained late (by 4-5 months), is not always reliable (with no good *in vitro* and *in vivo* correlation), and is based on a group of patients (i.e., re-treated patients) that is not considered a public health priority (priority is instead given to initial cases with smear-positive results).

The contribution of microbiology to the diagnosis of TB depends on the quality of the samples collected and the techniques employed. The present section reviews the basic protocols for collecting, transporting, and processing samples, followed by a review of the different techniques. In this section, only the conventional diagnostic techniques will be discussed, as these are the only options recommended on a routine basis in low- and middle-income countries. The remaining microbiological methods will be examined in Chapter 8. This latter set of techniques is not indicated for general use in low- and middle-income countries, except in very specific cases.

Importance of sample collection and processing

A series of norms have been established for the collection, storage, and shipment of samples. These guidelines must be followed consistently, since any deviation may affect the sensitivity of the different microbiological techniques used. The basic recommendations for the handling of samples can be summarised as follows:

- 1. Whenever possible, sampling should be carried out before starting chemotherapy.
- 2. Sampling is to be carried out in open areas or well-ventilated rooms, and away from other people.
- 3. Sputum and urine samples are to be shipped in clean, wide-lipped glass or plastic containers with airtight screw-on covers. Sterilisation is not necessary.
- 4. If the sample is obtained by direct methods (e.g., puncture of abscesses, cerebrospinal fluid, biopsy), an aseptic technique is required, placing the collected material in a sterile container.
- 5. The sample container should be labelled with the patient's initials before shipment to the laboratory. Identification preferably should be made on both the container and cover.
- 6. Serial studies are advised to ensure improved performance. In direct sputum smear microscopy, one sample will yield on average 85% of positive readings, while two samples will yield 95% (i.e., the second sample raises the possibility of positivity by 10%), and three samples

will afford 100%. This is why the collection (and shipment) of three samples per patient is advised. Sending of more than three samples would add no further benefit.

- Since the most viable sputum sample is that collected in the morning, 7. it is advisable, in the case of hospitalised patients, to obtain the three samples early in morning on 3 consecutive days. However, this procedure is problematic when sampling is performed at the peripheral level, since the patient would be required to present to the health care centre on 4 consecutive days: the first day being the consultation and the next 3 days to deliver the samples. Thus, under control programme conditions, the first sample should be obtained at the time of consultation, while providing the patient with a second container for collection of the second sample early the next morning. When the patient returns on the next day to deliver this second sample, the final third sample is collected. In this way, the patient is only required to report to the health care centre twice, and three samples are obtained, with one sample being an early morning sample. A similar procedure can be carried out in the case of urine sampling.
- 8. The collected samples are to be sent immediately to the laboratory. If this is not possible, the samples should be kept in a refrigerator. However, many peripheral health centres in poor countries lack these facilities. In such instances, it may be advisable to perform staining *in situ* and to send the slide, instead of the sample, for examination. If only a smear microscopy evaluation is requested, there is no problem storing the sample for 7 to 10 days before shipment. The problem only occurs when cultures are required. In which case, sample shipment should not be delayed for more than 4 to 5 days; storage in a refrigerator will be required. If this measure is not adopted, the sensitivity of the culture decreases considerably, since the bacteria tend to die and will be unable to grow in culture. Still, identification by direct smear microscopy remains possible.
- 9. The patient should be instructed on the correct way to collect sputum; if difficulties are encountered, induced sputum sampling can be attempted by clapping and/or using a physiological saline aerosol.
- 10. Efforts should be made to obtain the best sample possible. If the patient only expectorates saliva, the latter should never be discarded for two reasons. First, collection of the saliva reflects patient effort and also ensures that the programme adheres to the requirements concerning the collection of such samples. If this saliva is not collected, it becomes difficult to determine whether poor sample

quality is involved or whether this important step in the case detection procedure is failing. Second, while the diagnostic yield of saliva is very low, it is not negligible, and many studies have shown that it can contribute a percentage of positivity readings that, although low, should not be ignored. Sputum stained with blood should also not be rejected, since they yield some diagnostic information.

- 11. If the patient fails to expectorate, sputum can be induced with a physiological saline aerosol, with the procedure performed in open areas or well-ventilated rooms. Due to the high prevalence of TB in countries with low or middle incomes, when there is a clinico-radiological picture compatible with the disease but negative serial smear findings, the diagnosis can be assumed and treatment started—except when no serial smear microscopy evaluation has been made. Therefore, despite the acknowledged usefulness of bronchoalveolar lavage and other samples obtained via bronchoscopy, these techniques are not indicated in low- and middle-income countries, except in very specific cases, in particular, those in whom there are sufficient criteria for considering other alternative diagnoses. In any case, if these samples are obtained, they must be shipped and processed immediately since the lidocaine used in bronchoscopy inhibits the growth of *M. tuberculosis*.
- 12. In children who do not expectorate, gastric lavage can be performed on 3 consecutive days. The microbiological study of these samples has been shown to be useful, since in the course of the night children swallow their respiratory secretions. The major inconvenience here is that in order to obtain an adequate sample, gastric lavage must be performed when the child is awake, since subsequent intestinal peristalsis rapidly eliminates the secretions swallowed during the night. As a result, patient admission for sampling is necessary. Unless the clinical picture is serious, it is not necessary to hospitalise children simply to perform gastric lavage.
- 13. All biopsy samples should also be sent to the microbiology laboratory, without fixation and with only a few drops of distilled water added to prevent dehydration. Formalin is to be avoided, although it is appropriate for samples sent to the pathology laboratory.
- 14. In HIV-infected patients, in whom disseminated TB is much more frequent, collection of all possible samples (including sputum, urine, cerebrospinal fluid, biopsy specimens of different organs) to confirm the diagnosis should be considered. In patients with severe immune

deficiency and fever of unknown origin, three blood cultures for *M*. *tuberculosis* may be adequate.

Evolution of the microbiological techniques to diagnose tuberculosis

The evolution of microbiological diagnostic techniques since the time Robert Koch first used smear microscopy in 1882 has undergone four well-differentiated phases, during which progress has been very uneven. The first and very prolonged phase extended up until the mid-1970s and was characterised by few advances. Laboratories used conventional technology, with limitations involving the low sensitivity of smear microscopy and the excessively prolonged duration of culture, identification, and the performance of susceptibility testing. The lack of important developments during this period was attributable not only to technical limitations, which were only overcome recently, but also to the fact that since TB posed less of a problem in the developed world, there was no great pressure to develop new, faster, and more sensitive techniques. This phase, which has since ended in the developed countries, remains the norm in low- and middle-income countries. As a result, only a small proportion of the world population is currently benefiting from the advances characterising the rest of the evolutionary phase.

The second stage spanned the second half of the 1970s and was characterised by the introduction of a new culture technology that has still not been surpassed today: the so-called radiometric growth detection systems. The main limitation of this new technology was the need to work with radioactive isotopes, which was a major obstacle for many laboratories that did not possess the license to store and work with such materials.

The third phase took place during the 1980s and was characterised by two important events that greatly influenced the evolution of diagnostic techniques. One event was the advent of HIV, while the other was the accelerated development of new technologies. The appearance of HIV in the early 1980s led to an increase in TB cases and in disseminated infections caused by environmental mycobacteria in severely immunosuppressed patients. The evolution of diagnostic techniques during this period was characterised by the development of rapid non-radiometric culture techniques, the standardisation of effective systems for isolating mycobacteria from blood (blood cultures), and the development of rapid identification techniques (e.g., genetic probes, chromatography) as alternatives to traditional biochemical methodology. This period in turn gave way to the fourth phase, comprising the last 10 years, which involves the development of new genetic amplification techniques for the rapid diagnosis of TB.

Still, these important advances in microbiological diagnosis are used almost exclusively in the richest countries, since their cost and complexity render them unfeasible in those poorer countries with the greatest TB burden.

Conventional microbiological techniques for diagnosing tuberculosis

Conventional microbiological techniques for the diagnosis of TB are the only methods recommended for routine application in low- and middleincome countries. Only in exceptional situations, which will be analysed later, are other techniques justified.

The conventional microbiological diagnosis of TB is based on four successive stages: 1) sample staining for direct visualisation under the microscope (smear microscopy); 2) solid medium culture; 3) identification of the microorganism using biochemical techniques; and 4) drug susceptibility testing.

Smear microscopy

M. tuberculosis is a gram-positive or frequently colourless bacterium, as a result of which it is often not visualised in samples subjected to routine processing.

The detection of acid-fast bacilli in stained preparations examined under the microscope constitutes the first evidence of the presence of mycobacteria in a clinical sample. The acid-fast characteristics of the microorganism are attributable to the high lipid content of the bacterial wall (see Chapter 4). This technique is the easiest and fastest option available, and offers preliminary confirmation of the diagnosis, which, under the conditions of a TB control programme, allows confirmation of the case and implementation of treatment. It also addresses a public health concern by identifying infectious cases and offering the possibility of removing these infectious sources in the community through adequate treatment.

The recommended approach comprises the classic Ziehl-Neelsen technique, which reveals *M. tuberculosis* as small, red-coloured curved rods (bacilli) over a bluish background (Figure 11). This technique is simple, very economical, and reproducible in any setting. Visualisation is

carried out under x1000 magnification in immersion oil and should last for at least 10 to 15 minutes. When reporting the results of the microscopic examination, the microbiologist should provide the clinician with an estimation of the number of acid-fast bacilli detected. A decrease in the number of bacteria is indicative of the efficacy of treatment. The number of bacilli observed is preferably scored by means of the following crosssystem:

()	Absence of acid-fast bacilli/100 microscopic fields
(+)	1-9 acid-fast bacilli/100 fields. Report numerically
(++)	10-99 acid-fast bacilli/100 fields
(+++)	1-10 acid-fast bacilli per field (observation of only 50 fields
required)	
(++++)	>10 acid-fast bacilli per field (observation of only 20 fields
required)	



Figure 11. Direct smear microscopy based on the Ziehl-Neelsen technique. *M. tuberculosis* appears as small, dark-red rods over a bluish background.

Smear microscopy is the technique of choice for the diagnosis of TB in all settings, in view that it is still better than the other more sophisticated techniques in five areas: 1) simplicity and reproducibility in any setting; 2) speed; 3) low cost; 4) high specificity; and 5) the ability to delimit contagiousness. For this reason, any method that is to replace smear microscopy must at least offer these five characteristics, in addition to improving test sensitivity. The major limitation of smear microscopy is its relatively low sensitivity; indeed, the great majority of cases are detected at fairly advanced stages of the disease. The non-visualisation of acid-fast bacilli in a clinical sample does not rule out the diagnosis of TB, since the lowest detectable concentration of bacilli is 10,000/ml of sample. Thus, the technique only serves to detect very advanced and contagious cases of TB. For instance, if a sputum sample only contains 5000 acid-fast bacilli/ml-still a high figure-and 0.01 ml is extended on the slide, the latter will contain only 50 bacteria, i.e., a single bacterium per 200 microscopic fields. If the technician examines 100 fields, the probability of seeing a bacterium is only 50%.

In effect, the sensitivity of smear microscopy is relatively limited. This implies that a negative result does not exclude the disease, since many false-negative results may occur. This possibility of yielding false-negative results (sensitivity) can be influenced by three important factors. The first pertains to the stage of the disease. In this sense, sensitivity is high (80-90%) in a patient with TB who has a cavitary pattern on chest radiograph, but decreases in those who present with only TB infiltrates (50-80%), decreasing particularly in patients with nodular forms or masses (under 50%). The second factor pertains to sample quality and performance of the technique. As mentioned before, it is essential to obtain the best samples possible, and many studies have shown that the highest sensitivities are achieved with purulent sputum, followed by mucopurulent and mucous samples, and saliva. Sample quality is often not taken into account, but nevertheless it is an important consideration when trying to compensate for the low sensitivity of smear microscopy. The last factor involves the time spent by the technician or microbiologist in examining the sample under the microscope. It is known that 100 fields of the slide correspond to 1% of the smear, 200 fields to 2%, and 300 fields to 3%. In this context, the examination of 300 fields, which is the number needed to ensure a negative result with a high degree of certainty, would take 15 to 20 minutes. Persons studying the samples, however, often spend less time in examination. If the smear microscopy is clearly positive (++ or +++) (Figure 11), very little observation time is needed to confirm the result.
The problem of time mostly occurs with apparently negative results, which is the case with most slides. Therefore, not spending enough time to examine the sample can lead to a false-negative result. In turn, falsenegative results imply that contagious cases remain unidentified and are left untreated in the community. The need to spend adequate time in examining samples means that a technician working full time on smear microscopy can only examine a maximum of 25 to 30 slides in a day.

The acid-fast staining characteristics with smear microscopy are common to all species belonging to the genus *Mycobacterium*, as well as to some fungal species. As a result, the rest of the environmental mycobacteria appear the same under the microscope. Moreover, while lacking the typical bacillary shape, some fungi, *Nocardia* species, or even food particles, dirt, or scratches on the slide can mislead the inexperienced observer. This may slightly reduce the specificity of the technique. Still, in countries with high and medium burden of TB disease, over 99% of all cases of positive smear microscopy are effectively attributable to *M. tuberculosis*. For this reason, under the norms of a TB control programme, in countries with low- or middle-income levels a positive smear microscopy result in itself is sufficient to accept a case as having TB and to start treatment.

Sometimes the culture becomes negative before smear microscopy because the treatment provided to the patient makes the bacilli non-viable. The mycobacteria though continue to be eliminated by the host and continue to exhibit acid-fast staining characteristics. This situation gives rise to false-positive results owing to the existence of "non-viable bacilli". Despite these results, such patients have very little potential to infect, and their course is favourable. As will be commented later on, culture (not smear microscopy) is the only acceptable method for following up on a TB patient and for ensuring that cure has been effective. However, under TB control programme conditions, the logistical problems posed by culture examination make it necessary to resort to smear microscopy for patient follow-up, despite the fact that microscopy may not indicate the true course of the disease (e.g., smear-positive with negative culture results).

Mycobacterial culture

Mycobacterial culture is the only means of ensuring a definite diagnosis of TB (with the corresponding identification), and the only acceptable method available for assessing patient follow-up and confirming cure. For this reason, in countries with sufficient economic resources, all clinical

samples suspected of containing mycobacteria should be grown in adequate culture media. However, there are limitations that reduce the use of culture in low- and middle-income countries.

The results of culture are largely dependent on the previous steps of sample decontamination and digestion. Most clinical samples contain abundant commensal flora that grow faster than *M. tuberculosis*. These contaminating microorganisms must be eliminated from the sample since they would prevent the development of any mycobacteria present. It is also important to liquefy the organic remains (e.g., tissues, mucus, serum, and other proteinic materials) surrounding the microorganisms, so that the decontaminating agents can destroy the undesired bacteria. This would allow the mycobacteria to survive and access the nutrients contained in the medium. Mycobacteria are more resistant to strong bases and acids than are other microorganisms, as a result of which such digestion-decontamination techniques can be successfully used. However, decontamination should be performed with care because excessive or insufficient decontamination may adversely affect the viability of the mycobacteria in the sample, giving rise to false-negative results.

Culture offers several advantages that define it as the gold standard for the diagnosis and follow-up of TB cases. These advantages can be summarised as follows:

- 1. Cultures are much more sensitive than smear microscopy, and are able to detect as few as 10 bacteria per millilitre of sample.
- 2. Isolation in pure culture is necessary to correctly identify the isolated strains, since other mycobacteria appear identical to *M. tuberculosis* by smear microscopy.
- 3. Culture provides definitive confirmation of negative conversion and healing of patients with treatment. In poor countries where problems associated with treatment (e.g., suspected resistance) make culturebased follow-up necessary, the number of colonies obtained must be quantified, since this parameter is vital for monitoring of treatment and for assessing possible treatment failure. The same considerations apply to TB cases in rich countries.

However, the logistical problems posed by culture limit its use, particularly in poorer countries. The main inconveniences of culture can be summarised as follows:

- 1. The main limitation of conventional culture is related to the slow divisional capacity of *M. tuberculosis*. This causes the time elapsed from sample receipt to reporting of the result to be no less than 4 to 6 weeks in conventional solid media, and much longer in poor countries. This is too long a wait for establishing a firm diagnosis.
- 2. The cost of culture is far greater than that of smear microscopy, and specific media are needed, with subsequent storage in an oven. Moreover, more specific training of personnel is required to perform cultures.

In view of the above considerations, it is not possible to use culture at the most peripheral levels of health care, unlike with smear microscopy. Thus, when TB is clinically suspected and smear microscopy proves positive in this setting, treatment should be started and the patient registered as a TB case.

The indication for culture is therefore dependent on the extent of the endemic disease in the area, and on the available health care infrastructure and resources. It can generally be concluded that in industrialised countries, which for many years have been successful in diagnosing cases using smear microscopy among mildly ill patients, and which possess many health care centres and laboratories that do not have economic constraints, culture should be performed whenever a clinical sample is received from a patient suspected of having TB. However, in poor countries, where the main challenge continues to be access to smear microscopy evaluation for all symptomatic respiratory cases, culture is only indicated in special situations. Priority in these poorer countries must be given to smear microscopy and treatment. Culture would be reserved for cases of suspected resistance (although the need for culture in this situation depends on the availability of second-line drugs). Culture would almost never be included in the diagnostic algorithm of patients initially presenting with negative smear microscopy results.

An intermediate position is represented by middle-income countries, where patients with positive smear microscopy results are often dealt with adequately, and where the economic constraints are not as severe as in poorer countries. Here, culture examination should be performed whenever the initial patient course with therapy proves negative (e.g., suspected failure, defaulter, relapse). However, in patients with persistent respiratory symptoms following an initial negative smear microscopy result and with no clinical response to broad-spectrum antibiotic treatment, a new smear microscopy evaluation is required, with culture of one of the sputum samples, in addition to a chest radiograph (Figure 10).

Traditional culture has always been made in solid medium, using coagulated egg (e.g., Löwenstein-Jensen, Coletsos) or agar (Middlebrook 7H10 and 7H11) as a base. These should be the only media indicated for routine use in countries with low- or middle-income levels, with preference going to Löwenstein-Jensen medium (Figure 12). Some mycobacteria, many of which are associated with patients with AIDS, such as *M. haemophilum, M. malmoense, M. genavense,* and *M. avium subs. paratuberculosis*, require culture medium supplemented with special growth factors, such as hemin, blood, mycobactin, or ferric ammonium citrate. Incubation of the seeded media in an atmosphere enriched with 5% to 10% carbon dioxide favours the growth of *M. tuberculosis*.



Figure 12. Löwenstein-Jensen solid culture medium showing the growth of colonies (rough, breadcrumb appearance) of *M. tuberculosis*.

These solid media offer the advantages of increased culture simplicity, counting bacterial colonies (which is important in the follow-up of patients exhibiting a poor bacteriological course), detecting growths of more than one mycobacterium in the clinical sample, and cost-effectiveness. However, solid media have the inconvenience of slow bacterial growth and manual reading of results (which can lead to errors). These drawbacks have led to the search for faster and more sensitive techniques, and have yielded new culture media such as radiometric methods (Bactec[®] system), biphasic culture media (MB-Septi-Check[®]), and techniques for isolating mycobacteria from blood. These methods will be dealt with in detail in the chapter on non-conventional methods and new microbiological techniques for the diagnosis of TB.

Identification of mycobacteria

The mycobacteria comprising the *M. tuberculosis* complex can easily be differentiated using a set of biochemical tests, since these microorganisms are niacin positive, reduce nitrates to nitrites, and possess pyrazinamidase (which allows the distinction between *M. tuberculosis* and *M. bovis*) as well as a heat-sensitive (thermolabile) catalase. On the other hand, any identification strategy aiming to go beyond the simple separation of *M. tuberculosis* from the other mycobacteria entails the use of complex identification techniques capable of addressing a minimum of 20 differentiating features. The main limitations of biochemical techniques are their complexity, slowness, and lack of reproducibility, although they are considerably less expensive than other options. These limitations have led to the development of fast, alternative identification techniques, such as chromatography and genetic probes (see Chapter 8). However, only biochemical tests are indicated for routine application in countries with low or middle-income levels.

Species identification, while one of the steps in the microbiological diagnosis of TB, is of less importance in comparison with culture and, particularly, smear microscopy. As has been mentioned, in countries with high and medium rates of TB disease, about 99% of the cases of positive smear microscopy are attributable to *M. tuberculosis*. In this context, and from the logistical perspective, in these countries a diagnosis of TB can be accepted in all cases of positive smear microscopy, without the need for species identification.

In low- and middle-income countries, the cases of disease caused by environmental mycobacteria are so infrequent that they justify the existence of only one laboratory per country with the capacity to perform identification based on biochemical tests. Often, in countries where culture is indicated, it is only necessary to identify *M. tuberculosis*—this being simpler than having to identify the rest of the environmental mycobacteria. The identification of other types of mycobacteria would only be justified in patients exhibiting a poor clinical response to initial treatment and standard re-treatment. Nevertheless, the percentage of disease caused by these other mycobacteria is increasing in wealthier countries, particularly where AIDS is more prevalent. In such countries, techniques for speciation of mycobacteria can be justified in all TB patients with a positive culture because these countries often possess simpler automated techniques for identification and have a higher number of these cases.

In vitro M. tuberculosis susceptibility testing

M. tuberculosis susceptibility testing can be carried out directly with a concentrates specimen when abundant acid-fast bacilli are observed under the microscope (direct method) or on a bacterial culture in the exponential growth phase (indirect method). The standardised techniques for *in vitro* sensitivity testing are the Canetti proportions and multiple dilutions technique, the Meissner absolute concentration method, and the Mitchison resistance level procedure—with all cultures conducted in Löwenstein-Jensen medium. The Centers for Disease Control and Prevention (CDC) in the United States recommend the proportions technique, but using a semi-synthetic medium (Middlebrook 7H10). At present, sensitivity studies can be performed based on radiometric technology (Bactec[®]), using a simplified and adapted version of the proportions method developed by Canetti. Of all these options, the only one recommended for routine use in low- and middle-income countries is the Canetti proportions technique using Löwenstein-Jensen medium.

Susceptibility tests in Löwenstein-Jensen medium require 4 to 5 weeks, versus 2 to 4 weeks in semi-synthetic media (Middlebrook 7H10 or 7H11), and versus 5 to 8 days with the radiometric technique. These susceptibility tests require their respective periods of time because they are performed on the positive culture, and not on the direct sample (direct method). As a result, in addition to the above-mentioned durations, we must add the time required for the culture to yield a positive result: 3 to 6 weeks in the case of solid media, and 12 to 21 days for the Bactec system. Thus, a sensitivity test using the Bactec method can be completed in less

than 1 month, while in practice it takes more than 3 months when solid media are used.

The laboratory must inform the clinician of the amount of growth occurring in the media with antituberculous drugs, compared with in an untreated control medium. When susceptibility testing is performed correctly, the control will contain countable colonies. Thus, the counting of colonies in the medium containing drug and in the control will allow calculation of the proportion of resistant bacteria in the total population, expressed as a percentage. In general, when 1% or more of the bacterial population proves resistant to a critical concentration of a given drug, the drug should no longer be used as treatment, since the resistant population will become dominant in only a short period of time.

Here again, the indication for performing the technique will depend on the available resources and the extent of the disease in each area. Thus, in rich countries where there are few TB cases and a ready availability of second-line drugs, susceptibility testing is indicated at least in all patients exhibiting a poor microbiological course, as well as in cases of failure, relapse, and treatment default. Controversy exists over whether all initial patients should be subjected to susceptibility testing before starting treatment. The CDC has recommended this practice since 1994, owing to a marked increase in treatment resistance in the United States. However, many other industrialised countries consider this measure to be unnecessary, instead advocating periodic representative studies (ideally every 5 years) to monitor resistance rates. In the case of low- and middleincome countries, susceptibility testing would only be indicated in cases of treatment failure, relapse, and default, as well as for the periodic surveillance of resistance in the country. However, in some very poor nations where the problem of TB is very serious, it may be questioned whether it is advisable to spend markedly limited resources on susceptibility testing at the expense of other priorities, such as ensuring initial therapy and access to smear microscopy evaluation for all symptomatic respiratory cases.

In effect, susceptibility testing poses an important practical limitation for use in poor countries, since the information they afford is obtained late, is not always reliable, and is based on a group of patients (re-treated patients) that constitute a less important epidemiological priority (priority is given instead to initial cases with smear-positive results). The recommended practice is to perform drug susceptibility testing in Löwenstein-Jensen medium. It is necessary to take into account that this test may require 4 to 5 months before yielding results: 2 months for culture, 1 month for identification, 1 month for the actual drug susceptibility testing results, and as much as 1 extra month lost as a result of sample shipment delays and the preparation of reports. Furthermore, the *in vitro* (drug susceptibility result) and *in vivo* correlation (diseased host) is close to 100% only for isoniazid and rifampicin. In most disease TB cases, clinical management decisions must be taken many months before drug susceptibility testing becomes available.

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Imaging techniques

Section summary

Chest radiography is a highly sensitive technique for diagnosing pulmonary TB in immunocompetent individuals, even though it is unspecific, since TB generates no pathognomonic radiological signs, regardless of how suggestive the images may seem. Thus, while there are radiographic images highly suggestive of TB, these findings are only an inferential aid in the diagnosis and suggest that microbiological evaluations should be carried out to confirm the diagnosis. In the same way, the prognosis and response to treatment cannot be decisively assessed by the radiographic course, since lesion regression can take place in a period of 3 to 9 months.

The role of chest x-rays in the diagnostic algorithm of TB is again dependent on the available resources and on the prevalence of the disease in the population seen. In rich countries, radiographic evaluation, together with smear microscopy, is recommended in all cases of suspected TB. In poorer countries, radiographic evaluation would take a second place. Its use in poorer countries should be restricted to patients with two negative serial smear microscopy results in whom antibiotic treatment is unsuccessful. Here again, it should be remembered that the focus is on patients with smear-positive results who are easily diagnosed at the peripheral level by means of microscopy. In very exceptional cases, computed tomography and other imaging techniques are indicated for the diagnosis of TB. Their application on a routine basis should be discouraged.

Both pulmonary and extrapulmonary TB present no pathognomonic radiological signs. Thus, although there are radiological manifestations highly suggestive of TB (e.g., upper lobe cavitations), and the accompanying clinical picture may be compatible with the disease in the context of a favourable epidemiological setting, the diagnosis of TB should never be accepted merely on the grounds of a radiological study. In effect, chest x-rays suggestive of TB can only indicate the need for an opportune microbiological evaluation. On many occasions, TB is diagnosed based only on the chest x-ray findings in patients who actually do not have the disease (Figure 13). In the same way, the disease is often not diagnosed in patients who do have TB (Figure 14).



Figure 13. Chest x-ray of a 72-year-old patient diagnosed with active pulmonary TB on the basis of the present image, when in fact the subject did not have active disease (inactive residual TB).



Figure 14. Chest x-ray showing a large mass in the upper right lung lobe. The initial clinical suspicion was bronchogenic carcinoma, although the pathology study and transbronchial biopsy culture confirmed the diagnosis of TB.

In addition, the radiological course should not be used to decisively evaluate the prognosis and response to treatment, since lesion regression can take place in a period of 3 to 9 months. There may even be a paradoxical increase in the number of lesions in the first month of therapy, without the suggestion of treatment failure. In view of the above, all patients with uncomplicated TB should undergo only two radiological studies (even in places where the technique is readily available): at the start and the end of treatment. Radiographs should not be obtained on each control visit, unless complications develop.

A suspected diagnosis of pulmonary TB is mainly based on the existence of a suggestive chest x-ray. Thus, the technique is highly sensitive but it is also unspecific. For instance, a normal chest x-ray can be found in the context of pulmonary disease, only in patients with some

forms of primary TB, in few cases of very early detection of the disease, and in severely immune suppressed HIV-infected individuals. In the rest of cases, including almost all instances of adult TB, the presence of radiological abnormalities is a constant finding.

When primary TB manifests radiologically, it usually does so in the form of an alveolar infiltrate with or without adenopathies (hilar or within the mediastinum) (Figure 15), or as lymph node involvement without parenchymal lesions (Figure 16). Other possible presentations include normal chest x-rays, pulmonary consolidation, adenopathies (generally unilateral), cavitation (rare and isolated), pleural effusion (more frequent in young patients and adolescents), and even atelectasia of certain lobes secondary to compression by mediastinal adenopathies (more common in children and adolescents).



Figure 15. Chest x-ray of a 42-year-old patient showing typical lesions of primary TB, with right hilar adenopathies and parahilar infiltration. The diagnosis of TB was confirmed by culture.

In post-primary TB, lung parenchymal involvement is a common finding, most often involving the apical segments and posterior portions of the superior lobes, or the apical segment of the inferior lung lobes (Figures 17 and 18). Cavitation is frequent in such cases (Figures 17 and 18), as is bronchogenic spread to other parts of the lungs (Figure 19) and pleural effusion (Figure 20). Tuberculomas (nodules of variable size) with variations in morphology may preferentially be located in the upper lobes (Figure 21). However, while the above are the most commonly affected sites, any portion of the lung could be involved, with unusual or atypical radiographic findings (Figures 22 and 23). Among these atypical findings are parenchymal consolidations that are indistinguishable from those found in pneumonia of other infectious aetiologies (Figure 24). This fact often leads to delays in diagnosis, particularly if the clinical presentation is sudden rather than gradual. It is sometimes necessary to obtain chest x-rays with the patient in hyperlordosis, to better visualise small lesions present in both lung vertexes (Figure 25).



Figure 16. Chest x-ray of a 7-month-old boy with lesions typical of primary TB. A large, mediastinal, adenopathic mass is observed, causing collapse of the left upper lobe. Diagnosis of TB was confirmed by histopathological study and culture of the biopsy specimen obtained via bronchoscopy.



Figure 17. Chest x-ray of a 36-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes, with retraction of structures towards that zone. The diagnosis of TB was confirmed by smear microscopy and culture.



Figure 18. Chest x-ray of a 39-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes. There was evidence of bronchogenic seeding in the left lower lobe. The diagnosis of TB was confirmed by smear microscopy and culture.



Figure 19. Chest x-ray of a 52-year-old patient showing lesions typical of post-primary TB. Infiltrates with necrosis and cavitation are observed in both superior lung lobes, with retraction of structures towards that zone. There was evidence of bronchogenic seeding in the rest of the lobes in both lungs. The diagnosis of TB was confirmed by smear microscopy and culture.



Figure 20. Chest x-ray of an 18-year-old patient showing a large left pleural effusion. The diagnosis of pleural TB was based on histopathological study of the percutaneous biopsy specimen.



Figure 21. Chest x-ray of a 49-year-old patient showing an irregular nodule with poorly defined margins in the right upper lobe. Bronchogenic carcinoma was initially suspected, although sputum culture confirmed the diagnosis of TB.



Figure 22. Chest x-ray of a 24-year-old woman showing a cavitary lesion in the right lower lobe. The diagnosis of TB was confirmed by smear microscopy and culture.



Figure 23. Chest x-ray of a 73-year-old patient showing an irregular nodule with poorly defined margins in the right lower lobe. Bronchogenic carcinoma was initially suspected, although the histopathology and culture of the biopsy specimen obtained by thoracoscopy confirmed the diagnosis of TB.



Figure 24. Chest x-ray of a 24-year-old patient showing a segmental condensation in the right upper lobe. Community-acquired pneumonia was initially suspected, although smear microscopy and culture confirmed the diagnosis of TB.



Figure 25. Chest x-ray of a 42-year-old patient. The projection was made with the patient in hyperlordosis (lordotic radiography), which allowed the identification of a nodule in the right upper lobe that otherwise could not be visualised in either posteroanterior or lateral projection since the image of the nodule coincided with that of the collarbone. Sputum culture confirmed the diagnosis of TB.

Miliary TB can be a manifestation of both primary and post-primary TB. The typical radiographic findings consist of multiple fine nodules measuring less than 3 mm in diameter and generally very profusely distributed in the lower lung lobes (Figures 26 and 27). Visualization in these cases is greatly facilitated by identification of the retrocardiac space in the lateral chest x-ray projection (Figure 28). These nodules are sometimes better seen with systems that are able to magnify certain parts of the chest x-ray (Figure 29). It is important to know that these fine nodules may persist, even after the disease has been cured.



Figure 26. Chest x-ray of a 21-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.

In HIV-infected patients, the different radiographic findings that may be seen depend on the degree of immune suppression involved. If immune suppression is not severe and the patient was already infected with the TB in the past, endogenous reactivation of these bacilli is common, with the production of the typical lesions of post-primary TB (Figure 30). If immune suppression is severe, any exposure to a recent source of transmission, and even endogenous reactivation, will result in typical findings associated with primary TB, with frequent lymphatic involvement (Figure 31) and haematogenous dissemination. Among these findings, normal chest x-rays are common (Figure 32) and extrapulmonary involvement is frequent.



Figure 27. Chest x-ray of an 18-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.



Figure 28. Lateral projection chest X-ray of an 18-year-old female patient showing a disseminated miliary pattern. This miliary pattern can be better visualised in the retrosternal and retrocardiac space. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.



Figure 29. Amplification of the zone of the right upper lung lobe corresponding to a chest x-ray of an 18-year-old female patient showing a disseminated miliary pattern. The diagnosis of miliary TB was confirmed by the histopathological study and culture of the transbronchial biopsy specimen.



Figure 30. Chest x-ray of a 28-year-old female patient with AIDS showing a cavitary infiltrate in the right upper lung lobe. The CD4+ T-lymphocyte count was 468 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and sputum culture.



Figure 31. Chest x-ray of a 25-year-old patient with AIDS showing a large adenopathic mass in the region of the mediastinum and right hilum. No intraparenchymal lesions were observed. The CD4+ T-lymphocyte count was 84 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and culture of the specimen obtained by fine needle aspiration biopsy through the tracheal carina (bronchoscopy).



Figure 32. Chest x-ray of a 45-year-old AIDS patient showing no significant lesions. The chest x-ray can be considered normal. The CD4+ T-lymphocyte count was 43 cells/mm³. The diagnosis of pulmonary TB was confirmed by smear microscopy and sputum culture.

The pulmonary radiographic manifestations of environmental mycobacteria are very similar to those of TB when immunocompetent hosts are involved (Figures 33 to 35). The only apparent difference appears to be the absence of lesions suggestive of primary TB and the fact that some of these infections caused by environmental mycobacteria may manifest as solitary lung nodules (Figure 36). A relatively common presentation of lung disease caused by *M. avium* comprises small micronodules in the middle lung lobe, the visualisation of which may be greatly facilitated by computed tomography.



Figure 33. Chest x-ray of a 75-year-old female patient showing infiltration of the right lower lobe and middle lobe. Fibrous tracts are seen in the right upper lobe and lingula, with calcifications present in the zone. The initial positive smear microscopy study led to the diagnosis of TB, although the patient's symptoms failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. avium* complex.



Figure 34. Chest x-ray of a 48-year-old female patient showing a cavitary infiltration of the right upper lobe. The initial positive smear microscopy study led to the diagnosis of TB, although the patient's condition failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. kansasii*.



Figure 35. Chest x-ray of a 58-year-old female patient showing bronchial dilatations of the right lower lobe, suggestive of bronchiectasis. The initial positive smear microscopy study led to the diagnosis of TB, although the patient's condition failed to improve with the antituberculous treatment provided. Culture confirmed the involvement of *M. abscessus* complex.



Figure 36. Chest x-ray of a 52-year-old female patient showing a nodule in the left upper lobe. The patient was evaluated for haemoptysis, and bronchogenic carcinoma was initially suspected. However, bronchial aspirate culture confirmed the involvement of *M. abscessus* complex.

For a more correct assessment of the mediastinum, computed tomography is very useful, although apart from very exceptional cases, both computed tomography and other imaging techniques are not indicated for the diagnosis of TB. Their routine use is therefore not advised, although it must be stressed that TB can yield a series of characteristic images with imaging (Figures 37 to 39). Imaging can also be useful in detecting areas suitable for diagnostic puncture procedures in suspicious cases (Figure 40).

When deciding whether to include chest radiography in the diagnostic algorithm of TB, it is necessary to consider not only its good sensitivity and low specificity, but also to consider that it is more costly and less accessible than smear microscopy. For this reason, the inclusion of radiology in the diagnostic algorithm will vary according to the available resources and epidemiological characteristics of the region. Thus, in rich countries that have few TB cases, no restrictions in terms of resources, and radiological services available to the entire population, the ideal approach in the event of suspected TB would be to combine the good sensitivity of radiology with the high specificity of smear microscopy. The recommended approach in these countries whenever a suspected TB case is encountered is to always perform radiography and serial smear microscopy, with strict emphasis on the need to confirm the diagnosis by culture examination.



Figure 37. Computed tomographic scan of a 35-year-old patient showing a cavitary lesion with macronodules. TB was diagnosed from sputum culture.



Figure 38. Computed tomographic scan of a 28-year-old patient showing bronchogenic dissemination in the form of centrolobular nodules. TB was diagnosed with smear microscopy and culture.



Figure 39. Computed tomographic scan of a 32-year-old patient showing infiltration with cavitation. The arrow indicates the presence of so-called "tree-in-bud" lesions. TB was diagnosed with bacilloscopy and sputum culture.



Figure 40. Computed tomographic scan of a 56-year-old patient showing a cavitary lesion. TB was diagnosed by histopathological study and culture of the sample obtained by fine needle aspiration biopsy through the chest wall. Computed tomography was used to guide the needle biopsy.

However, in low- and middle-income countries, the primary focus should be on the smear microscopy-positive patient, who can be easily diagnosed at the peripheral health care level using microscopy. Moreover, these countries often have no access to radiographic equipment, and the cost of such studies allows these techniques only to be used in patients presenting with two negative serial smear microscopy results and in whom broad-spectrum antibiotic treatment has been ineffective (Figure 10).

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Tuberculin testing

Section summary

Tuberculin testing is of very limited value in the diagnosis of TB. However, in children, particularly those under the age of 5 years, in whom the prevalence of *M. tuberculosis* infection is very low, a positive tuberculin test indicates either very recent infection or actual disease. This is why the tuberculin test offers a high positive predictive value (PPV) for diagnosing tuberculous disease in this age group. The other group in which the test also yields a high PPV for diagnosing tuberculous disease is persons with severe immunosuppression. It should be noted, however, that a negative result never definitively rules out active disease.

The information relating to tuberculin testing in the diagnosis of tuberculous infection has been addressed in detail in the previous chapter. However, there are several points regarding the possible usefulness of the test in diagnosing TB. The positive predictive value (PPV) of the tuberculin test for diagnosing active tuberculous disease (i.e., the probability that a Mantoux-positive individual has active TB disease) is greatest in children, in whom the prevalence of TB infection is low, and in those with a positive tuberculin test result and a suggestive clinical picture of disease. The test can also be very useful in infected, high-risk patients, such as patients with HIV infection, silicosis, diabetes, or immunosuppressive disease; who use intravenous drugs; who suffer from malnutrition; or who are undergoing chronic immunosuppressive therapy.

In addition, it should be noted that a negative result does not necessarily rule out the diagnosis of TB disease, since the patient in question may have an impaired host response to the tuberculin (Table 4), as in the case of disseminated TB and pleural TB. It should also be taken into account that with the exception of HIV-infected individuals, elderly patients are the subjects who most often have negative tuberculin test results.

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Histopathological diagnosis

Section summary

In certain situations of negative smear microscopy, and when haematogenous dissemination, extrapulmonary TB, or neoplastic disease is suspected, biopsy samples may be required. Here, the diagnosis is based on the identification of caseating granulomas, although other disease processes can also produce caseating granulomas—particularly when other environmental mycobacteria or certain fungal species are involved. Consequently, the biopsy sample must always be sent to the microbiology laboratory for culture. Histopathology offers a high-probability diagnosis that justifies the start of treatment if the clinical and radiological pictures are suggestive, while waiting for culture confirmation.

In certain situations, TB is diagnosed on the basis of granulomas in samples collected via different organ biopsy techniques (e.g., bronchial, transbronchial pulmonary, thoracotomy pulmonary, hepatic, lymph node, bone marrow). These situations generally involve cases that are difficult to interpret, that have repeatedly negative microbiological findings (haematogenous dissemination, extrapulmonary TB), or that involve patients with suspected neoplastic disease. Thus, the diagnosis may be unexpected, as in the case where a solitary lung nodule was actually found to be TB (Figures 21 and 23).

The diagnosis is based on the identification of caseating granulomas with Langhans' cells (Figures 41 and 42), which are highly suggestive of TB. However, other disease processes can also produce a very similar histopathological picture, in particular other environmental mycobacteria and some fungal species. Moreover, such microorganisms can produce clinical and radiological pictures very similar to those associated with TB. In AIDS patients, however, it is very rare to find typical granulomatous lesions because of the changes in the immune system. As a general rule, the pathologist uses staining techniques to identify acid-fast bacilli (e.g., environmental mycobacteria, certain fungi). However, since this approach is not 100% reliable, a biopsy sample must be sent to the microbiological laboratory for culture whenever TB is suspected.



Figure 41. Microscopic view of a granuloma showing caseous necrosis typically associated with TB.



Figure 42. Microscopic view of a granuloma showing caseous necrosis typically associated with TB. Note the presence of epithelioid cells and Langhans' giant cells.

The detection of tuberculous granulomas in biopsy specimens in patients with clinical and radiological evidence suggestive of TB is sufficient indication to start treatment, while waiting for the culture results to confirm the diagnosis.

When a biopsy sample is obtained to identify the cause of death, macroscopic lesions highly suggestive of TB may also be identified. Such lesions may consist of cavitations (Figure 43), extensive bronchogenic disseminations (Figure 44), or even highly significant miliary TB lesions (Figure 45).

Recommended reading

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Figure 43. Macroscopic view of a lung affected by TB. Caseous necrosis is extensive, and a large cavitation can also be seen.



Figure 44. Macroscopic view of a lung affected by TB. Caseous necrosis is extensive, and significant bronchogenic dissemination is also observed.


Figure 45. Macroscopic view of a lung affected by miliary TB. The miliary pattern can be seen throughout the lung parenchyma.

Non-conventional methods for diagnosing tuberculosis: new techniques

This topic will be discussed in detail in the following chapter. However, it should be mentioned that despite the many techniques that have been developed in the past 20 years, almost none are currently indicated for the routine diagnosis of TB in low- and middle-income countries. Despite the advantages offered by some of these techniques, most are very expensive and complicated to use. Consequently, the conventional or routine diagnosis of TB must be based on the five major sections in this chapter.

Conclusions regarding the diagnosis of tuberculosis with conventional methods

Section summary

Diagnosis of TB:

- 1. *Certain*: positive sample culture with the identification of the *M*. *tuberculosis* complex.
- 2. *Highly probable*: justifies the start of treatment and acceptance of the case as constituting TB in the framework of an NTP:
 - Smear-positive results. No need for culture.
 - Caseous necrosis in a biopsy sample (must be cultured).
- 3. *Exclusion*:
 - Based on clinical, radiological, and laboratory criteria.
 - Samples must always be processed for smear microscopy and culture.

Based on the topics dealt with in this chapter, the only way to establish a firm diagnosis of TB is to obtain a positive culture, with identification of the mycobacteria as belonging to the *M. tuberculosis* complex. In countries with low or middle incomes, culture should be performed in solid media, preferably Löwenstein-Jensen medium, with identification using biochemical tests.

A highly probable diagnosis of TB comprises those cases offering smear-positive results, or patients who for different reasons have required the performance of a biopsy, with histological study showing granulomas with caseous necrosis suggestive of TB. However, on the basis of its high specificity, rapidity, simplicity, and low cost, a definite diagnosis may be accepted (within the framework of an NTP) if positive staining using the Ziehl-Neelsen technique reveals the presence of acid-fast bacilli. Thus, a smear-positive result represents a case of TB, and such a case should be registered with the NTP, and treatment is recommended.

In low- and middle-income countries, not all smear-positive samples should be subjected to culture since over 99% of such cases effectively correspond to TB, and culture examination would only add complexity and cost to case management at the peripheral level of health care. In these countries, culture is only indicated in those cases where several serial smear microscopy studies spaced at least 2 to 3 weeks apart prove to be negative, the symptoms persist, and the possibility of other diseases cannot be ruled out. Culture is also advised in the instance of suspected drug resistance or when the disorder may be caused by other mycobacteria. However, all biopsy specimens from cases of suspected TB should undergo culture examination.

Lastly, patients with clinical and radiological signs that are highly suggestive of TB may also be accepted as disease cases, even when microbiological studies have yielded negative results, other possible diseases have been ruled out, and the patient has been cured with antituberculous treatment. This constitutes what is known as an exclusion diagnosis, which requires microbiological confirmation, even if the results later prove to be negative. Such patients never exceed 10% of the total cases. In short, a diagnosis of TB should not be accepted based only on clinical and radiological criteria, without the performance of microbiological studies. Unfortunately, such diagnoses are still accepted too frequently.

Chapter 8. Non-conventional methods and new techniques for diagnosing tuberculosis

Chapter summary

The last 10 to 15 years have seen a great increase in research on the diagnosis of tuberculosis (TB), with the introduction of numerous new techniques. However, practically none of these techniques are indicated for the routine diagnosis of TB in countries with low- or middle-income levels. Despite the advantages afforded by some of these novel techniques, they have not been able to replace smear microscopy and culture in their respective indications. Further, most of these techniques are very expensive and complicated to perform.

Of all the methods described in this chapter, the following should be pointed out:

- 1. Smear microscopy involving fluorochrome staining (auramine) offers the advantage of rendering the bacilli fluorescent, allowing the mycobacteria to be seen at lower magnification levels and allowing the assessment of many more microscopic fields in less time. This time-saving benefit makes the technique cost-effective in laboratories that process more than 25 to 30 smears per technician each day.
- 2. Liquid culture media offer superior sensitivity versus solid media and, above all, faster detection of microbial growth, which can shorten the time to results by 2 to 3 weeks. However, their main limitations include increased contamination levels, difficulty in identifying mixed cultures, and inability to observe the morphology of the bacterial colonies.
- 3. While simple and fast, the new techniques developed for mycobacterial species identification are expensive. Moreover, 99% of all disease caused by mycobacteria is attributable to *M. tuberculosis*, and the identification of species other than those pertaining to the *M. tuberculosis* complex is of low epidemiological relevance in countries with a high prevalence of TB. As a result, such techniques are not indicated for use in low- and middle-income countries.

- 4. Many new methods have been developed for drug susceptibility testing, some of which yield rapid results and are simple to perform. However, their high cost, the lack of reproducibility, and the need for standardisation explain why none of them are recommended for routine use in countries with low- or middle-income levels.
- 5. The techniques based on the amplification of nucleic acids are fast (yielding results in less than 1 day) and highly sensitive, but they are also very expensive, may yield false-negative results, and require careful clinical interpretation in the event of a positive result.
- 6. Molecular biological studies can be useful in the field of epidemiology, especially for: i) determining the general epidemiological pattern of strains in a given population and in the control of epidemics; ii) differentiating between relapses and exogenous reinfections; and iii) studying cross-contamination in the laboratory. However, these are expensive and time-consuming techniques, and the results obtained must be evaluated in combination with conventional epidemiological practices.
- 7. The determination of adenosine deaminase in TB-affected serosal fluids has been sufficiently validated to recommend its use in middle-income countries, but not in the poorest parts of the world.

The present chapter will address the newer techniques for diagnosing tuberculosis (TB), although it may be more correct to refer to them as nonconventional techniques, since some of them have been in use for a number of years. Each technique will be reviewed in terms of its methodology, indications, advantages, and limitations that render it unsuitable for the routine diagnosis of TB. For increased convenience, the chapter has been divided into eight sections: seven addressing microbiological techniques—this perhaps being the field in which most research has been conducted—and one section on the remaining methods.

Reflecting the sequence used in the diagnosis of TB, the sections on new or non-conventional bacteriological techniques have in turn been divided as follows: smear microscopy, culture, identification, drug susceptibility testing, genetic amplification, serological diagnosis, and the potential contribution of these microbiological techniques to the understanding of the epidemiology of the disease.

Non-conventional smear microscopy techniques

Section summary

While fluorochrome staining (auramine) is comparable with the Ziehl-Neelsen technique in terms of advantages and limitations, it should only be indicated in laboratories that process more than 25 to 30 cases of smear microscopy per technician per day. The technique offers the advantage of rendering the bacilli fluorescent (more visible) and allowing the assessment of many microscopic fields at lower magnification levels and in less time.

Although the Ziehl-Neelsen method is the most widely used and is recommended as the standard procedure, fluorochrome staining (auramine) is equally effective and is based on the same principle as acid-fast staining. The advantage of this method is that the bacilli appear fluorescent (Figure 46); as a result, they can be seen much more easily and the examiner can work at lower magnification levels—allowing the assessment of many more microscopic fields in less time. While Ziehl-Neelsen staining requires operating at x1000 magnification and spending at least 10 to 15 minutes on observation, fluorescent staining makes it possible to work at only x200 to x400 magnification, as a result of which the preparation can be studied in only 2 to 3 minutes. However, positive readings with this technique must be confirmed by Ziehl-Neelsen staining, since Ziehl-Neelsen staining allows improved visualisation of certain details of bacterial morphology that, in the hands of an expert microbiologist, can provide clues to the identity of the *Mycobacterium* responsible for the disease.

Saving time is the main advantage of the fluorochrome technique. However, its limitations include the need for more costly technical equipment and materials that are difficult to maintain. A continuous electrical supply is also important, which can be a problem in very poor areas.

This fluorescence technique can be recommended for use in laboratories that process more than 20 to 30 smears per technician per day, where the resulting savings in time would compensate for the increased costs associated with equipment and materials. In practice, this would pertain to very few laboratories in countries with low- or middle-income levels.



Figure 46. Direct sputum smear microscopy using the auraminerhodamine technique. *M. tuberculosis* appears as small, fluorescent, yellow filaments over a green background.

The sample extensions can also be prepared with a cytocentrifuge, which allows mycobacteria to be concentrated in a smaller space on the slide. After staining, this affords increased sensitivity and observation convenience.

New mycobacterial culture methods

Section summary

The radiometric Bactec[®] system must be regarded as one of the most important diagnostic advances in the past 20 years. It offers important savings in time—both in detecting growth and in performing drug susceptibility testing—as well as superior sensitivity. Disadvantages include having to work with radioactive materials and the high cost. The new liquid culture methods have increased in usefulness, replacing the need for radioactive materials and affording full automatic performance, thereby facilitating manipulation. However, these methods are very expensive and would only be indicated for use in reference laboratories in wealthy countries. Although the biphasic systems are more sensitive than the radiometric systems and require no radioactive installations, they are not as time saving. This disadvantage, in addition to high cost and problems related to identification and drug susceptibility testing, largely limits their use. The use of blood culture techniques is not recommended in low- and middle-income countries, since their clinical manipulation is very specialised. Based on the high prevalence of TB in such areas, when TB is suspected (even if involving only fever of unknown origin) in an HIV-positive patient, the initiation of empirical antituberculous treatment is advised. In short, in places with limited resources, it is not acceptable to spend money on costly techniques considering the negligible public health benefit that may be derived.

In an attempt to overcome the main limitations associated with cultures, intense research in the last two decades has focused on the development of faster techniques that offer the added advantage of improved sensitivity performance. Consequently, three important advances were introduced in clinical laboratories: 1) liquid culture media; 2) biphasic culture media (MB-Septi-Check[®]); and 3) techniques for isolating mycobacteria from blood.

Liquid culture media

All such media offer the important advantage of increased sensitivity over solid media, particularly faster detection of bacterial growth (shortening the time to results by 2-3 weeks). However, their most important limitations are an increased contamination rate, the difficulty of identifying mixed cultures, and the inability to observe colony morphology.

Two liquid culture media systems have been developed: radiometric (Bactec[®] 460 TB) and non-radiometric (e.g., MGIT, ESP, MB/Bact).

Radiometric methods (Bactec[®] system)

For reference laboratories, the Bactec[®] system is undoubtedly the most useful diagnostic advance in clinical microbiology in the past 20 years. The technique automatically detects mycobacterial growth, measuring the amount of ¹⁴CO₂ generated by the metabolisation of ¹⁴C-radiolabelled substrate (fatty acids). The vials used contain 4 ml of Middlebrook 7H12 medium and the radiolabelled fatty acids, admitting inocula of up to 0.4 ml.

In comparison with the traditional culture systems, the Bactec method offers the following advantages:

- 1. Time saving (15-20 days) for the detection of growth.
- 2. Increased sensitivity both for detecting *M. tuberculosis* as well as for identifying other mycobacteria.
- 3. The possibility of identifying *M. tuberculosis* in 4 to 5 days, and of performing drug susceptibility testing for front-line drugs (isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide) in 3 to 6 days instead of the 21 to 42 days required by solid media. Pyrazinamide requires special Bactec medium.
- 4. The possibility of identification and drug susceptibility performance without having to perform subcultures.

However, the Bactec system also has several disadvantages, such as:

- 1. The need to use radioisotopes.
- 2. The high cost of equipment, reagents, and maintenance.
- 3. The need to use syringes, which can cause possible crosscontamination between samples, as well as the potential formation of aerosols.
- 4. Laborious performance, since a semi-automated system is used.

The main inconvenience of the Bactec system is the need for radiolabelled ¹⁴C-fatty acid, which means that the laboratory must obtain authorisation for the use and storage of radioactive material.

Based on the above considerations, use of the Bactec system appears to be justified only in reference laboratories in industrialised countries. In countries with low- or middle-income levels, the system is not warranted, since its main advantage (time savings) does not compensate for the increase in cost. Use of the Bactec system may be considered in reference laboratories in middle-income nations, but never in poorer countries, where the priority continues to be diagnosis by smear microscopy. A subject with smear-negative results and positive culture would already be receiving treatment, and a few additional weeks to confirm the diagnosis would have no effect on the treatment outcome of the patient or on the extent of the disease in the community. Non-radiometric automated systems

In recent years, and with the purpose of resolving the disadvantages of the Bactec system, new non-radiometric liquid media involving automatic reading have been designed and manufactured. Manipulation of the vials is simpler, since reading is totally automatic, thereby saving more time for the technical personnel. These new systems nevertheless retain all the advantages of the Bactec system, with the added benefit of continuous readings of mycobacterial growth (because of automated operation). However, they are associated with high cost, which limits their use in most low- and middle-income countries.

The systems that have been used most widely in recent years are the MGIT (Mycobacteria Growth Indicator Tube System[®]); the Bactec 9000 MB System[®]; the ESP Culture System II-Myco[®]; and the MB-BacT Mycobacteria Detection System[®]. All these systems are based on the Bactec methodology, with the exception that radioactive labelling has been replaced by the incorporation of a ruthenium compound (which emits detectable fluorescence as the partial O₂ pressure in the medium decreases as a result of microbial metabolism) or other substances. Culture positivity is therefore based on the detection of mycobacterial oxygen consumption, the decrease in atmospheric pressure within the vial, or CO₂ release from the culture medium.

Non-radiometric biphasic culture media (MB-Septi-Check[®])

A biphasic system was introduced in the market a few years ago for use with mycobacterial culture (MB-Septi-Check[®]), with the aim of affording a rapid and sensitive technique similar to the Bactec procedure, although without the need for radioisotopes. The system uses bottles containing 20 ml of Middlebrook 7H9 broth to which a device containing different solid media can be attached at the upper end.

This system has several advantages over the Bactec technique, such as the use of greater seeding inocula as well as somewhat greater sensitivity. Moreover, MB-Septi-Check allows growth in the solid phase, thereby allowing identification tests to be performed without having to re-seed, as well as allowing identification of mixed colonies. The main inconveniences are that growth detection is slower than with the Bactec system, *in vitro* sensitivity tests are not possible, and the presumptive *M*. *tuberculosis* identification system incorporated in the solid phase often fails.

Systems for mycobacterial culture in blood

The increase in disseminated infections caused by M. avium-intracellulare and M. tuberculosis in AIDS patients has prompted the development of techniques capable of detecting mycobacteria in blood. Among these systems, those best suited for this purpose are based on the lysiscentrifugation principle in the radiometric system, or on the new automated culture systems.

Both offer similar sensitivity. The main advantage of lysis centrifugation is that it allows the quantification of the number of bacteria per millilitre of blood, with serial control of the efficacy of the prescribed treatment. In contrast, direct seeding of blood in 13A medium, with subsequent control by the radiometric system, avoids many of the hazardous steps that are involved when using the lysis-centrifugation technique.

Use is indicated in AIDS patients with CD4+ lymphocyte counts of less than 50 cells/mm³ who present with fever of unknown origin, and only in high-income countries.

New techniques for identifying mycobacteria

Section summary

The relevance of identifying species other than those pertaining to the *M*. *tuberculosis* complex in regions with a high prevalence of disease, where over 99% of all mycobacterial diseases are caused by *M*. *tuberculosis*, is of little significance. Moreover, despite the simplicity and rapidity afforded by these new species identification techniques, their high cost and low clinical and public health utility make them unsuitable for use in countries with low- or middle-income levels.

The obvious limitations of the standard biochemical identification tests—complexity, slowness, and lack of reproducibility—have stimulated the development of rapid identification methods, including the NAP test in Bactec 12B, chromatography, and identification based on molecular techniques.

NAP test in Bactec 12B

NAP (*p*-nitro-alpha-acetylamino-beta-hydroxypropiophenone) is a precursor of chloramphenicol synthesis that inhibits the growth of *M. tuberculosis* complex, but not of the environmental mycobacteria. The test strain is inoculated in two vials: one corresponding to Bactec 12B and the other to Bactec 12B containing NAP. If the mycobacteria grow in the Bactec 12B vial, but not in the vial that also contains NAP, then the test microorganism effectively belongs to the *M. tuberculosis* complex. The test result is obtained in less than 1 week, without the need for re-seeding in solid media for conducting standard biochemical identification tests.

Chromatography

As has been commented, mycobacteria are characterised by a cellular wall with an extraordinary high complex lipid content (e.g., wax, mycolic acids, long-chain fatty acids, glycolipids). The lipid content of the wall is stable and specific to each mycobacterial species.

Among the most extensively studied mycobacterial lipids, mycolic acids and other cell wall fatty acids should be mentioned because of their taxonomic value. Mycolic acids can be separated with relative ease in the form of methylic esters via thin-layer chromatography (TLC) in silica gel. The TLC-based study of mycolic acids divides mycobacteria into groups, and definitive identification is achieved by gas chromatography.

Despite the high specificity of chromatography and the fact that there already are automatic systems that can perform the technique, both the equipment and materials are very expensive. As a result, they can only be recommended for use in reference laboratories in industrialised countries with experience in the field.

Genetic probes

Advances in molecular biology have allowed the identification of DNA or RNA sequences specific to each mycobacterial species. In order to allow hybridisation with these sequences, genetic probes have been developed, comprising fragments of complementary nucleic acids labelled with radioactive isotopes or chromogenic substances.

A probe is a biological reagent composed of a DNA fragment possessing a base sequence complementary to that of a genomic fragment of the microorganism. The probes are in turn labelled with different indicators that are easy to detect: radioactive isotopes (hot probes) or chromogenic substrates (cold probes).

When the nucleic acid of a microorganism is freed, and the released DNA is subjected to denaturalisation (i.e., the two DNA molecular strands are separated by physical procedures at a temperature of 90-140°C), the probe is able to bind (hybridise) with its homologous fragment, provided such fragment exists. Probe hybridisation with its homologous fragment is easily detected via means of the incorporated marker.

At present, several cold probes are available on the market, such as Gen-Probe[®] and Syngene[®], for the identification of *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. gordonae*. There are no commercially available probes yet for the other mycobacterial species.

The main advantages of genetic probes include simplicity of manipulation, adaptation to any laboratory, rapidity of the technique (identification in only 2 hours), and high specificity. The major disadvantages are that these methods do not identify species within the M. tuberculosis complex, and they are associated with high cost. Consequently, their use is limited to reference hospitals in industrialised countries.

Other molecular techniques

Polymerase chain reaction (PCR), which involves multiplication of a given DNA fragment millions of times, can be used for species identification in three ways:

- 1. Amplification with the appropriate primers, followed by detection of a specific fragment of a specific species via electrophoresis and ethidium bromide staining.
- 2. Amplification of a DNA fragment common to all mycobacterial species, followed by recognition of the product amplified with species-specific probes.
- 3. Amplification of a DNA fragment common to all mycobacterial species, followed by lysis of the product with restriction enzymes, and visualisation of the restriction fragments in agarose gel after staining with ethidium bromide. This methodology, known as PCR with restriction fragment length polymorphism analysis (PCR-RFLP or PRC), is very useful for rapidly identifying species belonging to the

genus Mycobacterium.

Characterisation of the hypervariable regions of the 16S ribosomal RNA molecule specific to mycobacterial species has allowed the development of a mycobacterial identification protocol based on the sequencing of their nucleic acids. After amplifying a genomic fraction via PCR, sequencing is performed, and comparison of the results identifies the species obtained with the known specific regions of each mycobacterial species. With this technology it has been possible to identify and describe new species belonging to the genus *Mycobacterium*.

New techniques for drug susceptibility testing

Section summary

All methods presented in this section are only indicated for use in very few reference laboratories in industrialised countries. Despite the attractiveness of many of these methods, and the fact that they are fast and sometimes also easy to use, their high cost, frequent lack of reproducibility, and need for standardisation exclude them from use in low- and middle-income countries. Once again, it is necessary to emphasise that the priority concern of these countries is the diagnosis and treatment of cases with positive smear microscopy findings. As will be addressed in the chapter on retreatment (Chapter 10), in these regions the management of cases with possible resistances must be included in the standardised treatment plans of the tuberculosis control programmes. Under these circumstances, a savings of 2 to 3 weeks spent in determining the drug susceptibility results affords no benefit, because in many instances the logistical problems also lead to important delays. In view of the above, re-treatment, when necessary, will have to be based on a detailed history of the drugs administered in the past (discussed in Chapter 10).

The World Health Organization and the International Union Against Tuberculosis and Lung Disease recommend the method of proportions or other standardised methods, with the antibiotic concentrations established for each medium. They also advise the establishment of internal quality controls (control of each medium and antibiotic batch, with the use of reference strains) and international controls (the minimum requirement being a standard concordance for isoniazid and rifampicin of 90%).

The reference drug susceptibility testing methods involving solid media were developed in the 1960s. There were no major advances until the 1980s, with the introduction of the Bactec[®] system. However, the advances in the 1990s have been impressive, partly because of the HIV epidemic

and the need to detect resistances in such patients as quickly as possible. The list of these methods is extensive and includes flow cytometry, rRNA detection, redox indicators, MGIT, MB redox, latex-alpha antigen, E-test, sequencing, ESP Mycosystem, PCR-SSCP, MycoBact/Alert 3D, Inno-Lipa, ATP detection, heteroduplex PCR, luciferase gene expression, and chromatography.

However, most of these techniques have not yet been adequately validated, and further studies are needed to evaluate them in comparison with the reference methods. To date, only four methods are accepted as references: three are based on solid media (resistance ratio, absolute concentrations, and proportions method), with visual reading after 21 to 28 days, while the fourth uses a liquid medium (the Bactec 460 radiometric technique), with semi-automatic reading and results after 3 to 5 days. To these times we must also add the time required to obtain a sample culture. The proportions method is the most widely used reference technique and is the only recommended option in low- and middle-income countries. Its main limitation is slowness in yielding results. Industrialised countries tend to employ faster techniques involving liquid media, although to date only the semi-automatic Bactec 460TB system is accepted as offering good correlation with the reference method. The fully automatic (nonradiometric) systems, which are as fast as the Bactec method, should be regarded as still being in the development phase. They represent the future trend in countries that do not have economic restrictions.

M. tuberculosis drug susceptibility studies can be made based on phenotypic or genetic techniques, as will be described below.

Drug susceptibility testing based on phenotypic techniques

The study of *M. tuberculosis* resistance based on phenotypic methods can be made using solid or liquid media, or using new technologies that are still in the evaluation phase.

Drug susceptibility testing of *M. tuberculosis* in solid medium

The methods most widely used in solid medium are:

1. *Method of proportions in Löwenstein-Jensen medium*, via either the indirect technique (culture of various centesimal dilutions) or the direct technique (in samples with high bacterial loads). This is the only recommended option in low- and middle-income countries.

- 2. Sensitivity testing in Middlebrook media (7H10 or 7H11), using plates with Middlebrook semi-synthetic media. Results can be obtained after 2 to 3 weeks.
- 3. *E-test system.* This method involves the use of strips impregnated with increasing concentrations of different antimicrobial agents. The mycobacterial suspension is distributed over plates containing Middlebrook 7H11 medium. This is a convenient and rapid method (5-10 days), although it is not always easy to interpret the minimum inhibitory concentration (MIC) and plate contamination is relatively easy. Further evaluation of the method is needed to determine whether it correlates well with the standard methods.

Drug susceptibility testing of *M. tuberculosis* in liquid medium

The great advantage of this type of medium is the savings in time (results in 4-14 days) and its simpler manipulation. However, its high cost, the use of radioactive material (in the case of the Bactec system), and the fact that most of these techniques have not been sufficiently compared contraindicate their use in low- and middle- income countries. The most widely used methods involving liquid media are the following:

- 1. *Bactec 12B system*. This is an adaptation of the solid-medium proportions technique. It is the only liquid-medium procedure to have undergone extensive evaluation and that has been validated in relation to the reference techniques. The method is widely used in industrialised countries because of its rapidity, simplicity, reproducibility, and concordance with the classic proportions technique.
- 2. Other liquid systems (*ESP II, MB-BacT, MGIT, Bactec 9000MB*). Despite their very promising future, most of these techniques have not been sufficiently compared or validated.
- 3. *Broth microdilution method.* Some authors have proposed broth microdilution methods to determine the MICs of chemotherapeutic agents against *M. tuberculosis*, and have suggested the use of Alamar blue or diphenyltetrazolium bromide to facilitate MIC reading. This allows the detection of resistance to rifampicin in only 3 days.
- 4. *Macrodilution method*. This is an adaptation of the Bactec 60 system to determine the MIC and minimum bactericidal concentration of the strains of *M. tuberculosis* and other mycobacteria in relation to each of

the drugs analysed. The system is easy to use in drug-association synergy studies.

In vitro susceptibility studies of M. tuberculosis using new technologies

The appearance of multidrug-resistant TB has increased the need for methods capable of rapidly determining resistance to antituberculous drugs. These technologies, which are still in the evaluation phase and thus have not been adequately validated, comprise the following:

- 1. Luciferase reporter phage assay. In this method, live mycobacteria are infected with TM4 mycobacteriophages expressing the luciferase gene. After adding luciferin substrate, light photons are emitted. The amount of light is proportional to the number of viable bacilli present. If the mycobacteria come into contact with an antituberculous drug to which they are sensitive, bacterial destruction will result and the phages will be unable to infect them, thereby resulting in the cessation of light emission. Results are obtained in a few days.
- 2. Phage amplified biologically (PhaB) system. This technique uses a mycobacteriophage presenting a lytic cycle within *M. tuberculosis* and *M. smegmatis*. The advantages of this method are its simplicity, rapidity (4 days), reduced cost, and biosafety (since many mycobacteria are destroyed by the phage), and it does not require the use of costly equipment. At present, the concordance of the technique with the proportions method is good for rifampicin (95%), but insufficient for isoniazid (85%).
- 3. *Flow cytometry.* The advantages of this system are its rapidity (24-30 hours), simple performance, and use of inexpensive reagents. However, its disadvantages include the high cost of the flow cytometer and the need to work under high biosafety conditions. The system has been used with good results in *in vitro* studies of environmental mycobacteria susceptibility (*M. avium, M. marinum, M. fortuitum,* and *M. gordonae*) to many antibiotics.

Drug susceptibility testing based on genetic techniques

These methods, especially those relating to genetic polymorphisms, require the availability of a specialised infrastructure that is very costly, and thus tests are restricted to research laboratories in industrialised countries. The most frequently used genetic techniques are described below.

DNA probes

The use of commercial DNA probes has been proposed in the study of drug resistance of M. *tuberculosis* strains to rifampicin and isoniazid. The sensitivity and specificity of this test after 5 days of incubation is 95% for isoniazid and 100% for rifampicin.

At present, attempts are being made to develop techniques that can allow early identification of resistance to different drugs based on the detection (either directly from the clinical sample or based on culture) of the point mutations responsible for generation of resistant phenotypes. This requires extensive knowledge of the genetic targets involved in the resistance mechanisms. The most important targets of such resistance to isoniazid and rifampicin are reviewed in Chapter 11.

Single-strand conformation polymorphism

The study of resistance based on cultures has been proposed, focusing on four target genes: *katG*, *inhA*, and *ahpC* for isoniazid, and *rpoB* for rifampicin. The methodology is based on the use of the single-strand conformation polymorphism technique, which allows the detection of a single mutation in a region of single-strand DNA from its altered secondary structure with respect to the single-strand DNA of wild strains. At present, this method can be standardised and can be used in laboratories possessing the required infrastructure.

INNO-LipA solid-phase hybridisation

Of the methods based on genetic procedures developed in the last few years, special mention should be given to Innogenetic's INNO-LipA solidphase hybridisation kit, which can detect over 80% of the mutations responsible for resistance to rifampicin in the *rpoB* gene. The system uses nitrocellulose strips containing a series of probes that, upon hybridisation with the amplified product, perform two functions: 1) verification that the strain belongs to the *M. tuberculosis* complex; and 2) full coverage of the *rpoB* gene, thereby confirming the existence of up to 86% of all mutations responsible for resistance to rifampicin. The technique can be applied directly to the clinical sample or culture, and offers excellent correlation with the traditional methods. This is a rapid method that, in the event of resistance to rifampicin, allows us to suspect that the strain is multidrug resistant, since 90% of all strains resistant to rifampicin are also resistant to isoniazid.

Diagnosis of tuberculosis using genetic amplification techniques

Section summary

The basis of genetic amplification techniques is to produce millions of identical copies of a specific and known nucleic acid sequence (DNA or RNA). These techniques are rapid (results available in less than 1 day) and highly sensitive, although they also have several limitations.

In smear-positive samples, these techniques offer very high sensitivity (>98%) and specificity (>98%), although in such cases the diagnosis has already been established by staining. Variability is greater in samples with smear-negative results, with the techniques that amplify RNA offering increased diagnostic possibilities. However, in these cases, a positive amplification result does not confirm the diagnosis of TB (1-5% false-positive results), and a negative reading does not rule out the diagnosis (sensitivity, 50-80%). Therefore, the result must be interpreted for each case in such situations.

The main disadvantages of these amplification systems are the falsenegative results generated by reaction-inhibiting substances—particularly in non-respiratory samples—as well as the clinical interpretation of a positive result. Based on these limitations and considering the high cost of these techniques, their use is absolutely contraindicated in countries with low- or middle-income levels.

In the past decade, a series of molecular biological techniques have been developed that are able to amplify the specific DNA and RNA sequences of the *M. tuberculosis* complex. Based on a single copy of DNA or RNA, and using an enzymatic process, they can generate millions of copies of the target nucleic acid, thereby facilitating the detection of *M. tuberculosis*. This technology has made it possible to overcome the main problems inherent in conventional microbiological techniques, allowing rapid diagnosis (between 2-8 hours) and improving the sensitivity of the traditional culture methods.

After solving some of the problems prevalent in the early days of these techniques, it is now possible to find many commercially available amplification systems that provide all the reagents needed and that are able to function under standardised conditions. The development of PCR technology has been followed by the development of second-generation genetic amplification systems, such as strand displacement amplification, ligase chain reaction (LCR), Q beta replicase, and transcription-mediated amplification. In general, these systems involve three fundamental steps: 1) preparation of the sample to eliminate inhibitor substances of amplification; 2) amplification of the nucleic acid specific to the *M. tuberculosis* complex, with different formats capable of amplifying both mycobacterial DNA and RNA; and 3) detection of the amplified product using various methods.

The evaluations made with clinical samples involving smear-positive results have yielded excellent results in terms of sensitivity (>98%) and specificity (>98%) for both DNA and RNA amplifying systems. Variability is greater in samples with smear-negative results, with the techniques that amplify RNA offering increased diagnostic possibilities. This finding is easily explained, since by amplifying ribosomal RNA, these techniques increase the amplification target 2000 to 3000 times, whereas in the case of techniques that use DNA, the target is present in the form of only a few copies in the microbial genome. Thus, with the AMTDT-2 system (one of the most extensively evaluated RNA amplification systems), sensitivity ranges from 83% to 85% in samples with smearnegative microscopy, and from 65% to 77% in non-respiratory clinical samples. In turn, PCR and LCR have been the most widely evaluated DNA amplifying systems. The studies based on PCR have yielded sensitivities of 50% to 60% in samples with smear-negative microscopy, with a significant reduction in the case of non-respiratory samples, owing to the number of reactions that are inhibited. This phenomenon does not occur with LCR, thus reflecting improved sample processing. The sensitivity and specificity performances of LCR in respiratory samples with negative smear microscopy findings vary from 53% to 72%, and from 71% to 78% in non-respiratory samples.

Based on the above, these techniques would have excellent applications in samples with smear-positive results, where, owing to high sensitivity and specificity, a positive reading would establish the diagnosis of TB, whereas a negative reading would strongly support the existence of mycobacteria not belonging to the *M. tuberculosis* complex. However, it should be pointed out that in these samples the diagnosis of TB has already been established by a more economical and simpler technique (i.e., smear microscopy). Moreover, over 99% of positive smear microscopy findings correspond to *M. tuberculosis* in countries with a high prevalence of TB

disease, causing such sophisticated techniques to lose their potential use in low- and middle-income countries. Further, in samples with negative smear microscopy results, a positive amplification reading does not ensure the diagnosis of TB (1-5% of false-positive results), while a negative reading does not rule out the diagnosis (sensitivity of 50-80%). In these situations, the result must be interpreted with caution for each case.

One main disadvantage of these amplification systems is the falsenegative results generated by interference by substances that inhibit reactions, particularly in non-respiratory samples. Another limitation is the clinical interpretation, which may involve a positive reading. For example, in most studies evaluating these systems, a small proportion of samples (1-5%) have a positive amplification result lacking culture confirmation and without clinical justification. In such cases, the presence of very few bacteria can result in a positive reading. In sites such as the pleura and meninges, a positive reading is always indicative of disease, although this is not the case in the lungs, where persons with inactive residual TB or healthy infected individuals always carry a significant number of bacilli. These false-positive readings due to the different amplification reactions reflect the problem posed by such sensitive techniques, and they are considered a major limitation. Another limitation is the fact that many of these techniques are not fully automated, thereby complicating work and increasing the risk of contamination by amplicons during manual processing of the samples.

Based on these limitations, it must be accepted that these techniques simply represent one more available diagnostic tool, whose results must be interpreted in the context of each individual patient—as a result of which they cannot be introduced for the routine diagnosis of TB. Moreover, their high cost only allows them to be used in rich countries; utilisation is absolutely contraindicated in low- and middle-income countries.

In summary, the amplification techniques currently available for the diagnosis of TB can be classified according to the nature of the amplified nucleic acid component (DNA or RNA).

Techniques that amplify mycobacterial DNA

Polymerase chain reaction

PCR has been by far the most widely used DNA amplification technique for diagnosing TB. The method involves the serial repetition of three well-differentiated steps (Figure 47): 1) denaturalisation of the double-strand

DNA molecule to form single-strand DNA; 2) alignment of the primers (synthetic oligonucleotides) to their complementary sequences; and 3) extension from the primers of a DNA chain complementary to the template DNA region to be amplified. This reaction is mediated by a DNA polymerase (TaqDNA polymerase). These three steps represent a single amplification cycle, and the repetition of each cycle implies an exponential increment in the amplification product (Figure 47). In order to perform this technique, it is necessary to have: 1) the DNA fragment to be amplified (present in the clinical sample); 2) primers (synthetic oligonucleotides) that bind to the denaturalised DNA and frame the sequence to be copied; and 3) TaqDNA polymerase, which is very stable at high temperatures. Specific probes have been developed for the detection of *M. tuberculosis*, *M. avium*, and *M. intracellulare*.

An automatic PCR technique has been developed, offering not only the attractive feature of automation, but also two new features: the introduction of an internal amplification control, and the use of magnetic particles bound to the specific probe for the capture of amplicons.

Ligase chain reaction

LCR comprises the same steps as PCR, although the technique uses four initiators or primers designed to flank the region to be amplified, arranging them in adjacent positions. Thus, the probes can be joined enzymatically via DNA ligase, after the action of DNA polymerase, to form the amplified product, which in turn serves as a template for subsequent amplification cycles (Figure 48). LCx MTB is a semi-automatic system that detects the amplified product via a microparticle enzymoimmunoassay technique.



Figure 47. DNA amplification by PCR.

Strand displacement amplification

This system uses specific primers, DNA polymerase, and a restriction endonuclease to yield approximately 10-fold amplification of the original target DNA after 2 hours of reaction. Although the system is complex, the reaction steps take place individually and, once the reaction has started, require no further monitoring. The technique offers the advantage of being isothermal (except for the initial denaturalisation step at 95°C), and requires no specialised laboratory. Moreover, it can be applied to a single or double DNA strand.

Techniques that amplify mycobacterial RNA

Transcription-mediated amplification

The AMTDT-2 system (Amplified *M. tuberculosis* Direct Test) is based on the amplification of ribosomal RNA via the synthesis of complementary DNA and RNA, using an enzyme mixture composed of reverse transcriptase and RNA polymerase (Figure 49). This is an isothermal and autocatalytic process designed to amplify mycobacterial ribosomal 23S RNA. The system is completely manual, requires no sophisticated instruments or installations, and can be easily used in any laboratory. Rapid results are obtained (in less than 4 hours), with high sensitivity and specificity. In turn, the AMTDT-3 system, similar to the above, offers the advantage of full automation of the amplification and amplified product detection processes, as well as the incorporation of an internal amplification control.

Initially described in 1988, this technique is based on the incorporation of an oligonucleotide designed to bind specifically to the target nucleic acid (23S rRNA). The system is fully automated, and detection of the amplified product is carried out via fluorimetric testing—the amount of fluorescence generated being proportional to the amount of RNA amplified.

Nucleic acid sequence-based amplification

This system constitutes a commercial development of the previously described transcription-mediation amplification method, and is very similar to self-sustained sequence replication. Amplification (under isothermal conditions) of ribosomal 16S RNA is achieved by the concerted action of three enzymes: reverse transcriptase, RNA polymerase, and RNA ase.



Figure 48. Schematic representation of DNA amplification mediated by L Q beta replicase.



Figure 49. Schematic representation of DNA/RNA transcription–mediated amplification. RT = reverse transcriptase.

Serological diagnosis of tuberculosis

Section summary

There is no justification for spending scarce resources on serological tests in poor countries. Despite the many studies published on the subject, the attempts to develop a serological technique to help in the diagnosis of TB have failed. Major problems related to sensitivity (similar to those of smear microscopy), specificity, and the interpretation of a positive result (inactive residual TB and disease by other atypical mycobacteria) have prevented the technique from being recommended for standardised use in the diagnosis of TB, even in countries with more economic resources. Only in very specific cases of TB with smear-negative results, extrapulmonary TB, and TB in children can this methodology be somewhat useful in diagnosis (despite its low sensitivity), although the result should always be interpreted in the clinical context of the patient involved.

Although more than 100 years have passed since the first attempt was made to diagnose TB using blood tests, and despite the many advances in microbiology in recent years, it must be recognised that failure has beset the field of TB serology. Studies evaluating different antigens and techniques have increased in the last two decades, and yet it has not been possible to resolve the problems of sensitivity and specificity. No doubt there have been several significant advances, but progress has been for the most part unsatisfactory. In short, serological testing has so many limitations that it cannot be used for the routine diagnostic assessment of TB.

The factors that have contributed to the recent advances in the serological diagnosis of TB include the use of more sensitive techniques and more specific purified antigens. From the technical perspective, testing by enzyme-linked immunosorbent assay (ELISA) appears to be the most useful, since it is rapid, can be automated, provides reproducible results, and offers optimum sensitivity performance. The best-known and most widely used purified antigens in recent years have been of a protein and lipid nature.

Current data indicate that the diagnosis of TB in the context of clinical practice, using ELISA with the best antigens available, would require a specificity of close to 100% in order to ensure good test predictive values. To this effect, it would be necessary to adopt cut-off points (to yield a

positive result) equal to the averages obtained in the serum of normal individuals plus 3 standard deviations—in which case the sensitivity usually does not exceed 50%.

At present, knowledge is still limited with regards to the dynamics of the appearance of immunoglobulins and their half-lives in the course of TB, and to the antigens that may prove most useful. It is also not clear why some patients with active TB do not have detectable antibody titres at the time of diagnosis. Until these questions are answered, the usefulness of serology in the diagnosis of TB will remain uncertain and the technique will be inapplicable in the context of general use.

Although many of the studies published on this subject have served to define antigens capable of affording very high specificity, the corresponding sensitivity depends on the site and severity of the disease. The best sensitivity corresponds to pulmonary TB with positive smear microscopy (65-85%), but this performance is only slightly better than that afforded by staining. Moreover, sensitivity decreases considerably in the presence of negative smear microscopy results (<50%) and in patients with extrapulmonary TB (<25-30%). Despite such low sensitivity, the best performance could correspond to cases in which smear microscopy cannot contribute to obtaining a rapid diagnosis. Likewise, in children, serological testing has very low sensitivity (<25%), but may be useful in the few cases in which the technique proves positive—particularly since smear microscopy will almost always be negative. Moreover, a positive result in children almost always indicates the presence of TB disease.

The interpretation of a positive result is complicated. It is necessary to emphasise the number of positive results recorded in healthy individuals with inactive residual TB—regardless of whether they have received treatment or not—and in those with mycobacteriosis. The former group of patients is extremely common in TB endemic regions (comprising all patients healed in the past), in whom a positive result indicates that the disease had existed and has been healed. This phenomenon can be explained by the dynamics involving the appearance and disappearance of TB antibodies. Up to as long as 20 years after TB healing, circulating antibodies may persist with the capacity to respond to serological testing. The same applies to the other mycobacteria involving antigenic determinants crossed with those of *M. tuberculosis*. Thus, the existence of a large pool of infected individuals with inactive latent TB further limits the possibility of employing serological diagnostic tests in low- and middle-income countries.

Microbiological techniques as an aid in tuberculosis epidemiology

Section summary

Recent studies involving molecular biological techniques have been very beneficial with regards to certain aspects of the epidemiology of TB. The most widely used marker, in view of its great discriminative capacity, has been the study of restriction fragment length polymorphism (RFLP), using the insertion sequence IS6110. The genome of *M. tuberculosis* contains on average 5 to 20 copies of IS6110, located at various positions along the chromosome. These studies have contributed valuable information for: 1) determining the general epidemiological pattern of TB strains in a given population; 2) outbreak investigations and TB control; 3) differentiating between relapses and exogenous reinfections; and 4) studying cross-contamination in the laboratory. The results obtained with this technique must always be assessed in combination with the information afforded by conventional epidemiology.

The high cost and complexity involved, as well as the high TB transmission rates found in low- and middle-income countries, limit the use and indication of this technique in these regions.

Traditionally, the study of the epidemiology of TB has been complicated by the absence of adequate strain markers. The only marker used until recently—the study of susceptibility to mycobacteriophages—has significant limitations owing to its low discriminating capacity. Other methods investigated, such as the enzyme type, serotype, and drug susceptibility pattern, have been unable to find practical application.

At present, the application of molecular techniques in epidemiology makes it possible to precisely determine the strains circulating in a given population. Such techniques include genomic enzyme restriction followed by field electrophoresis, restriction-hybridisation with probes complementary to repeated sequences in the genome of *M. tuberculosis*, and PCR-mediated amplification polymorphism. The most widely used, in view of its excellent discriminative capacity, is the study of restriction fragment length polymorphism (RFLP), using the insertion sequence IS6110. A standardised protocol has been developed, allowing comparisons of the results obtained from different laboratories, as well as the establishment of large-scale databases. This marker can be used for: 1) determining the general epidemiological pattern in a given population; 2) controlling epidemics; 3) differentiating between relapses and exogenous reinfections; and 4) studying cross-contamination in the laboratory.

The genome of *M. tuberculosis* contains a large number of copies (between 5-20 copies) of the IS6110 insertion sequence, located at various positions along the chromosome. Strains that are not related epidemiologically present their own restriction-hybridisation patterns and exhibit a high degree of polymorphism. In contrast, strains that are related epidemiologically exhibit identical patterns, and a clonality relation can easily be established. In strains with low or no presence of the IS6110 insertion sequence, it is necessary to use other molecular markers associated with increased polymorphism. These studies can be very useful for establishing the transmission dynamics in the community. However, the results obtained must be interpreted in the context of the study setting, and must always be assessed with the information provided by conventional epidemiology methods. Despite the attractiveness of these methods, they are very expensive to carry out and require expertise for interpretation. This disadvantage, together with the high rate of TB transmission in countries with low or middle incomes, make the application of these techniques impractical in these geographical settings.

Other non-microbiological techniques

Section summary

Of all the new techniques that have been developed in recent years in relation to the analysis of serosal fluids from patients with suspected TB, adenosine deaminase determination has shown great usefulness, with a sensitivity and specificity in excess of 95% in studies from highly endemic regions. The technique has been sufficiently validated to allow it to be used in cases of uncertainty (i.e., pleural TB) in middle-income countries, but not in poorer parts of the world.

In countries with high disease burdens, when pleural or other serosal TB disease is suspected, antituberculous treatment should be initiated if performing a biopsy is not possible. A series of simple laboratory tests applied to the fluid may also be helpful.

Many of the studies of pleural fluid and other serosal components in patients with TB serve to guide diagnosis. With few exceptions, unless there is positive smear microscopy of sputum or pleural fluid, a biopsy is needed to confirm the diagnosis of pleural TB. Samples obtained by this method must always be processed for smear microscopy, culture, and histological study.

Interpretation of pleural fluid testing

Serosal fluid affected by TB can be characterised as an exudate with a high protein concentration and glucose levels generally in excess of 60 mg/dl (3.3 mmol/l). Low glucose levels are associated with concomitant empyema, rheumatoid arthritis, delayed diagnosis, or advanced disease with pleural fibrosis and pachypleuritis. The fluid is generally acidic, and the total leucocyte count is usually less than 5000 cells/l. The typical finding involves more than 50% of mature lymphocytes, although polymorphonuclear cells may predominate in the early stages of the disorder. Mesothelial cells are scarce, with generally fewer than 10 cells per 1000 leucocytes. This absence of mesothelial cells has been attributed to the fibrinous layer covering the pleural surface, which prevents these cells from desquamating.

Adenosine deaminase

The determination of adenosine deaminase (ADA) in pleural fluid and other serosal fluids has been shown to be very effective in diagnosing pleural TB in recent years. This enzyme, which is involved in purine catabolism and whose main physiological activity takes place in lymphoid tissue, affords great sensitivity and specificity (>95% in highly endemic countries), although false-positive results have been detected in subjects with metapneumonic effusion, empyema, rheumatoid arthritis, lupus erythematosus, or lymphoma.

The usefulness of determining this enzyme has been sufficiently validated by numerous studies, thus allowing it to be incorporated in the diagnostic algorithm for suspected TB effusions. The sensitivity of microbiological techniques with regards to serosal TB is very low; as a result, the diagnosis of TB can be accepted in the case of a patient with suggestive clinical manifestations, radiographic evidence, and a positive ADA assay. This is particularly valid in places where a pleural biopsy cannot be performed, or when meningeal, peritoneal, or pericardic TB is suspected. The positivity cut-off points for this technique must be established by the laboratory performing the test, and depend on the origin of the fluid. Thus, the cut-off points for pleural or peritoneal fluid usually

range between 40 to 45 international units, whereas in the case of cerebrospinal fluid the range is usually 9 to 10 international units.

The technique is neither complex nor expensive, as a result of which certain reference centres (depending on the population) in middle-income countries may benefit from using the procedure. The technique is not indicated in poorer regions where, because of the limited resources available and the high endemic rates, the initiation of empirical TB treatment is recommended instead.

Other determinations in serosal fluid with suspected tuberculosis

Another test that has been shown to be useful in studying pleural TB is the determination of lysosyme (muramidase) in the fluid, and especially the ratio of lysosyme to the equivalent assay in serum. A ratio of over 1.2 has been shown to afford excellent sensitivity and specificity, with some false-positive results attributable to empyema and rheumatoid arthritis. However, further studies are needed to validate the technique before it can be recommended for routine use.

Since in tuberculous pleural fluid the proportions and absolute numbers of T lymphocytes are considerably increased versus blood values, the number of lymphokines caused by these cells will also be increased. One such lymphokine, interferon, has been shown to be a useful parameter in the diagnosis of pleural TB.

Lastly, there is evidence suggesting that levels of some tumour markers, such as immunosuppressive acidic protein (IAP) and alpha₁-acid glycoprotein, are significantly higher in tuberculous pleural fluid compared with in neoplastic pleural effusions. However, the measurement of these markers is not indicated in low- and middle-income countries.

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Chapter 9. Treatment of tuberculosis

Chapter summary

The treatment of tuberculosis (TB) is based on two bacteriological considerations: the combination of drugs to avoid the selection of drug resistance, and the need for prolonged treatment to ensure that all bacteria in their different phases of metabolic growth are effectively destroyed. Clinical and microbiological research between 1950 and 1970 led to the conclusion that the best treatment for a patient with sensitive TB is 2HRZ/4HR. However, considering that in many parts of the world pharmacological treatments have been used indiscriminately, resistance to isoniazid (H) is usually more than 4%. Thus, to prevent treatment failure and further drug resistance in this setting, ethambutol (E) should always be used during the first 2 months of therapy. It has also been demonstrated that if strict supervision of observed therapy is not established for the full duration of treatment, there is a high risk of developing resistance to rifampicin (R). For this reason, in areas where supervision of drug administration cannot be guaranteed in the second phase, ethambutol should be prescribed instead of rifampicin during the second phase, and treatment should be extended to 8 months. These inconveniences with rifampicin are clearly obviated if several drugs are used in combination (especially H+R and H+R+Z), at fixed doses, and incorporated in the same tablet—an approach that should always be recommended. Thus, the treatment scheme advised for all initial patients should be 2HRZE/4HR or 2HRZE/6HE.

Although all first-line drugs (isoniazid, rifampicin, pyrazinamide [Z], ethambutol, and streptomycin) are well tolerated, personnel treating and caring for patients should be aware of the few associated side effects. It is important to know how to proceed in the event of side effects, to deal with food-drug interactions, and to manage patients presenting with special conditions, such as renal failure, severe liver impairment, or pregnancy. All these aspects of management will be analysed in detail in this chapter, and must always be dealt with by specialist physicians in the field.

Lastly, it must be emphasised that extrapulmonary TB should be treated in the same way as pulmonary TB, and that it is essential to know when surgery is indicated, as well as when the use of corticoid therapy is warranted. Hospitalisation of patients with TB disease should be based on the severity of the disease, and occasionally to ensure adherence to therapy; hospitalisation should never be based only on the patient having TB.

A brief history

Section summary

The history of TB treatment comprises two major periods: the chemotherapeutic period, corresponding to the last 50 years during which TB became a curable disease, and the period covering all previous attempts to overcome this terrible disease. The pre-chemotherapeutic period of TB management made use of many different resources over the centuries, although practically none were particularly efficacious. The early years of the chemotherapeutic period gave rise to the currently accepted bacteriological bases for the treatment of TB, including the need to combine several drugs to counter the development of drug resistance. During the very early periods of the chemotherapeutic era (the late 1940s), it became apparent that resistance to antituberculous drugs was the result of flawed patient management on both the individual and community level.

Chapter 3 provides a detailed explanation of the efforts to combat TB throughout history. Up until the introduction of chemotherapy, most of these attempts were futile. All this began to change with the introduction of streptomycin by Waksman and Schatz in 1943, and its use against TB since 1944. This antibiotic led to clinical and radiological improvement, with negative conversion of smear microscopy in diseased individuals. Its only disadvantages were toxicity (as the drug was not very purified at the time) and the fact that after 2 to 3 months of treatment a significant proportion of patients suffered a relapse with TB that was no longer susceptible to streptomycin. Drug resistance, another means of M. *tuberculosis* to defend itself, was becoming known. Thus, the main disadvantage associated with streptomycin was the appearance of bacilli resistant to the drug in patients who had already received streptomycin therapy for some time (e.g., routinely for 2 to 3 months).

In 1944, therapeutic testing began with para-aminosalicylic acid, which was shown to have similar efficacy as streptomycin, in addition to the same toxicity and resistance problems. However, in 1949 it was seen that para-aminosalicylic acid, combined with streptomycin, also delayed or prevented the development of resistance to streptomycin. Still, a treatment offering efficacy, low cost, easy administration, and no side effects
remained elusive until the introduction of isoniazid, or isonicotinic acid hydrazide, a substance that had been first synthesised in 1912 but that was not experimentally tested against TB until 1951. In view of its advantages, isoniazid was referred to as the "miracle drug" against TB, and up until today there is no drug that surpasses it. However, it was soon realised that isoniazid alone could not resolve the problem of TB, and bacteria resistant to the drug rapidly appeared. Treatment was then shifted towards combining isoniazid with streptomycin and para-aminosalicylic acid in 1955. This long-term combination therapy became for the first time the only treatment capable of completely curing TB, without encouraging the emergence of drug-resistant mutants.

Based on the above considerations, the first and most important microbiological principle for TB treatment was established: the combination of drugs to counter the development of drug resistance. It also became evident that very prolonged treatment was required to prevent disease relapse. This second major microbiological principle was established a few decades later, based on universally accepted theoretical models. It is therefore essential to review these microbiological considerations in order to understand the current management requirements recommended for TB.

Microbiological bases for the treatment of tuberculosis

Section summary

The microbiological fundamentals of TB therapy were established between 1950 and 1970. The first principle attempts to respond to the large number of multiplying bacilli present in the tissue of a diseased host, and to the ability of *M. tuberculosis* to mutate after multiple divisions. Thus, to counteract this property of the tubercle bacilli, it is essential to know that several drugs must always be used in combination in order to avoid the development of drug-resistant mutants that can undermine the efficacy of a medication.

The second principle attempts to respond to the variable growth capacity of *M. tuberculosis* in different locations within lesions, which varies depending on metabolic status. For this reason, extended treatments are needed, to allow treatments to act upon the latent bacterial populations that divide very little during treatment because the prevalent surrounding environmental conditions are not conducive for proliferation.

At present, it is widely accepted that TB chemotherapy should be based on two important microbiological considerations: the combination of drugs to avoid the development of resistance, and the need for prolonged chemotherapy to prevent disease relapse.

Prevention of resistance: the need for drug combinations

If treatment is started in a patient with cavitary TB using only one drug, the patient experiences a first phase during which most of the bacteria are eliminated and the symptoms improve. This initial phase is followed by a second phase in which treatment selects the resistant bacteria, which in a short time become the dominant microbial population-the "fall and rise" phenomenon. In addition, the drug will lose its efficacy for the rest of the life of the patient, since TB resistance is chromosomal, definitive, and irreversible. In effect, although all the bacilli present in a colony originate from a single cell, the bacilli do not show a homogeneous behaviour against the different antituberculous drugs. Thus, beyond a certain number of microorganisms, spontaneous natural mutants arise during the successive bacillary divisions, which will be resistant to some of the drugs used. Such mutation is random and independent of the environment involved, but is closely related to the number of bacilli present and the type and concentration of the medication administered. The approximate number of bacilli needed for the appearance of a natural mutant resistant to each of the drugs is shown in Table 5. Table 6 describes the bacterial populations calculated for each of the different types of TB lesions. Thus, in a culture of *M. tuberculosis*, spontaneous natural mutation gives rise to one microorganism resistant to isoniazid for every 10^5 to 10^6 bacilli. This mutation is independent for each of the different drugs used, since different genetic targets are involved. The probability that resistance to two drugs may develop is equal to the product of their respective mutation rates.

Isoniazid (I)	$1 \ge 10^{5} - 10^{6}$ bacilli
	7 0
• Rifampicin (R)	$1 \ge 10^{7} - 10^{\circ}$ bacilli
• Streptomycin (S)	$1 \ge 10^5 - 10^6$ bacilli
• Ethambutol (E)	$1 \ge 10^5 - 10^6$ bacilli
• Pyrazinamide (Z)	$1 \ge 10^2 - 10^4$ bacilli
Quinolones	$1 \ge 10^5 - 10^6$ bacilli
• Others	$1 \ge 10^3 - 10^6$ bacilli

Table 5. Number of bacilli required for the appearance of a mutant resistant to different drugs.

Thus, all monotherapeutic regimens (real, or masked by combination with drugs to which resistance has previously been established or which prove ineffective) inevitably lead to treatment failure and to the development of resistance. When two or more drugs are administered, the risk of resistance is practically zero, since the volume of this bacillary population is not usually attained in the human body $(10^{13} \text{ for H+R and } 10^{19} \text{ for H+R+E})$.

Table 6. Estimated bacterial populations within different TB lesions.

•	Smear-positive TB	10^7 - 10^9 bacilli
•	Cavitary	10 ⁷ -10 ⁹ bacilli
•	Infiltrating	10^4 - 10^7 bacilli
•	Nodules	10^4 - 10^6 bacilli
•	Adenopathies	10^4 - 10^6 bacilli
•	Renal TB	10 ⁷ -10 ⁹ bacilli
•	Extrapulmonary TB	10^4 - 10^6 bacilli

The need for prolonged treatments. Bacillary populations of M. tuberculosis

As has been mentioned, *M. tuberculosis* is a strict aerobe whose growth and metabolic activity is proportional to the surrounding oxygen partial pressure and pH. The ideal conditions for the bacilli comprise a pH of 7.40 and an oxygen pressure of 110 to 140 mm Hg. Based on the different characteristics of the environment in which *M. tuberculosis* is found, four tubercle bacilli growth modalities have been established that condition the bases of the currently used drug combinations and the duration of treatment. The bacillary populations can be described as follows: Metabolically active and under conditions of continuous growth

This population is also referred to as emergent flora and represents most of the bacilli, with a population of 10^7 to 10^9 . These bacilli are easily detected in the expectorations of diseased individuals, and are located within the cavitary walls, where the oxygen pressure and pH are ideal for growth. These bacilli are located extracellularly and are responsible for the failure of anti-TB treatment and for the development of resistance if they are not homogeneously eliminated. This population is rapidly exterminated by the bactericidal action of isoniazid, and less rapidly by streptomycin and rifampicin. The ability of a treatment regimen to eliminate a particular bacterial population is referred to as its bactericidal activity, which can be assessed by the percentage of negative conversion of cultures at the end of the second month of treatment. Early bactericidal activity refers to the capacity of the drug to kill bacteria within the first 2 months of therapy. Some consider that negative conversion of cultures after 2 months may also be an indication of the sterilising capacity of the drug (see below).

Bacilli in the acid-inhibition phase

This is a scantly numerous population of about 10^3 to 10^5 bacilli. Their growth is inhibited by the acid medium contained within the phagolysosomes of the macrophages in the case of bacteria located intracellularly, or by the acidity in the necrotic inflammatory zones of the cavitary wall. The deficient oxygenation in the necrotic tissue also helps to inhibit their growth. Thus, since these bacilli lack metabolic activity, they are unlikely to be eliminated by the administered drug. For this reason, this population and the bacteria in sporadic multiplication phase constitute the so-called persistent bacterial flora, which represent the main source of microbiological TB relapse. The most active drug against this particular bacterial population is pyrazinamide. The action of isoniazid and rifampicin decreases by almost 50% when the pH of the environment decreases from 6.6 to 5.4, whereas the activity of pyrazinamide increases with increased acidity. The ability of drugs to eliminate this bacillary population and its sporadic multiplication is referred to as its sterilising activity, and can be quantified by the number of relapses that follow treatment. The sterilising capacity of pyrazinamide has been shown to reduce the duration of treatment to 6 months.

Bacilli in the sporadic multiplication phase

This population comprises approximately 10^3 to 10^5 bacilli, often located in solid caseum, where the pH is neutral. These bacteria undergo long dormant periods, with occasional and brief metabolic periods that last for hours. As a result, the administered drug is only able to destroy these bacteria during the brief metabolic periods, which may not occur during the course of therapy. Therefore, this population, together with bacilli in the acid-inhibition phase, are responsible for microbiological relapses after the conclusion of therapy. On the other hand, the limited and occasional activity of these bacteria prevents them from developing resistances. The drug of choice for eliminating this population is rifampicin, because of the rapid onset of its sterilising action (15-20 minutes, versus 24 hours as in the case of isoniazid).

Persistent or totally dormant populations

These bacteria lack metabolic activity, as a result of which anti-TB treatment is not effective against them. Probably only the individual host defence mechanisms are able to have some effect on this population. One hypothesis is that these bacteria are one of the populations responsible for relapse in patients with severe immunodeficiency.

Rationale for an ideal initial treatment regimen

Section summary

The microbiological bases for TB treatment indicate that the combination 2HRZ/4HR is ideal in all initial cases of the disease where sensitivity to all the drugs can be guaranteed. However, the high initial resistance rate to isoniazid (H) that is found in many parts of the world makes it necessary to add a fourth drug (ethambutol [E]) to this initial phase of therapy. Indeed, this resistance to isoniazid is found in almost all low- or middle-income countries, and these nations should recommend the use of a fourth drug in their respective National Tuberculosis Control Programmes (NTPs). Moreover, administering these drugs either daily or 2 to 3 times a week has equivalent efficacy, which makes the 2HRZE/4H₂R₂ or 2HRZE/4H₃R₃ regimens equally recommendable. However, in order to be able to recommend regimens with rifampicin (R) in the second phase, it is essential to ensure adherence to treatment in both phases, for in the event

of non-compliance, there is a serious risk of developing resistance to rifampicin—which is the drug requiring protection at the present time.

The regimen 2HRZE/6HE (or 2HRZE/6HT in areas where the prevalence of HIV infection is very low), although somewhat less effective than 2HRZE/4HR, should be recommended as the initial regimen in low-and middle-income countries where strict supervision of administration cannot be guaranteed in the second phase—a situation that is much more frequent than admitted by the different NTPs.

Drug formulations containing H+R, H+R+Z, H+R+Z+E, H+E, and H+T, in fixed-dose combinations in the same tablet, should be recommended for standard use in the context of control programmes. These formulations facilitate the supervision of patient adherence to therapy, improve compliance, and especially prevent partial default of treatment. This last factor is associated significantly with the emergence of drug resistance. The other drug combinations for which bioavailability studies are not available should be avoided.

Therefore, unless strict supervision of the administration of the entire treatment in the first and second therapeutic phase is guaranteed, the ideal regimen for use in low- and middle-income countries is 2HRZE/6HE. Only when such supervision can be guaranteed can 2HRZE/4HR be considered, regardless of whether the second phase includes daily or intermittent administration. Protection against the selection of resistance to rifampicin, and the possible invalidation of this drug, are the reasons for these differences in treatment recommendation.

The need to combine drugs in the fight against TB has already been mentioned. The drugs that selectively act upon the different bacterial populations are isoniazid (H), rifampicin (R), and pyrazinamide (Z); these three drugs should constitute the basis for an effective TB treatment regimen. The best treatment for TB tested to date uses isoniazid, rifampicin, and pyrazinamide during the first 2 months of treatment, followed by isoniazid and rifampicin for another 4 months. This regimen offers potent bactericidal and sterilising action, with few relapses (less than 1-2%) and side effects. Pyrazinamide should only be administered for 2 months, since after this period the great majority of lesions and cells presenting acidic conditions would have disappeared (i.e., the conditions of preferential action for this drug).

Thus, in all cases of initial TB in which sensitivity to all the drugs can be guaranteed, the ideal treatment is 2HRZ/4HR. However, there are important conditioning factors—microbiological (possible initial resistance to some of these drugs), logistical (impossibility of guaranteeing supervision of administration), and cost (some drugs are very expensive)—that make it necessary to consider a series of variables in relation to this theoretically ideal regimen. These recommendations can be outlined using a series of questions:

Why the need for a fourth drug in the initial phase?

Isoniazid and streptomycin (S) have been used extensively worldwide in the past decades, although sometimes inappropriately (e.g., frequent or masked monotherapies). This has caused the initial resistance rates to these two drugs to be high in many parts of the globe. The situation particularly affects isoniazid, which remains the basis for initial treatment. In the event of initial resistance to isoniazid, and considering the high proportion of natural mutants resistant to pyrazinamide that is in all bacterial populations (Table 5), it can be assumed that rifampicin is the sole remaining agent that can be used against very large microbial populations. For this reason, in areas with a high initial resistance to isoniazid, a fourth drug must be added during the first 2 months of treatment when the bacillary population is very high. By the second treatment phase, this population would have been reduced to such low levels that even with initial resistance to isoniazid, the number of surviving bacilli would be too small to generate a mutant resistant to rifampicin (Table 5). At present, unless the initial resistance rate to isoniazid is known (based on well-designed and conducted studies) and is shown to be less than 4%, a fourth drug should always be added during the first 2 months of therapy. This criterion is applicable to practically all low- and middle-income countries, as well as to many wealthier nations.

What is the ideal drug to add to isoniazid, rifampicin, and pyrazinamide in the initial phase?

Having accepted the need to add a fourth drug in the first phase of TB treatment, the choice may centre on streptomycin or ethambutol (E). The latter is preferable for both microbiological and practical reasons. The microbiological reason is that streptomycin has been as extensively used as isoniazid and its initial resistance rate in much of the world is therefore also high. Since the reason for adding a fourth drug is to prevent the emergence of resistances, streptomycin should be rejected in favour of ethambutol, a drug to which very few initial resistances have been

described. In turn, the practical reason is that streptomycin must be administered via the intramuscular route, which requires the availability of a nurse to perform injections. This complicates the administration of treatment at the most peripheral levels of health care, i.e., in areas where the person responsible for treatment may be a less qualified health worker or perhaps someone who does not work in health care (e.g., community leaders, teachers). Furthermore, in very poor areas where disposable syringes are unavailable, the risk of HIV transmission must also be considered.

Can the drugs be administered only 2 to 3 times a week? Intermittent treatments

At present, unless the initial resistance rate to isoniazid is known (based on well-designed and conducted representative studies) and is shown to be less than 4%, a fourth drug should always be added during the first 2 months of therapy. M. tuberculosis multiplies very slowly (approximately once every 14-24 hours), which allows drugs to be effective even when administered in a single daily dose. Moreover, for over two decades it has been known that the administration of a single dose of isoniazid is able to inhibit bacterial growth for 4 days, as a result of which it is equally effective to administer either two weekly doses or a daily dose. However, when the interval between doses exceeds 4 days, efficacy decreases. With rifampicin and ethambutol, growth inhibition is found to persist for more than 8 days, although the bactericidal action of rifampicin makes the latter much more effective. In any case, the efficacy of these two drugs is seen to be similar when administered daily or once a week. Similar considerations apply to pyrazinamide, which, at a pH of 5.6, inhibits mycobacterial growth for 9 days, following 24 hours of bacterial exposure to the drug. This inhibitor effect, which pertains to isoniazid, rifampicin, ethambutol, and pyrazinamide, is not applicable when the combination includes ethionamide (Eth) or thiacetazone (T). Although streptomycin is bactericidal and the other two drugs exert bacteriostatic effects, when the three are combined efficacy decreases dramatically when not administered on a daily basis, particularly in the case of thiacetazone. Therefore, if regimens including isoniazid, rifampicin, ethambutol, and pyrazinamide are recommended, an intermittent style of administration, in the form of two weekly doses, can be used with the same therapeutic safety margin as with daily dosing-the sole requirement being an increase in the amount of isoniazid, ethambutol, and pyrazinamide in each dose (Table 7). The

amount per dose of rifampicin should not be increased. However, in order for efficacy to be the same, a minimum of two doses per week is required. Consequently, National Tuberculosis Control Programmes (NTPs) that recommend such intermittent regimens should first ensure strict supervision of administration of the medication.

The fact that some programmes recommend administration 3 times a week (when it has been determined that twice a week is enough) can be attributable to practical reasons. It is in the second phase of therapy (with daily dosing in the first phase) when nonadherence to treatment increases. Hence, if a single weekly dose were to be missed, 2 weekly administrations would suffice to ensure therapeutic efficacy. However, if the problem of missing a dose occurs in the twice-weekly treatment regimen, the patient in effect would have received only a single weekly dose. This is a very dangerous situation, since rifampicin inhibits mycobacterial growth for 8 days and isoniazid does so for only 4 days. In effect, a single weekly dose of these two drugs means that the patient is actually receiving sequential monotherapy with rifampicin for 3 days of the week, with the risk of selecting for rifampicin-resistant mutants.

Furthermore, the use of an intermittent treatment regimen makes supervision of treatment adherence easier. In this case, supervision is necessary only twice a week instead of daily. This option is much less expensive (in the case of rifampicin, the most costly drug, it is not necessary to increase the dose at each administration), and toxicity is similar to that associated with daily dosing. Moreover, it has been suggested that by achieving greater peak concentrations in blood, the selection of resistant mutants is less likely.

Although the drugs could be administered intermittently from the start of treatment (inhibition of mycobacterial growth being achieved from the first dose), it is generally advised to begin therapy with a daily administration phase lasting 1 to 2 weeks, when the maximum bactericidal action takes place. Nevertheless, some studies have shown intermittent administration to be effective from the start, although involving four drugs in initial phases.

Should regimens without rifampicin be used in the second phase? When are they advisable?

The ideal therapeutic regimen for all initial TB cases comprises 2 months with isoniazid, rifampicin, ethambutol, and pyrazinamide, plus 4 additional

months with isoniazid and rifampicin—2HRZE/4HR. However, rifampicin and isoniazid are among the most suitable drugs for treating TB. Considering that the initial resistance rates to isoniazid are already high in many parts of the world, a top priority at present is to protect rifampicin from the development of mutants resistant to it. As has been explained in the previous section, if intermittent medication is provided in the second phase of treatment, each failure in dosing conveys a potential danger of rifampicin monotherapy for several days. If this happens occasionally, no problems are likely and the treatment will still be effective. However, the reality is that the most frequent practice among noncompliant patients is not that of abandoning treatment completely, but that of intermittent discontinuation of one or more of the drugs, which poses the serious risk of the development of resistances to rifampicin. For this reason, rifampicin should never be administered in the second phase of treatment unless strict dosing supervision can be guaranteed.

Economic and operational considerations led the International Union Against Tuberculosis and Lung Disease (IUATLD) to advocate a second treatment phase of 6 months with isoniazid and thiacetazone, administering both drugs in the same tablet, without the need for direct supervision of dosing. This regimen (2HRZE/6HT) is not only much less expensive than the previously mentioned treatment regimen, but also protects rifampicin against possible resistances (since rifampicin is administered only in the first phase, along with three other drugs). This scheme offers a highly bactericidal first phase requiring strict dosing supervision, and a selfadministered second phase that could be described as sterilising. Its extensive use in many parts of the world has confirmed its efficacy under TB control programme conditions.

Still, in the past 10 years serious problems have been reported as a result of the frequent and severe toxicity of thiacetazone in AIDS patients. As a result, thiacetazone has been replaced with ethambutol in treatment recommendations. This change still protects rifampicin from resistances, although the initial cost advantage is now lost because the new regimen is similar in cost to the one using rifampicin in the second therapeutic phase.

Should preparations of various drugs at fixed doses in the same tablet be recommended?

For years it has been discussed whether the bioavailability of drugs administered together in the same tablet is the same as when each drug is given separately. The controversy has been particularly intense in relation to rifampicin. Many studies have shown that the combinations of H+R, H+R+Z, and H+T can be used without any problems. The combinations of H+R+Z+E and H+E have also recently been manufactured, and bioavailability studies have reported favourable results thus far.

These formulations, which contain several drugs at fixed doses in the same tablet, should be recommended for use under TB control programme conditions, since they facilitate the supervision of patient adherence to therapy, improve compliance in patients for whom strict supervision is not possible, and especially prevent selective discontinuation of one or more drugs. This last point, in addition to causing treatment failures, promotes the selection of drug-resistant mutants. In theory, the problem of selective discontinuation of treatment should never occur if strict supervision of therapy is ensured. The achievement of strict patient adherence to therapy should therefore be a goal of all NTPs.

Drug combinations other than those specified above should be avoided. In some countries, it is common to find combinations, prepared by local companies, that are different from those specified. These products should strictly be avoided since they lead to the development of resistance owing to the low bioavailability of some of their active substances. The best strategy is to ensure that each country only purchases the drugs and combinations recommended by the respective NTPs, avoiding the acquisition of other products, even in private practice. While this objective is difficult to achieve in many free market–oriented economies, it should constitute a priority aim.

What are the recommended regimens for new TB cases?

Based on all the above considerations, the ideal treatment regimen should always include a first phase of 2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol. The approach in the second phase will depend on whether or not strict supervision of administration is possible. If adherence to treatment can be guaranteed, then the second phase should comprise isoniazid and rifampicin for 4 months, administered on a daily basis or intermittently to facilitate supervision. The recommended regimens would therefore consist of 2HRZE/4HR, 2HRZE/4H₂R₂, or 2HRZE/4H₃R₃. However, it must be re-emphasised that the use of these regimens requires strict supervision of drug dosing, which calls for the existence of a well-established, well-run NTP.

If adherence to treatment cannot be guaranteed, then the second phase should consist of 6 months of isoniazid and ethambutol combined in the same tablet. This prevents the selection of rifampicin-resistant mutants, as well as lowers the risk of augmenting the problem of multidrug resistance.

First-line antituberculous drugs: action and side effects

Section summary

The first-line drugs for the treatment of TB are isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin. This section discusses these drugs, which are the most effective and well-tolerated treatment options available, and which are associated with fewer adverse reactions as well as lower costs. Other drugs should not be used prior to using these first-line agents. Moreover, in order to facilitate patient adherence and improve tolerance, these medications should be administered together in a single daily dose.

Table 7 describes the mechanism of action, target bacterial population, most common side effects, and most frequent pharmacological interactions of the five first-line antituberculous drugs: isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), and streptomycin (S). These medications are the most effective and well-tolerated treatment options available. They are associated with fewer adverse reactions or side effects, and they are also the least expensive. As a result, the use of other drugs is not justified without first using these five agents.

These five drugs are to be administered simultaneously in a single dose, in order to facilitate patient adherence (and therefore efficacy), as well as to ensure improved tolerance and less toxicity. The only exception to this rule is represented by rifampicin and pyrazinamide, which should be administered at least eight to eleven hours apart. **Table 7**. First-line antituberculous drugs: target bacterial population, dosage, frequent side effects, and pharmacological interactions.

Drug	Daily dose	Twice- weekly dose	Side effects	Control	Interaction s	Action
Isoniazid	5 mg/kg Up to 300 mg/kg	15 mg/kg	Neuritis Hepatitis Hypersensitivity	SGOT SGPT	Phenytoin	Extra- + intracellular bactericide
Rifampicin	10 mg/kg Up to 600 mg/kg	10 mg/kg Up to 600 mg/kg	Hepatitis Febrile reaction Purpura	SGOT SGPT	Inhibits oral contraceptiv es Quinidine	Bactericidal all populations Sterilising
Pyrazinamide	15-30 mg/kg Up to 2 g	50 mg/kg	Hyperuricaemia Hepatitis	Uric acid SGOT SGPT		Intracellular bactericide Sterilising
Ethambutol	15-20 mg/kg	50 mg/kg	Optic neuritis	Red-green discriminati on Visual acuity		Extra- + intracellular bacteriostatic
Streptomycin	15-20 mg/kg Up to 1 g	25-30 mg/kg Up to 1 g	VIII cranial nerve damage Hypersensitivity	Vestibular function Audiogram Creatinine	Neuromuscu lar blocker	Extracellular bactericide

Alternative regimens when first-line drugs cannot be used

Section summary			
In all treatment regimens, attempts should be made to use as many first-			
line drugs as possible. If a drug that has been rendered ineffective is			
correctly identified, then the following regimens can be recommended:			
- If pyrazinamide (Z) cannot be used:	2HRE/7HR		
- If isoniazid (H) cannot be used:	2REZ/10RE		
- If rifampicin (R) cannot be used:	2HEZ(S)/10HE		
- If ethambutol (E) cannot be used:	2HRZS/4HR		

Before starting this section, it should be commented that treatment becomes greatly complicated when any of the first-line drugs cannot be used. For this reason, patients in whom these drugs cannot be used must only be cared for by specialist physicians with extensive experience in the use of second-line drugs and combinations. Unfortunately, many specialist physicians lack this knowledge and experience, and should therefore avoid treating such patients, since there is the risk of worsening the problem as a result of increased intolerance or resistance. Consequently, this section should only serve as a guide for physicians who are experts in the field. The first premise in this section should be the advice to use as many first-line drugs as possible in any treatment regimen. An account is provided of the regimens that should be recommended in the event that only one first-line drug cannot be used. These schemes constitute an effective guide for resolving the problems associated with serious adverse reactions to antituberculous drugs and demonstrated resistance to a single drug. Most adverse reactions are attributable to a single drug, and can be resolved by designing a treatment regimen to exclude that agent.

Prior to the introduction of rifampicin (R), the different drug combinations had to be administered for at least 18 to 24 months to ensure cure. The duration of treatment can now be shortened to 9 months owing to the bactericidal and sterilising action of rifampicin. With the subsequent "rediscovery" of pyrazinamide (Z), this duration has been shortened further, to only 6 months.

If, for some reason, pyrazinamide cannot be used in the initial treatment phase (e.g., due to a lack of availability, serious adverse reactions, or demonstrated resistance), a 9-month regimen should be advised with isoniazid and rifampicin, with supplementation with ethambutol (E) during the first 2 months of therapy (2HRE/7HR).

If isoniazid (H) cannot be used, because of toxicity or demonstrated resistance to the drug, then rifampicin and ethambutol should be given for at least 12 months, with supplementation with pyrazinamide during the initial 2 months (2REZ/10RE).

On the other hand, if rifampicin cannot be used, then the isoniazid and ethambutol regimen is again advised for a minimum of 12 months, likewise with pyrazinamide supplementation during the first 2 months (2HEZ/10HE). In countries with high initial resistance rates to isoniazid, supplementing with streptomycin (S) may also be appropriate in the first 2 months.

In turn, if ethambutol cannot be used, the treatment should be the same as that previously mentioned for 6 months, but using streptomycin instead of ethambutol in the 2 months of the first treatment phase (2HRZS/4HR).

The inability to use streptomycin does not affect the initial treatment scheme, since this should be the same as that indicated above, i.e., 2HRZE/4HR.

In all these treatment regimens, the drugs can be administered intermittently in the second phase, correcting only the dose per administration. The doses to be administered for each drug, in both the daily and intermittent dosing options, are the same as those specified in Table 7.

Management of adverse antituberculous drug reactions

Section summary

The risk groups for adverse antituberculous drug reactions are the elderly; malnourished patients; pregnant or nursing women; alcoholics; patients with chronic renal or liver failure, HIV infection, disseminated and advanced TB, atopy, anaemia, or diabetes mellitus; patients with a family history of adverse anti-TB drug reactions; patients receiving irregular TB treatment; and individuals who in addition to TB treatment are taking drugs for other disorders. In all these groups, close follow-up is required during treatment, with periodic clinical controls and laboratory tests.

A physician with extensive experience in the field should always carry out the management of adverse reactions. Such therapy cannot be standardised; each case must be dealt with individually. Initially, in the event of mild or moderate reactions, attempts should not be made to suppress treatment, although in the case of serious or severe toxic reactions, urgent hospitalisation is indicated, with suspension of the entire treatment until improvements in clinical and laboratory tests are observed. Subsequently, attempts should be made to reintroduce the treatment, except in the case of serious adverse reactions, such as purpura, anaphylactic shock, acute renal failure, severe hepatitis, haemolysis, retrobulbar optic neuritis, exfoliative dermatitis, and agranulocytosis. In such an event, a different treatment scheme should be designed, excluding the drug implicated in the observed reaction. In the rest of cases, such progressive drug reintroduction should be attempted, beginning with those substances least likely to have been implicated in the reaction. The ideal approach is to begin administering a sixth of the total dose, followed by gradual increments of one sixth each day, thereby ensuring full reintroduction in 1 week.

At the start of this section, emphasis should again be placed on the recommendation that only expert physicians with extensive experience should deal with such patients. If no such personnel are available, it is best not to become involved in the management of these complicated cases.

Although generally well tolerated, the first-line antituberculous drugs may cause side effects or adverse reactions, as described in Table 8. Some of the reactions may be serious and, in exceptional cases, can even lead to death. Much has been written about the possible fatal outcomes of liver toxicity associated with isoniazid.

Drug	Adverse reaction	Recommended action
Isoniazid	Symptomatic hepatitis	Treatment interruption and evaluation of transaminase and bilirubin levels
	Peripheral neuropathy	Supervision and pyridoxine administration (B ₆) 25-50 mg/day
	Skin hypersensitivity (rare)	If serious, interrupt treatment Desensitise if drug is essential for treatment
	Pellagra	Treat with nicotinamide
Rifampicin	Asymptomatic hepatitis:	Supervision
	transient bilirubin elevation without cell damage and no transaminase level increase; subsides spontaneously and rapidly	Control especially in patients with chronic liver disease
	Symptomatic hepatitis	Drug suspension
	Skin hypersensitivity and photosensitivity Gastrointestinal alterations	Drug suspension and supervision Symptomatic treatment
	Reduced efficacy of oral contraceptives, anticoagulants, and oral hypoglycaemic drugs	Proceed according to medical criterion
	"Flu" syndrome similar to influenza, presenting after 3-6 months	More frequent in intermittent treatment. Often corrected by daily treatment
	Thrombocytopenic purpura	Symptomatic treatment and supervision. Drug suspension
	Dyspnoea similar to asthma	Immediate and definitive drug suspension
	Haemolytic anaemia	Drug suspension
	Acute renal failure	Drug suspension
Pyrazinamide	Arthralgia	Suspend if arthralgia is intense
	Gout	Definitive suspension
	Nausea and anorexia	Symptomatic treatment

Table 8. The most important adverse antituberculous drug reactions and basic actions recommended in each case.

Drug	Adverse reaction	Recommended action
	Symptomatic hepatitis	Symptomatic treatment
(asymptomatic hyperuricaemia—very frequent—does not require treatment suspension)	Hypersensitivity	Symptomatic treatment
,	Hypersensitivity, skin, and generalised reactions	Drug suspension
Streptomycin	Vestibular alterations, deafness. (These reactions may be permanent; most often found in young children and in patients over age 45 years)	Drug suspension
	Vertigo and numbness (related to serum concentration)	Total and definitive suspension
	Aplastic anaemia, agranulocytosis (rare)	Total and definitive suspension
Ethambutol	Optic neuritis	Symptomatic treatment Drug suspension, if permanent
	Nausea	Symptomatic treatment
	Peripheral neuropathy, hypersensitivity (rare)	Medical criterion (drug should be avoided in patients with severe renal problems during pregnancy). In serious cases, total and definitive suspension

General considerations regarding adverse drug reactions

Since adverse antituberculous drug reactions can be serious and even life threatening, early detection is essential and will clearly affect the associated morbidity and mortality. In this context, the application of strictly supervised TB therapy will also allow earlier clinical control and prompter detection of adverse drug reactions. Once again, it must be emphasised that physicians with expertise in the field should manage these difficult cases.

When a person experiences a serious adverse reaction, all treatment should be suspended until the affected organ or system is normalised (usually within 2-3 weeks). During this period, it will be necessary for the patient to undergo adequate clinical evaluation and for the physician to assess the risk-benefit ratio of these drugs.

The continuation of treatment in a patient who has suffered an adverse reaction cannot be standardised, and each situation must be evaluated individually. With regards to the antituberculous treatment regimen following an adverse reaction, as many first-line drugs should be tried as possible, since these are more effective and less toxic than the second-line agents.

Risk groups for adverse drug reactions. Action recommendations

Different groups at risk of adverse reactions have been identified (Table 9). Such patients must be specifically evaluated according to their risk profile.

Table 9. Risk groups/factors for adverse antituberculous drug reactions.

- Advanced age
- Malnutrition
- Pregnancy or nursing
- Alcoholism
- Liver failure
- Chronic renal failure
- HIV infection
- Disseminated and advanced TB
- Atopy
- Anaemia
- Diabetes mellitus
- Family history of adverse antituberculous drug reactions
- Patients receiving irregular antituberculous treatment
- Patients receiving medication for other disorders, in addition to antituberculous drugs

In all the groups listed in Table 9, and in patients who in the course of treatment develop signs and/or symptoms suggestive of adverse reactions, careful evaluation must be carried out, with close follow-up and strict laboratory test assessments. This will facilitate the early detection of adverse reactions.

The reasons contributing to the possibility of adverse antituberculous drug reactions in the groups listed in Table 9 are the following:

- 1. *Ageing*. Ageing is responsible for changes in drug metabolism and excretion, and for increased interindividual variability.
- 2. *Malnutrition*. A high prevalence of fatty liver exists in such situations, resulting in a reduction in hepatocyte glutathione. Consequently, there is little neutralisation of the toxic metabolites originating from drug acetylation. Likewise, hypoalbuminaemia is observed, which causes

an increase in the plasma free (i.e., unbound) fraction of drugs, thus increasing the risk of drug-induced toxicity.

- 3. *Pregnancy*. As in the case of malnutrition, fatty liver with hypoalbuminaemia may be observed during pregnancy. Moreover, the use of aminoglycosides has been associated with the development of hearing problems in newborns of women taking this class of medication. Growth cartilage abnormalities have been associated with the use of quinolones in pregnant women. Likewise, ethionamide should be avoided, since it has been shown to have teratogenic effects in animals.
- 4. *Liver or kidney dysfunction*. Anti-TB drugs can cause liver or kidney toxicity; consequently, special caution should be exercised when administering such substances to patients with impaired function of these organs.
- 5. *Treatment with other drugs*. The cytochrome P450 has been frequently associated with the production of hepatotoxic reactive metabolites. Its involvement in drug metabolism and effects on bioavailability should be considered when combinations of drugs are used.
- 6. *Disseminated or advanced TB*. In this setting, adverse reactions are probably a consequence of malnutrition or liver deterioration attributable to the disease itself.
- 7. *Patients previously subjected to antituberculous therapy*. There is an increased risk of damage attributable to rifampicin, which in turn is associated with hypersensitivity reactions that lead to the release of immune complexes.
- 8. *Atopy*. Atopy has been linked to the existence of other cases of adverse anti-TB drug reactions in family members; thus, the clinical history of the patient should be carefully evaluated before treatment is started.
- 9. *Sex.* Women are at a comparatively greater risk of developing druginduced liver reactions. This effect must be considered when providing TB treatment.
- 10. *HIV infection*. Adverse reactions are more frequent in HIV-infected patients. The risk of such reactions increases with the degree of host immunosuppression.

In groups at risk of developing adverse reactions, baseline examinations should be requested, adapted to the clinical picture. Routine supervision based on laboratory tests is not necessary in patients given antituberculous treatment. The baseline examinations to be requested should include measurement of the following: a complete blood count with haematocrit, platelet count, prothrombin time, glucose, urea, creatinine, uric acid, transaminases, total bilirubin, conjugated and unconjugated bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase. Hepatitis serology should also be performed in areas endemic for viral hepatitis.

Controversy persists regarding the role of fast acetylators in the metabolism of isoniazid. It is known that the rate of acetylation of isoniazid and monoacetylhydrazine is genetically determined and there is a bimodal distribution of persons who acetylate them either slowly or rapidly. Since individuals acetylate isoniazid at the same speed that they convert monoacetylhydrazine to diacetylhydrazine, which is not toxic, it is very likely that this pattern does not influence hepatotoxicity reactions.

Drug dosage should be adjusted to body weight in all cases, and the personnel, patient, and relatives must be instructed on the possibility of developing an adverse reaction, highlighting the need to report any such reactions immediately to the physician.

In special cases, the patient at high risk of adverse reactions may require hospitalisation; unnecessary prolonged stays should be avoided.

The management approach to adverse drug reactions

Success in the management of an adverse reaction depends on the establishment of an early and correct diagnosis. The patient and relatives must be instructed on how to recognise the most common adverse effects and to report them to the health personnel. It is equally important to question the patient about possible adverse reactions each time the patient reports for treatment.

The first step involves evaluating the severity of the adverse reaction and establishing whether it is dose dependent (a very common situation), and making the necessary dose adjustments. If the adverse effect is due to some factor other than dose, then the physician should advise suspending treatment with all drugs, or only the drug suspected of causing the reaction, and should assess if treatment suspension be permanent or temporary. It is important to try to establish a cause-effect relation between the adverse event and the suspect drug. The management of any adverse anti-TB drug reaction must always be carried out by physicians with great expertise in the field.

In situations of mild or moderate adverse reactions, the initial consideration is to not suspend the medication. The recommended approach is to provide symptomatic treatment for the reaction, adjust the drug dose, or change the timing of administration. If these measures prove to be unsuccessful, suspension of treatment should then be considered.

However, in patients with serious or severe toxic reactions, urgent hospitalisation is required, with suspension of the entire treatment until clinical and laboratory test improvement is achieved. Once the reaction has improved, the patient should be discharged, and after clinical and laboratory test amelioration, a waiting period of approximately 2 to 4 weeks is advised, to allow stabilisation of the immune system. During this time, the patient should be monitored for TB evolution. After 2 to 4 weeks of normalised clinical and laboratory test results, reintroduction of therapy may be considered, with possible desensitisation attempts. However, if during these 2 to 4 weeks the patient suffers a serious complication or develops a more severe form of TB, urgent hospitalisation is necessary, with the evaluation of an alternative treatment scheme—which may involve second-line drugs—or the reintroduction of therapy with immediate desensitisation.

Reintroduction of treatment and desensitisation

The reintroduction of therapy and desensitisation should not be attempted in patients who have developed severe toxic reactions, such as purpura, anaphylactic shock, acute renal failure, haemolysis, retrobulbar optic neuritis, severe hepatitis, exfoliative dermatitis, or agranulocytosis. In such situations that seriously compromise the patient's life, a new treatment scheme should be used, excluding the drug implicated in the reaction. Treatment reintroduction is likewise contraindicated in HIV-infected individuals.

Before attempting to reintroduce treatment and perform desensitisation, a plan should be established on how to proceed in the event the adverse reaction reoccurs. Some experts recommend that the patient should receive prednisone (1-2 mg/kg body weight) 3 days before reintroduction of antituberculous treatment, continuing with this dosage for up to 2 weeks after reintroducing treatment, followed by gradual tapering of the dose. Concomitantly, the attending physician may consider using an H₁-blocker (e.g., loratadine 10 mg/day) or an H₂-blocker (e.g., ranitidine 300 mg/day), administered preferably at 18:00, in view of the relation of gastric acidity to the circadian cycle.

The subsequent steps indicated for reintroducing treatment and desensitisation are detailed below:

- 1. Based on a detailed clinical history and pertinent laboratory tests, attempt to identify the possible drug responsible for the observed reaction.
- 2. Determine whether it is necessary to continue administering the drug suspected of causing the adverse reaction.
- 3. Identify what other additional drugs are to be used in the reintroduction of antituberculous treatment.
- 4. Reintroduce the treatment drug by drug, in progressively increasing doses.
- 5. Progressive drug reintroduction should begin with the substance that is least likely to have been implicated in the observed reaction. This serves to increase physician and patient confidence, and the possibility of a new adverse reaction is postponed. This should be followed by the continued reintroduction of drugs in order of their likelihood of being related to the adverse reaction (from least to most likely).
- 6. If it is not possible to identify the culprit medication before reintroducing therapy, referral to the adverse anti-TB drug reaction frequency studies made in the country is required, in order to decide the best approach.
- 7. The timing of reintroduction of each drug will depend on the severity of the adverse drug reaction and on patient tolerance.
- 8. The ideal approach is to begin administering a sixth of the total dose, followed by gradual increments of one sixth each day, thereby ensuring full reintroduction of the drug in 1 week—this length of time being too short for a monotherapeutic regimen to select mutants resistant to that particular drug.
- 9. Once one of the antituberculous drugs has been reintroduced in the course of 1 week, an additional drug should be reintroduced each week in the same way as the first drug. Since a single week is not enough for the selection of resistances to the drugs, this recommendation decreases the likelihood of resistance development.
- 10. In relation to management after drug reintroduction, it must be taken into account that the regimen designed cannot be subject to the treatment norms established for the majority of patients. The patient subjected to treatment reintroduction must complete therapy according to the regimen designed, and the doses must be rigorously adjusted to body weight. The second phase of such treatments must always be administered on a daily basis.

11. It is extremely important for all severe adverse reactions to be managed and supervised by a specialist physician with extensive experience in these cases.

Drug and food interactions in tuberculosis

Section summary

The most important drug interactions that may be encountered with antituberculous medication occur at the level of absorption and metabolism of these agents. Isoniazid, rifampicin, and ethambutol require an acid medium for absorption; as a result, their absorption will be worse in the presence of drugs that increase the gastric pH, or in patients with achlorhydria (a common situation in HIV-infected patients).

Particularly important is the effect of food on the absorption of antituberculous drugs. Isoniazid, rifampicin, and cycloserine must be administered with the stomach empty, whereas rifapentine, clarithromycin, clofazimine, and para-aminosalicylic acid are better absorbed with food. In turn, the bioavailability of pyrazinamide, ethambutol, ethionamide, rifabutin, and fluoroquinolones is only minimally affected by food, and can therefore be administered with meals.

The rifamycins (rifampicin more so than rifabutin) induce cytochrome P450 enzyme activity, as a result of which they may produce interactions with drugs such as oral anticoagulants, contraceptives, glucocorticoids, oral antidiabetic drugs, immune suppressors (cyclosporine), and methadone—thereby reducing their therapeutic efficacy. In the case of rifampicin, the maximum inducing effect appears after 9 to 12 days. Rifampicin also interacts with protease inhibitors, the latter being the basis of HIV treatment, significantly reducing their plasma concentrations.

Drug interactions consist of alterations in the response of a drug due to the action of some other drug, food, beverage, or environmental contaminant. Such interactions may have beneficial effects (e.g., increasing the plasma concentrations of low bioavailability drugs) or deleterious consequences, which lead to treatment inefficacy (e.g., increased plasma concentrations with the risk of toxicity, or a reduction of such concentrations).

Interactions may take place:

- At the drug absorption level.
- At the distribution level. This is generally of little importance, unless the metabolism of the drug involved is also inhibited, or its renal elimination is reduced.
- At the metabolic level. These are the most important interactions.

- Pharmacodynamic interactions. These are modifications in target organ response and may give rise to addition phenomena, drug synergy, or antagonism.

As mentioned above, the most important drug interactions are those taking place at the metabolic level, although in the case of antituberculous therapy, those occurring at the drug absorption level can also be important—particularly in view of the possible effect of food on these drugs. TB can cause physiological and immune alterations that may affect drug absorption, metabolism, and binding to plasma proteins, and thus alter the magnitude of the interaction.

Interactions at the drug absorption level. The effect of food

These interactions may consist of an alteration in drug absorption rate, changes in the total amount of drug absorbed, or both. The causes of such interactions can be attributed to changes in the pH of the gastrointestinal contents, effects on gastric emptying and gastrointestinal motility, the fixation or chelation of drugs to form soluble complexes, and transport via glycoprotein P.

Isoniazid, rifampicin, and ethambutol require an acid medium for absorption; as a result, their absorption is worse in the presence of drugs that increase the gastric pH, or in patients with achlorhydria (a common condition in HIV-infected patients). It is therefore advisable to administer these drugs at least 1 hour before taking antacids.

Food exerts important effects on the absorption of antituberculous drugs. Table 10 details the most important interactions of all antituberculous drugs (first- and second-line) with food and antacids. Isoniazid, rifampicin, cycloserine, and azithromycin should be administered with the stomach empty, whereas rifapentine, clarithromycin, clofazimine, and para-aminosalicylic acid are better absorbed with food. In turn, the bioavailability of pyrazinamide, ethambutol, ethionamide, rifabutin, and fluoroquinolones is only minimally affected by food, and these medications can therefore be administered with meals. However, in the case of fluoroquinolones, it is important to avoid administration with dairy products, since the high calcium content of dairy products can reduce drug absorption as a result of the formation of complexes. In turn, isoniazid is unstable in the presence of sugars such as glucose and lactose, as a result of which drinks or drugs containing substantial amounts of these

sugars (e.g., zalcitabine) should be avoided when taking isoniazid. In such situations, it is advisable to space both administrations at least 1 hour apart.

On the other hand, HIV-infected individuals may present with malabsorption due to gastrointestinal mucosal disorders (the *M. avium* complex), infectious diarrhoea (cryptosporidiosis or microsporidiosis), hypochlorhydria or achlorhydria, lactose intolerance, pancreatic insufficiency, or mucosal atrophy due to caloric-protein malnutrition. Such malabsorption has been shown to affect certain drugs, including rifampicin and ethambutol. In turn, isoniazid and pyrazinamide are well absorbed in patients with HIV infection, although absorption decreases in the presence of diarrhoea.

Interactions at the drug metabolism level

Metabolism-inducing drugs reduce the amount of substrate, with the potential risk of treatment failure. This action can be countered by increasing the substrate dose or removing the enzyme inducer. In contrast, metabolism-inhibiting drugs increase the substrate concentration, as a result of which toxicity may be enhanced.

Table 10. The most clinically significant interactions ofantituberculous drugs with food and antacids.

Drug	Effect of food	Effect of antacids
Isoniazid	To be administered on an empty stomach, since absorption is reduced by 57% in the presence of food, particularly carbohydrates. Avoid administering with liquids containing abundant glucose or lactose. Isoniazid can inhibit monoamine oxidase, as a result of which the drug should not be administered with food containing abundant tyramine or alcohol, since disulfiram-type reactions may occur, with an increased risk of liver toxicity.	Antacids reduce AUC by 0- 19%. Contradictory information. Avoid combined administration as a measure of caution.
Rifampicin	To be administered on an empty stomach, since absorption is reduced by up to 26% in the presence of food.	Can be administered with ranitidine, although coadministration with antacids should be avoided.
Pyrazinamide	Food exerts minimum effects on bioavailability.	Can be administered with antacids.
Ethambutol	Food exerts minimum effects on bioavailability. C_{max} is reduced by 16%, with no changes in AUC.	Reduces C_{max} by 28% and AUC by 10%. Avoid combined dosing.
Ciprofloxacin	Food exerts minimum effects on bioavailability. However, absorption is reduced by foods with abundant calcium (dairy products) and other ions; administration should thus be made 1 h before or 2 h after meals	Important reduction in absorption. Avoid combined dosing.
Ethionamide	Food exerts minimum effects on bioavailability.	Can be administered with antacids.
Cycloserine	Food reduces C_{max} by 30% and prolongs T_{max} 3.5-fold. Orange juice (and probably also other acid beverages) reduces C_{max} by 15%. If possible, administer with water and between meals.	Antacids do not significantly modify AUC or C _{max} .
Para-aminosalicylic acid	Acidic drinks or yoghurt prevent release in stomach, reducing the incidence of nausea. Food	Antacids do not significantly modify AUC or C_{max} .

Drug	Effect of food	Effect of antacids
	increases its absorption (-52% AUC). Administer with water, orange juice, or fatty foods.	
Clofazimine	Fatty foods increase C _{max} .	Can be administered with antacids.
Rifabutin	Food exerts minimum effects on bioavailability.	Not known. Didanosine does not affect absorption. Avoid administering with antacids until further information is available.
Levofloxacin	Food exerts minimum effects on bioavailability.	Important reduction in absorption. Avoid combined dosing.
Clarithromycin	Bioavailability increases by 25%.	Although data are limited, combined dosing seems possible.
Azithromycin	Administer 1 h before or 2 h after meals. Food reduces absorption by 50%.	
AUC = area under the curve	; $C_{max} = maximum$ concentration; T_{max}	ax = time to maximum
concentration.		

The rifamycins (rifampicin more than rifabutin) induce cytochrome P450 enzyme activity; consequently, they may reduce the therapeutic efficacy of drugs such as oral anticoagulants, contraceptives, glucocorticoids, oral antidiabetic drugs, immune suppressors (cyclosporine), and methadone, among other substances. In the case of rifampicin, the maximum inducer effect occurs after 9 to 12 days. Rifampicin also interacts with cotrimoxazole, reducing the area under the curve for trimethoprim by 63% and for sulphamethoxazole by 23%.

Protease inhibitors, the basis of anti-HIV therapy, generally act as metabolism inhibitors. These drugs constitute CYP3A4 substrates, and when combined with inducers of this enzyme (e.g., rifamycins), their plasma concentrations can be reduced significantly—thus compromising their therapeutic efficacy and facilitating the development of resistance. This is why the combination of rifampicin with protease inhibitors is contraindicated, with the exception of ritonavir, the combination of ritonavir and saquinavir, and the non-nucleosides delavirdine and nevirapine—due to the risk of causing treatment inefficacy and promoting resistances to these drugs. When rifampicin is used initially, at least 2 weeks must elapse after suspension of this drug before starting protease

inhibitor therapy. Rifabutin is easier to use in patients requiring antiretroviral therapy; it can be combined with most of these drugs. Due to the mechanism of action and difficult use of these substances, such treatments must always be prescribed by specialist physicians with the required expertise.

Due to the type of metabolism involved, it is unlikely for interactions to occur between protease inhibitors and isoniazid, pyrazinamide, ethambutol, aminoglycosides, para-aminosalicylic acid, and quinolones. Isoniazid can increase the concentrations of phenytoin and carbamazepine, probably as a result of inhibition of the liver metabolism of these antiepileptic drugs. Isoniazid can also alter the metabolism of ethanol and paracetamol, increasing the production of a toxic metabolite of the latter. High paracetamol doses should therefore be avoided when administering isoniazid. In turn, clarithromycin demonstrates clinically important interactions with carbamazepine and theophylline, as a result of which the plasma levels of these drugs should be monitored.

Pharmacodynamic interactions

Drugs that tend to worsen renal function, such as the aminoglycosides, can reduce the elimination of antiretrovirals, which are mainly eliminated through the kidneys, such as 3TC, d4T, and ddC.

Pyrazinamide can induce episodes of gout in patients at risk, since it competes with uric acid for renal elimination. This effect is more evident in patients receiving allopurinol, since allopurinol reduces the elimination of the main metabolite of pyrazinamide, which also reduces uric acid secretion.

Ethambutol can cause optic neuritis, while rifabutin can cause uveitis. Patients who simultaneously receive several drugs capable of causing ocular toxicity must be closely monitored.

In patients administered aminoglycosides on a continuous basis, periodic hearing evaluation is indicated, particularly among those individuals receiving other ototoxic agents in combination (clarithromycin, ethacrynic acid, furosemide). Aminoglycosides can enhance the effects of muscle blockers and can trigger neuromuscular block in patients with myasthenia gravis.

Cycloserine should be administered with caution to patients with a history of depression or psychosis, in view of its adverse effects on the central nervous system. Ethionamide can cause peripheral neuritis; consequently, caution is advised when combining it with antiretrovirals that also exhibit such toxicity. It can also cause liver toxicity and goitre, with or without hypothyroidism. Periodic monitoring of the concentrations of thyroidstimulating hormone is required. Para-aminosalicylic acid can enhance such toxicity at the thyroid level. Para-aminosalicylic acid can cause diarrhoea, affect the pharmacokinetics of other drugs, as well as cause different forms of malabsorption (e.g., steatorrhoea, vitamin B_{12} , folic acid, xylose, and iron absorption).

Clofazimine can cause changes in skin colour, while amiodarone and rifabutin can worsen this side effect.

In patients who do not respond adequately to antituberculous treatment, monitoring of plasma concentrations may be helpful. A number of studies have reported low plasma concentrations of antituberculous agents in HIV-infected patients undergoing antiretroviral therapy.

Therefore, emphasis must be placed on the importance of closely monitoring the possible side effects caused by these different drugs.

Special situations in the treatment of tuberculosis

Section summary

It is not necessary to modify the initial treatment regimens in HIV-infected patients, individuals with some form of immune deficiency, pregnant or nursing women, children, or infants. The only requirement is to adjust the corresponding doses and ensure close follow-up. When malabsorption is involved, or the patient is unable to take medication orally, the same regimens are provided, although via the parenteral route.

In advanced liver failure, the administered regimen should always include ethambutol (E) and streptomycin (S), with a third drug that should be isoniazid (H) if laboratory evidence indicates cholestatic conditions (2HES/10HE), or rifampicin (R) in the event of necrosis (2RES/10RE).

In patients with advanced kidney disease, it is best to avoid (or at least monitor the blood levels to ensure dose adjustment) all drugs with potential nephrotoxic effects, such as ethambutol, streptomycin, kanamycin, cycloserine, viomycin, and capreomycin. The 2HRZ/4HR regimen, with close supervision and follow-up, should suffice.

In patients with immune deficiency, very close patient follow-up is necessary, with each patient evaluated on an individual basis, even though the standard treatment regimens indicated above should be used. Studies involving large series of diabetic or patients with malignancies, or those who have undergone gastrectomy, have reported similar results as in healthy individuals.

In HIV-infected subjects, the same treatment regimens are indicated, with the only additional requirement of closer follow-up because of the increased risk of adverse reactions, intolerance, and drug interactions.

During pregnancy, no changes to the recommended regimens are necessary, for although isoniazid (H) and rifampicin (R) cross the placental barrier, they have not been associated with teratogenic actions. However, due to its potential foetal toxicity, streptomycin (S) should be avoided, along with ethionamide (Eth) and the other aminoglycosides. During nursing, all antituberculous agents can be used. Although part of the dose is secreted in human milk, the amount is insufficient to either have a therapeutic effect or cause adverse effects.

In children and infants, the same regimens as in adults apply, although maximal caution should be exercised and the dose should be adjusted according to body weight.

In patients with malabsorption problems, or who are unable to take medication orally, the same regimens defined above apply—employing the same doses, but administered parenterally.

In patients with advanced chronic liver failure, the recommended treatment regimen should always include those first-line agents that are preferentially eliminated by the kidneys (i.e., ethambutol [E] and streptomycin), taking care to avoid drugs selectively metabolised in the liver (pyrazinamide [Z], ethionamide, and para-aminosalicylic acid [PAS]). The drug to be added to ethambutol and streptomycin in turn depends on the predominant biochemical alteration involved:

- When the patient exhibits cholestatic alterations (increased bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase levels), isoniazid should be added—thus yielding the regimen 2HES/10HE, with frequent monitoring of liver function.
- When the observed pattern is necrotic (increased liver transaminase levels), it is better to add rifampicin, since liver toxicity generally occurs in combination with isoniazid (rifampicin being an enzyme inducer). The recommended alternative regimen is therefore 2RES/10RE, likewise with frequent liver function tests.
- When the predominant biochemical pattern is not defined, it is better to use 2RES/10RE.

• In patients undergoing antituberculous therapy who have druginduced liver toxicity, the points detailed above should be followed when reintroduction of all the drugs is not possible, and depending on the predominant biochemical pattern observed.

In cases of advanced kidney disease, it is advisable to avoid (or at least monitor) the blood levels to adjust the doses of all drugs with a potential for nephrotoxicity or that are eliminated by the kidneys. These drugs are ethambutol, streptomycin, kanamycin, viomycin, capreomycin, and cycloserine. Thus, the regimen 2HRZ/4HR would suffice, provided there is very close monitoring and follow-up since there is a possibility of selection of resistances attributable to not being able to administer a fourth drug in the initial phase of treatment. In such cases, it is advisable to administer the second treatment phase on a daily basis. All these patients must be subjected to full clinical evaluation, with a 24-hour urine creatinine clearance test. Based on the test result, it may be advisable to reduce the dose or increase the dosing interval. If creatinine clearance exceeds 50 ml/min, no such changes in dose or dosing interval are needed. However, if clearance is less than 50 ml/min, the therapy specified in Table 11 is indicated. In no event is it necessary to modify the dose or dosing interval of rifampicin. In patients undergoing dialysis, treatment should be provided at the end of the dialysis session.

Table 11. Dose adjustment for antituberculous drugs in patients with chronic renal failure, according to the 24-hour urine creatinine clearance test results.

	Creatinine clearance (ml/min)					
	<50	<20			<10	
Drug	Dose	Interval	Dose	Interval	Dose	Interval
H, E, Z, S Aminoglycosides	< Reduce to half	Every 48 h	-	-	Reduce to 1/3 dose	Every 72 h
Ciprofloxacin	-	-	Reduce to half	Every 18-24 h		
E = ethambutol; $H =$ isoniazid; $S =$ streptomycin; $Z =$ pyrazinamide.						

Treatment of extrapulmonary tuberculosis

Section summary

Under conditions of a TB control program, there should be no differences in the treatment of extrapulmonary TB versus pulmonary TB.

Regarding the treatment of extrapulmonary locations of TB, the trials conducted to date have not been as thorough as treatment trials for pulmonary TB. Nevertheless, there appear to be no theoretical or practical bases for not using the same treatment regimens as those indicated for pulmonary TB (in terms of the drug associations and durations involved). Thus, the localisation of the disease should not imply any exception to treatment, and the treatment regimens described above should be used. Some authors and scientific societies recommend extending the duration of therapy to 9 months in patients with meningeal, osteoarticular, and lymphatic TB—although there is no firm evidence supporting this recommendation.

The drugs with the greatest distribution in cerebrospinal fluid are pyrazinamide (80% of the plasma concentration), isoniazid (50%), and ethambutol (20%). Only streptomycin and rifampicin cross the blood-brain barrier and afford therapeutic drug levels in the presence of meningeal inflammation.

Surgery, corticoids, and other therapeutic measures

Section summary

Surgery is only indicated in specific cases for managing the sequelae or complications of pulmonary TB, and in very exceptional cases of multidrug-resistant TB in which the lesions are localised and there are no other drugs to treat the disease. In patients with extrapulmonary TB, surgery may be acceptable for obtaining samples for study and for treating certain situations such as constrictive pericarditis, vertebral abscesses that may compress the spinal cord, or superficial and accessible abscesses in cases of osteoarticular TB.

Corticoid treatment should only be contemplated in four situations: meningeal TB, serious miliary TB, pericardial TB, and TB involving gangliobronchial perforation. At present, surgery lacks indications in the management of TB. In lung TB, surgery should be passed over in view of the excellent performance afforded by pharmacological treatment. Only some believe that surgery should be considered in the treatment of localised lesions in which the bacterial population is resistant to practically all drugs. This condition is very rare—particularly regarding localised lesions with good lung function. Often, when surgery is performed in such patients, the procedure is used more because of economic constraints or lack of access to effective second-line drugs than because of any genuine surgical indication. Moreover, even when radiographs reveal the presence of localised lesions, it should be remembered that TB is in fact a disseminated disease.

A different situation is presented in the management of TB complications or sequelae, including massive haemoptysis, bronchiectasis, and bronchopleural fistulae, where surgery may indeed prove useful.

In extrapulmonary TB, surgery may be useful, particularly for obtaining samples for diagnostic purposes, and for treating certain conditions, such as constrictive pericarditis or vertebral abscesses that may compress the spinal cord. Surgery should also be evaluated in cases of superficial and accessible abscesses in the context of osteoarticular TB. Apart from unique cases, surgery is not indicated for use in peripheral adenopathies affected by the disease. Surgery should only be considered in the event of mechanical complications and possible sequelae.

In turn, corticotherapy should only be considered in three situations:

- 1. *Meningeal TB*. Some paediatricians recommend corticotherapy to prevent the development of an internal hydrocephalus.
- 2. *Miliary TB*. Use in seriously ill patients to provide symptom relief, although corticotherapy appears to have little effect on the prognosis.
- 3. *Pericardial TB*. To reduce the risk of constrictive pericarditis and the need for subsequent surgery.

The use of corticosteroids is not justified in pleural TB, where it has been shown that while these drugs shorten the time to symptom and effusion resolution, they have no effects on preventing possible complications. Similar considerations apply to endobronchial TB, where it is not clear whether corticoids reduce complications. However, in the IUATLD's recommendations regarding children with TB, corticosteroids are advised when gangliobronchial perforations are present.

Lastly, vitamins and protein supplements are not helpful. Pyridoxine can interfere with the activity of isoniazid, whereas nicotinamide can interfere with pyrazinamide. Such substances should only be used in the setting of deficiencies, which is a fairly common situation in poorer countries.

Hospital admission criteria

Section summary

At present, only five conditions warrant hospitalisation:

- Disease severity. Admission is due to the seriousness of the patient's condition, not due to the fact that the patient has TB.
- Complications of the disease or its sequelae. Admission is likewise due to complications, and not due to merely having TB.
- Management of serious adverse drug reactions.
- Re-treatment of TB with second-line drugs.
- Due to social reasons (rare).
- Uncomplicated initial TB is not a criterion for hospital admission.

Rest and sanatorium care are no longer used, both from the treatment and the patient isolation perspective. Thus, there are presently no indications for admission of initial TB cases simply due to the fact that the patient has the disease. However, in some instances admission is warranted—because of the seriousness of the patient's condition (e.g., respiratory failure, massive haemoptysis, severe malnutrition, terminal condition) or because of complications of the disease or its sequelae (e.g., empyemas, bronchopleural fistulae, haemoptysis, respiratory failure). It is necessary to point out, however, that admission in these situations is due to the seriousness of the patient's condition, not to the fact that the patient has TB.

Additional indications for admission are: 1) serious adverse drug reactions, which may be life threatening; and 2) the first few weeks of retreatment with second-line drugs, due to the increased risk of intolerance and adverse antituberculous drug reactions.

Lastly, social factors may sometimes also support admission, such as extreme poverty, excessive distance between the home of the patient and the health care centre (a relatively common situation in very poor countries), and repeated treatment default.

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Chapter 10. Re-treatment of tuberculosis

Chapter summary

The re-treatment of tuberculosis (TB) constitutes one of the most difficult challenges in the management of the disease; consequently, in theory it should only be carried out by expert physicians. This aspect is all the more important considering that in many instances, re-treatment is the last chance for the patient to get cured. Unfortunately, however, many professionals with limited knowledge of TB re-treatment undertake such management, thereby making the problem worse.

The reasons for re-treatment comprise a variety of factors as distinct as microbiological relapse, pharmacological failure, patient abandonment, and poor adherence to therapy.

The truly worrying aspect is the selection of resistant mutants to the drugs used—a situation almost always seen in treatment failure and partial abandonment of therapy. These are situations that must be well known to the physician facing the problem. The health professional must also be aware of the limited utility of drug sensitivity testing, and of the great importance of a detailed history of the drugs used in the past, which are essential when devising a re-treatment strategy. With a sound knowledge of all the drugs offering action against TB, a re-treatment scheme can be designed that includes a minimum of three drugs that have never been used by the patient. There are presently 13 drugs for which such action has been demonstrated; consequently, the problem is often more a matter of limited drug availability in a given country than of a true lack of alternative second-line drugs.

However, re-treatment is extremely expensive, particularly when second-line drugs are needed. This is why priority should be given to the management of initial patients, both in view of the superior costeffectiveness of initial TB therapy and because correct management of the initial disease is the only way to prevent the need for re-treatment. Hence, at least in theory, no resources should be spent on re-treatment until it is confirmed that all initial disease patients have been granted free access to a short-duration treatment regimen.

Once the above has been taken into account, even the poorest countries with the greatest TB problems should attempt to offer at least one standard 8-month re-treatment regimen involving first-line drugs $(2HRZES/1HRZE/5H_3R_3E_3)$. This regimen is inexpensive and very practical. Not only does it heal over 90% of patients included in National Tuberculosis Control Programmes (NTPs) as re-treated cases, but it also identifies the true failures (i.e., patients with drug resistance) who may thus be able to receive more individualised care at a higher level within the health care system. The great majority of countries with low-income levels should not spend further resources on re-treatment.

However, in middle-income countries and in some low-income countries, the acquisition of a second-line drug reserve may be advisable in order to offer a standard treatment regimen with these agents: 3(Z, Kn, Eth, ofloxacin)/15(Z, Eth, ofloxacin). Such drug reserves should always be managed by the Central Unit of the corresponding NTP.

The possibility of individualised re-treatment according to drug susceptibility results is perhaps only advisable in richer countries, and in some middle-income countries.

As its name indicates, the term tuberculosis (TB) "re-treatment" refers to treatment in a patient who has already been treated with antituberculous drugs in the past. These therefore are patients who have already had the opportunity to receive treatment and cure their disease. Accordingly, in most patients in whom re-treatment is being considered, there were errors in the administration of the previous treatment. In short, "the best retreatment is the therapy that should have been administered several years earlier, with the application of a good initial treatment scheme." This statement would only exclude specific cases of disease relapse, a concept that will be dealt with later on. Thus, in order to minimise the number of individuals requiring re-treatment, two fundamental premises apply: the need to prescribe standardised initial treatment protocols, and the use of strict supervision measures to guarantee that patients adhere to treatment.

TB re-treatment is often the last chance the patient has of cure, particularly in low-and middle-income countries where the availability of second-line drugs is limited. For this reason, re-treatment schemes must always be managed by experts in the field, even in the case of those schemes that are administered on a standard basis in the context of a National Tuberculosis Control Programme (NTP).

This chapter describes the bases for developing a re-treatment regimen, and discusses its possible use according to the resources available in different countries.

Different situations requiring re-treatment: relapse, failure, default, and poor adherence to therapy

Section summary

The most important consideration when dealing with a patient subjected to re-treatment for TB is to attempt to identify whether disease relapse, treatment failure, partial or total treatment default, or poor patient adherence to medication is involved. It is essential to try to determine the reason for re-treatment because subsequent treatment is specific to each situation. However, the process of assessing the different situations requiring re-treatment is complex, and often times cannot be accomplished at the peripheral levels of the health care system.

Relapse is the appearance of new microbiological evidence of disease in a patient who has completed correct treatment and has been cured. Such situations do not usually condition the selection of drugresistant mutants. Failure is defined as the appearance or persistence of microbiological activity in a given patient in the course of antituberculous treatment, either following initial negative conversion of the cultures or without negative conversion. In general, failure implies the selection of resistant mutants to all the drugs that are being used. On the other hand, if a patient completely abandons treatment, a situation similar to relapse will develop, with the isolates continuing to be sensitive to the drugs used. If abandonment is only partial, the resulting situation is more akin to treatment failure, with the possible selection of resistant mutants.

The first and most important consideration when dealing with a patient requiring re-treatment for TB is to determine whether the case corresponds to bacteriological relapse or to pharmacological failure, since the therapeutic connotations and prognoses differ greatly from one setting to another.

Bacteriological relapse is defined as the appearance of microbiological evidence of disease in a patient who has completed appropriate treatment and who has been cured. However, the truth is that only in cases of directly supervised treatment can it be ensured that the patient has effectively taken all the necessary medications. While relapse may occur in the early or later stages of the disease, it most often takes place during the first 12 to 24 months after the end of therapy. Such relapses are usually attributable to the persistent or dormant bacterial population that did not divide during the

administration of initial treatment, and that therefore has not found the conditions required to favour drug-resistant mutants. This is why it is generally possible to restart treatment in these patients using the same initial therapeutic scheme. If relapse occurs several years after the initial treatment, the true situation may correspond to reinfection rather than to relapse—although this is difficult to demonstrate. In any case, relapse does not change the indication or affect the repeated use of the initial therapeutic regimen. Although, as has already been mentioned, microbiological relapse usually occurs at the expense of sensitive bacteria, and it is advisable to perform sensitivity tests of the first-line antituberculous drugs in these patients.

The concept of relapse and the presumed sensitivity similar to that found in the initial disease process is only applicable when it is certain that the patient has received an appropriate treatment regimen. In the field, when drug resistance studies are performed, relapses tend to be associated with a significant increase in resistance compared with the available initial susceptibility results. This may be explained by poor patient adherence to therapy, or because patients presenting in the relapse group already had an initial primary resistance. This is why patients who relapse are assigned a reinforced longer primary treatment under the conditions of a TB control programme.

Pharmacological failure is the appearance or persistence of microbiological activity in a patient during the course of antituberculous treatment and following initial negative conversion of the cultures. Failure is also accepted in patients in whom there is no such negative conversion of cultures during the course of therapy. In the initial 6-month treatment course recommended in the present Guide (i.e., 2HRZE/4HR), failure can be considered when the sputum cultures at the end of the fourth month remain positive. In the 8-month treatment regimen (2HRZE/6HE), it is necessary to wait until the end of the fifth month to confirm possible failure. In this situation, treatment failure occurs at the expense of the bacillary population, which has been metabolically active and has divided during treatment, favouring the selection of naturally resistant mutants. For this reason, failure almost always involves resistance to all the drugs being administered. Hence, the essential requirement in such cases is drug susceptibility testing.

Drug resistance is the theoretical explanation underlying treatment failure. In actual practice, however, up to 50% of all failures show sensitivity in the *in vitro* tests, and implementing a new or reinforced first-line drug regimen ultimately cures such patients. The so-called programme

failures, i.e., failed treatment due to treatment irregularities, should be resolved if the standard therapeutic guidelines of the NTP are followed. Another influencing factor of treatment failure is the lack of correlation between the laboratory drug susceptibility test results and the clinical evolution of the patient.

Chronic TB patients who have undergone multiple treatments often represent multidrug resistance (MDR) and are the main therapeutic problem. In an important percentage of such patients, the 'culprit' in the development of drug resistance is the physician who recommends incorrect treatment regimens. When patients show poor adherence to therapy, they typically abandon all the drugs at an early stage, as a result of which the subsequent relapses occur at the expense of the initial bacterial population that has not developed drug resistance. In these situations, it suffices to reintroduce the initial treatment regimen to achieve cure. These cases are similar to relapse cases, with the same resistance behavioural pattern. Only when the patient selectively abandons treatment (as in the case of masked monotherapies) does the risk of developing drug resistance occur. This risk does not exist when the patient ceases taking all medications, which is a more common occurrence.

Based on the above considerations, it may be accepted that a patient undergoing re-treatment, without drug resistance, either corresponds to a case of microbiological relapse or to a case of complete treatment default. When drug resistance is demonstrated, the problem is almost always attributable to the physician who recommended the wrong initial treatment regimen or, infrequently, to partial or selective patient default of therapy.

Methods for detecting drug resistance

Section summary

A detailed history of the drugs taken in the past is the best method for detecting drug resistance and for designing a re-treatment regimen. In practice, this approach is superior to sensitivity testing, which, although compulsory in all patients subjected to re-treatment for TB, affords very late information. Drug susceptibility test results must always be compared with the corresponding history of drugs taken by the patient.

When dealing with a patient requiring re-treatment for TB, the first requirement is to determine whether the patient has any drug resistance. Above any other method, review of the medical record is critical, as it will contain information indicating if the patient had relapsed, abandoned treatment completely, experienced treatment failure, or partially defaulted on treatment. In the case of relapse or treatment abandonment, it may be accepted that there are no drug resistances, and that it would suffice simply to reintroduce the initial treatment regimen, with close monitoring of the microbiological course.

However, if the case involves treatment failure, partial abandonment of therapy, or a chronic patient with multiple previous treatments (the most frequent situation), then it is very possible that there is resistance to one or more drugs. In these cases, a detailed and thorough history of the drugs previously used by the patient should be established, with evaluation of all the doses administered and the drug combinations used in each treatment protocol.

If this detailed and exhaustive history of the drugs used in the past is prepared by expert personnel and considerable time is spent on compiling the history (no less than 30-60 minutes), a chronological representation can be obtained of the introduction and withdrawal of drugs in the past (Figure 50)—thus making it possible to identify either real or masked past monotherapies. It becomes possible to determine (with good accuracy) drug resistances, which should indicate which drugs should not be used again in the re-treatment regimen.

In practice, this drug history is superior to drug susceptibility testing, which may take more than 3 months to yield results (when conventional methods are used) and which can fail because of insufficient culture development. Moreover, it is important to consider that while the *in vitro* and *in vivo* correlation of susceptibility testing is very reliable for isoniazid and rifampicin, and even for streptomycin, the same cannot be said for the remaining drugs, in which reliability is far less. Drug resistance, as defined by susceptibility test results, refers to the inefficacy of a particular anti-TB drug in the culture medium. Sensitivity determined by such testing does not imply that the drug will be effective in a new treatment regimen. The patient drug history must be evaluated in these cases.

Drug susceptibility testing, while compulsory in all patients undergoing re-treatment, affords very late information. Thus, it is not acceptable to wait so long before starting re-treatment. Drug susceptibility results must always be compared with the corresponding history of drugs taken by the patient. Drug susceptibility tests have been addressed in detail in Chapter 7.

A drug resistance detection system based on the history of drugs taken in the past becomes all the more important in low- and middle-income countries, where sensitivity testing must be performed using the proportions technique in solid culture media, which implies a delay of about 4 to 5 months before information is received. This major inconvenience makes a detailed history of the drugs taken in the past essential for evaluating a re-treatment protocol.

Bases for choosing a re-treatment regimen

Section summary

When selecting a re-treatment regimen, a series of 10 premises must be taken into consideration. Treatment should include three drugs that the patient has never taken. At least two of these drugs must be maintained for 18 to 24 months if neither isoniazid nor rifampicin can be used. If use of these drugs is possible, then treatment for 12 months will suffice. Re-treatment must be carried out by specialised personnel with experience with re-treatment regimens, since management with the great majority of second-line drugs is complicated and side effects are more frequent than with other drugs. At present, 13 drugs have been identified that offer activity against *M. tuberculosis*. An effective re-treatment regimen needs only three. The most important limitations are determined by the availability of these drugs and the experience in designing adequate treatment regimens.

After establishing the past drug history of the patient, a re-treatment scheme adhering to the 10 premises reflected in Table 12 must be developed. Treatment should include three drugs that the patient is certain to have never taken. At least two of these drugs must be maintained for 18 to 24 months if it is not possible to include isoniazid or rifampicin. If such inclusion is possible, then treatment for 12 months will suffice. Re-treatment can be carried out in the ambulatory setting, with close outpatient follow-up by specialised centres and involving personnel with experience in the field, since the use of appropriate intensive treatment regimens with second-line drugs is often complicated and involves toxic adverse effects. If there is no adequate infrastructure or social environment for careful outpatient monitoring, initiate re-treatment with the patient admitted to a reference centre.

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Figure 50. Sample form for recording a detailed history of the antituberculous drugs used in the past. This drug history should serve as a basis for preparing an individualised re-treatment regimen. Complete one sheet for each patient and year.

 Table 12. Guidelines for the elaboration of a TB re-treatment regimen.

- 1. Initiate re-treatment in the ambulatory setting if there is adequate infrastructure and social environment for outpatient monitoring. Otherwise initiate re-treatment with the patient admitted to a reference centre.
- 2. The scheme should be designed by personnel with extensive experience in the handling of second-line drugs.
- 3. It is very important to establish a detailed history of the drugs used by the patient in the past.
- 4. Associate at least three drugs that have never been used by the patient or for which no drug resistance exists (i.e., well associated in earlier treatment regimens).
- 5. Use the maximum possible number of bactericidal drugs.
- 6. Always include an aminoglycoside or capreomycin.
- 7. Caution is required due to possible cross-resistance among drugs, especially:
 - Aminoglycosides*: streptomycin \rightarrow kanamycin \rightarrow amikacin
 - All quinolones
- 8. Minimum treatment time:
 - 18 months if isoniazid and rifampicin cannot be used
 - 12 months if isoniazid or rifampicin can be used
- 9. Strict supervision of treatment administration is required.

10. Never add a single drug to an ineffective or failing regimen.

*Resistances in aminoglycosides are considered to be unidirectional, and such drugs should be used sequentially as shown in the table. See explanation in text below.

Among the drugs to be included in a re-treatment regimen, and in relation to some of the premises indicated in Table 12, the following should be noted:

- 1. If feasible, combine the maximum possible number of available bactericidal drugs that act upon different targets in the microorganism. If few such drugs are available, an aminoglycoside must always be included. Although capreomycin does not belong to this group, it possesses a similar mechanism of action and should therefore be included in this group when selecting an aminoglycoside. Moreover, since capreomycin is less toxic and is well tolerated, many experts recommend its use when streptomycin cannot be used.
- 2. It is important to evaluate the possibility of cross-resistance with incorrectly administered drugs. Drug resistance among aminoglycosides is considered to be unidirectional, and such drugs should be used sequentially, as reflected in the Table. Thus, if a drug is resistant to streptomycin, it is very likely to remain sensitive to the

rest. If it is resistant to kanamycin, it is very likely to remain sensitive to the rest, except streptomycin (to which it most likely will be resistant). Cross-resistance also exists between rifampicin and the ansamycin antibiotics or rifamycins.

- 3. Do not administer drugs with confirmed or suspected drug resistance. Their combination should only be evaluated in the treatment regimen if no other drugs are available.
- 4. Never add a single drug to, or associate it with, a regimen that has already been found to be ineffective. To do so would only create the conditions for masked monotherapy.
- 5. Careful assessment is required of the selected drugs, taking into account that they have important toxicities and side effects, and that they must be administered to persons who have already undergone multiple treatments in the past.

The list of all antimycobacterial drugs that can be used in a retreatment regimen is provided in Table 13; the great majority of these second-line agents are very expensive and difficult to obtain. As can be seen in Table 13, there are 13 drugs with demonstrated anti-TB efficacy, although involving different bactericidal and sterilising capacities. For this reason, attempts should always be made to include as many first-line drugs as possible (isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide), for in addition to being more effective, they are better tolerated and much easier to acquire. As has already been pointed out, all TB patients can be cured with at least three never previously used drugs. A sufficiently large therapeutic arsenal exists to ensure that cure can be achieved. Ultimately, the most important limitations are determined by the availability of second-line drugs and the ability to acquire them. Success of re-treatment will depend on the experience in designing adequate treatment regimens and in the use of these drugs, which have increased toxicity and poorer tolerance profiles.

 Table 13. Drugs with demonstrated activity against *M. tuberculosis*.

- 1. Isoniazid
- 2. Rifampicin
- 3. Pyrazinamide
- 4. Ethambutol
- 5. Streptomycin
- 6. Capreomycin
- 7. Kanamycin
- 8. Amikacin
- 9. Ethionamide prothionamide
- 10. Cycloserine
- 11. Para-aminosalicylic acid
- 12. Quinolones
 - Ciprofloxacin
 - Ofloxacin
 - Levofloxacin
 - Sparfloxacin
 - Moxifloxacin
- 13. Thiacetazone

Second-line antituberculous drugs

Section summary

The so-called second-line drugs include capreomycin, kanamycin, amikacin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, and the second-generation fluoroquinolones (ofloxacin and ciprofloxacin). All are more expensive, more difficult to obtain, less effective, much more toxic, and less tolerated than the first-line drugs. For this reason, attempts should always be made to introduce as many first-line substances as possible in the re-treatment scheme. The side effects and intolerances involved must be well known in order to ensure effective management of patients undergoing re-treatment.

Table 14 details the mechanism of action, target bacterial population, dosage, common side effects, and drug interactions of the most common second-line antituberculous drugs. In addition, the following should be considered:

1. Thiacetazone should be administered at a dose of 150 mg in a single administration. Its main complications comprise blood dyscrasias with the appearance of bleeding and petechiae due to thrombocytopaenia.

Aplastic anaemia has also been described. In HIV-infected patients, dermatological alterations are frequently seen, some of which can be fatal (Steven-Johnson syndrome). The use of thiacetazone is therefore contraindicated in such patients.

- 2. While capreomycin is not exactly an aminoglycoside, its mechanism of action is similar. It is less toxic, however, and may therefore be the drug of choice in re-treatments when streptomycin cannot be used.
- 3. All drugs should be administered in a single dose and simultaneously, since this not only offers superior results, but in general also ensures improved tolerance with fewer toxic effects. The only exception is represented by rifampicin and para-aminosalicylic acid, which should be administered at least 8 to 11 hours apart. In the case of ethionamide, para-aminosalicylic acid, and cycloserine, dosing in two to three administrations should be considered because of the possibility of gastric intolerance.
- 4. Prothionamide is an ethionamide derivative; it possesses the same action but offers improved gastric tolerance. The dosage indicated is the same as for ethionamide. However, since there are more data on ethionamide, ethionamide is preferred.
- 5. The fluoroquinolone derivatives, dealt with more extensively below (under the section on new antituberculous drugs), are perhaps the drugs on which most research in TB re-treatment has been conducted in the last decade. These agents constitute one of the possible options in patients with MDR-TB.
- 6. Clofazimine is an antileprosy drug that is also used in MDR-TB, although its efficacy against TB is very low.

Table 14. Daily dosage, side effects, and drug interactions of the major second-line antituberculous drugs.

Drug	Daily dose	Side effects	Side-effect control test	Interaction	Action		
Capreomycin	15-30 mg/kg. Up to 1 g	VIII cranial nerve lesion, nephrotoxicity	Vestibular function, audiometry, BUN	Neuromuscular blocker	Extracellular bactericide		
Kanamycin	15-30 mg/kg. Up to 1 g	VIII cranial nerve lesion, nephrotoxicity	Vestibular function, audiogram, BUN	Neuromuscular blocker	Extracellular bactericide		
Ethionamide	15-30 mg/kg. Up to 1 g	GI alterations, hepatotoxicity	SGOT SGPT		Extra- + intracellular bactericide		
PAS	150 mg/kg. Up to 12 g	GI alterations, hepatotoxicity	SGOT SGPT		Extracellular bactericide		
Cycloserine	10-20 mg/kg. Up to 1 g	Psychosis, seizures, rash	Psychological tests	Alcohol	Extra- + intracellular bactericide		
BUN = blood urea nitrogen; GI = gastrointestinal; PAS = para-aminosalicylic acid; SGOT = serum							
glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvate transaminase.							

New drugs: future therapeutic perspectives in tuberculosis

Section summary

Despite the many studies continuously conducted to discover new drugs or therapies for TB, the great majority of possible options are still in the research stage and for the time being cannot be recommended in clinical practice. These alternatives include new antibiotics or derivatives of previously developed antimicrobials with known antimycobacterial properties. The drugs that are most widely used and that have demonstrated the best effects are the rifamycin derivatives, fluoroquinolone derivatives, some macrolides, the oxazolidinones, and the nitroimidazoles.

Of all the new antibiotics and other future therapeutic possibilities in TB treatment that are analysed in the present chapter, low- and middleincome countries can consider the incorporation of the second-generation fluoroquinolones (ciprofloxacin or ofloxacin) for the management of patients with MDR-TB as they are less expensive, well tolerated, and easily obtained. The remaining options have no current indications in these countries. Many studies—the majority of which are in the experimental stage—have been carried out in an attempt to discover new drugs or therapies for *M. tuberculosis* disease. While most efforts have focused on the development of new antibiotics or derivatives of previously developed antimicrobials with known antimycobacterial properties, other substances or therapies have also been investigated. The present section will be divided into two parts: research in relation to new antibiotics, and future directions in TB management.

New antibiotics with activity against M. tuberculosis

Some agents have already been tested in clinical trials, while others have only been evaluated in animals or in *in vitro* studies. The level of knowledge gained is greatest in the case of the antimicrobials belonging to pharmacological groups similar to those already used, such as the derivatives of the rifamycins (e.g., rifabutin, rifapentine, FCE 22250, GCP 29861, R-76-1), isoniazid, clofazimine, thiacetazone, and mitronidazole. A second major group is represented by families of new antimicrobials, such as the quinolones (e.g., ofloxacin, ciprofloxacin, sparfloxacin, levofloxacin, moxifloxacin), the macrolides (e.g., roxithromycin, clarithromycin, azithromycin), folate reductase inhibitors, novel betalactams, and mycoplanecins, among others.

The drugs that are the most widely used and that have shown the greatest action have been the rifamycin derivatives, fluoroquinolone derivatives, some macrolides, oxazolidinones, and nitroimidazoles.

Rifamycin derivatives

Within the family of the rifamycins (and apart from rifampicin), rifabutin and rifapentine deserve mention since they have mechanisms of action and minimum inhibitory concentrations (MICs) very similar to those of rifampicin. However, rifabutin presents cross-resistance with rifampicin in 70% of cases, while rifapentine does so in 100% of cases. This eliminates their possible use in the treatment of patients with TB resistant to rifampicin.

Rifabutin has become a good drug for replacing rifampicin in the initial treatment of patients with HIV infection who need protease inhibitor therapy, since the drug causes less active induction of the cytochrome P450 pathways in the liver. It has also been found to be useful as one of

the basic drugs in the treatment of disease caused by environmental mycobacteria.

The main advantage of rifapentine is that it is the rifamycin with the longest duration of action (24 hours, or five times longer than that of rifampicin), and it exhibits an increased macrophage penetration potential (25- to 50-fold that of rifampicin). Thus, rifapentine could be used in highly intermittent treatment schemes. This drug has been studied in a number of randomised clinical trials, almost all involving a single weekly dose of 600 mg, and always with isoniazid. Results from these studies have shown that regimens containing rifapentine demonstrate the same toxicity, tolerance, compliance, and therapeutic failure profile as those containing rifampicin, although the relapse rate is higher. This suggests that the weekly 600-mg dose is probably suboptimal. Regimens involving 900-mg and 1200-mg doses are currently being evaluated in patients without HIV infection, since in patients infected with the virus the failure rate has been found to be comparatively greater. Despite its possible future application, the role of rifapentine in the treatment of TB remains to be defined.

With regards to the other rifamycins, such as FCE 22250, GCP 29861, and R-76-1, there is little experience with them to date. Until there are further studies to assess their possible roles, none of them can be recommended.

Fluoroquinolone derivatives

These agents are carbonic acid derivatives, and their antituberculous action has been found to involve the inhibition of DNA gyrase. Since all the agents in this class act at the same level, they all present cross-resistance to each other. Consequently, if one of them cannot be used because of established bacterial resistance, the rest of the group is likewise rendered ineffective. However, not all members of the family present crossresistance with the rest of the antituberculous drug groups.

The first-generation fluoroquinolones, such as norfloxacin, are of little use, since they need to be given in very high doses. However, the second-generation drugs, such as ofloxacin and ciprofloxacin, have shown very good sensitivity *in vitro*, and there is sufficient clinical experience to propose them as one of the drug options of choice in TB re-treatment regimens—particularly because they are very well tolerated over the long-term and are easily available almost anywhere in the world. However, no randomised clinical trials have yet been conducted of these drugs.

In turn, some third-generation (e.g., levofloxacin) and fourthgeneration (e.g., moxifloxacin) fluoroquinolones offer superior MICs than do the second-generation drugs, and may also have additional advantages. For example, it has been found that the sterilisation capacity of moxifloxacin in the lungs of infected mice is superior when the drug is used with isoniazid than when either isoniazid or moxifloxacin is used alone. However, these drugs are very expensive, and there is no information yet on their long-term tolerance and toxicity. Consequently, for the time being, the second-generation fluoroquinolones are preferred, as they are less expensive, well tolerated, and easily obtained.

Macrolide derivatives

Although some macrolides, such as roxithromycin, clarithromycin, and azithromycin, have demonstrated *in vitro* antimycobacterial activity with good MICs, this is not the case in all instances, and there is insufficient evidence to recommend their use in the clinical setting. Accordingly, because both clarithromycin and azithromycin have shown such good activity against the rest of environmental mycobacteria, they currently constitute the basis for treating diseases caused by practically all these mycobacteria.

Oxazolidinone derivatives

The oxazolidinones are antibiotics that are administered orally. Their mechanism of action involves early protein synthesis disruption. Both the most widely used representatives in this class (e.g., linezolid) and the rest of its members (e.g., U-100480 and esperezolid) have demonstrated antituberculous activity *in vitro*. Linezolid also demonstrates antituberculous activity *in vivo*, and has been used on an experimental basis in patients with MDR-TB. Little, however, is known about its toxicity, particularly when administered for prolonged periods of time. To date, the main toxic effects identified involve induced anaemia and thrombocytopaenia, and peripheral neuropathy. These agents do not present cross-resistance with the antituberculous agents. At present, the lack of knowledge about their possible toxicity and their very high price excludes these drugs from possible use, even as rescue medication.

Nitroimidazole derivatives

These compounds, which are related to metronidazole, have been shown to be bactericidal when used against *M. tuberculosis*, both *in vitro* and *in vivo*. Experiments involving the drug referred to as PA-824 have shown efficacy similar to that of isoniazid, although its spectrum of action is very restricted (highly specific for TB). Both isoniazid and PA-824 affect bacterial cell wall lipid synthesis, although at different stages of the process. PA-824 also inhibits protein synthesis. In the same way as isoniazid, PA-824 acts on the rapidly multiplying bacterial population. However, in a static anaerobic culture model, the drug has also appeared to act on bacteria that are not in the replication phase. PA-824 has been shown to be effective against *M. tuberculosis* strains that are resistant to the first-line drugs. Moreover, it appears to be less toxic than isoniazid. Although further studies are needed, this agent shows promise as a good alternative to the first-line medicines. Until then, it cannot be recommended for use.

Other future therapeutic possibilities in tuberculosis

Apart from the antimycobacterial agents, other substances that are not antibiotics have also been developed. These substances, which show inhibitory effects on mycobacterial growth, include the derivatives of vitamin K or coenzyme Q (gangamycin).

Research is also being conducted on substances that interfere with the biosynthesis of vital components of mycobacteria, such as the mycoside C synthesis inhibitors, arabinogalactan synthesis inhibitors, transmethylation inhibitors, magnesium chelating agents, membrane cation flow inducers, substances interfering with mycobactin synthesis, membrane receptor blockers, trehalose phosphate synthetase inhibitors, analogues of mesodiaminopimelic-D-alamine, mycobactin analogues, and inhibitors of muramic acid enzymatic glycosylation.

Another line of treatment research refers to the so-called products for re-sensitising previously resistant bacterial strains, such as membrane permeators, beta-lactamase inhibitors (amoxicillin with clavulanic acid), and inhibitors of aminoglycoside-inactivating enzymes.

Lastly, an important chapter in the development of antimycobacterial treatment comprises immunotherapeutic or immune-modulating agents, which, together with the above-mentioned substances, might improve TB

therapy. Monoclonal antibodies, new vaccines, substances that improve opsonisation, cytokines, and immune-enhancing microbiological agents are the options that are currently being investigated the most intensively.

Another future therapeutic possibility is the use of antimicrobials contained within liposomes. These are phospholipids (double- or multilayered phospholipid vesicles) that can encapsulate drugs and macromolecules (size: 0.2 nm to 2-3 nm). These liposomes are avidly phagocytosed by macrophages, which may help direct drugs towards the macrophage population and the rest of the organs of the reticuloendothelial system. Liposomes are administered via the intramuscular route, which would help to reduce the dose and number of injections required, as well as (hopefully) toxicity and cost. The effect may persist for up to 5 weeks after administration, which might facilitate improved patient adherence to therapy. Studies involving streptomycin have been conducted in mice, as well as with rifampicin and isoniazid contained in cholesterol and phosphatidyl choline as vehicles. Intracellular action has been demonstrated, particularly in the liver, spleen, and kidneys, and, to a lesser extent, in lung tissue and lymph nodes.

Surgery for the management of multidrug-resistant tuberculosis

Section summary

Removing diseased portions of lung tissue for the management of MDR-TB is practically never indicated and should be rejected systematically. Lung surgery should only be contemplated in exceptional cases. Unfortunately, surgery is often ultimately used because of a lack of experience in the management of these patients or because of a lack of second-line drugs in certain countries. In such situations, it would be necessary to decide which approach is more reasonable: surgery in the face of such practical difficulties, or establishment of an adequate second-line drug bank to avoid surgical treatment.

With all the therapeutic resources and premises considered above, it is clear that efforts to remove diseased lung tissue for the management of MDR-TB should almost never be performed. The special circumstances that would warrant surgery include the presence of localised lung lesions, patients with good respiratory function, and the unavailability of drugs needed to design a re-treatment regimen. It should be emphasised that lung surgery is associated with high morbidity and mortality; further, the procedure is not certain to cure the disease. Thus, ultimately, TB retreatment is a matter of adequate management of second-line drugs.

Re-treatment as a strategy in an NTP: possibilities in lowand middle-income countries

Section summary

No resources should be spent on re-treatment until it is certain that all initial disease patients have been granted free access to a short-duration treatment scheme.

Once the above has been taken into account, even the poorest countries with the greatest TB problems should attempt to offer at least one standard 8-month re-treatment regimen involving first-line drugs (2HRZES/1HRZE/5H₃R₃E₃). This regimen is inexpensive and practical. It has been shown to heal over 90% of the patients included in NTPs as re-treated cases. It also identifies patients who have drug resistances, allowing them to receive more individualised care at a higher level within the health care system. The great majority of low-income countries should not spend further resources on re-treatment.

However, in middle-income countries, as well as in some low-income countries, the acquisition of a second-line drug bank may be advisable in order to offer a standard treatment regimen with these agents: 3(pyrazinamide, kanamycin, ethionamide, ofloxacin)/15(pyrazinamide, ethionamide, ofloxacin). Such drug banks should always be managed by the Central Unit of the corresponding NTP.

Individualised re-treatment according to the sensitivity results is only advisable in the wealthier countries and, as a last resort, in some middleincome countries.

It should be emphasised that prevention—with good initial drug schemes and strict supervision of treatment—is the best way to avoid having to deal with the complex problem of re-treatment.

The topics covered in this section offer a theoretical update in the field, and point to easy applicability, provided the best economical, health care, and epidemiological conditions are available. This is indeed the situation in richer countries, which have few cases of TB and which have abundant economic and health care resources. In these countries, there are more specialist physicians than there are patients requiring re-treatment. This makes it possible for each patient to be cared for by expert personnel, with the added support of all the necessary diagnostic methods (rapid sensitivity testing) and therapeutic options, since there are no economic

limitations. These expert physicians are able to identify relapses and cases of complete treatment abandonment, and can recommend returning to the same initial treatment scheme. They are also able to identify failures and cases of partial abandonment, and design re-treatment schemes that involve the appropriate second-line drugs. Thus, in the industrialised parts of the world, re-treatment is customised for each specific case. Nevertheless, these individualised re-treatments cost between US\$5000 and US\$10,000, and may even exceed US\$100,000 if the costs of prolonged hospitalisations are included.

Need for a standard re-treatment regimen with first-line drugs at the peripheral level in low-income countries

The situation described for industrialised countries does not apply to most regions in the world. In TB endemic countries-all of which have limited economic resources-even with the institution of good NTPs it is estimated that 10% to 15% of initial patients will ultimately return to the programme after some time. This means that in a country where 10,000 cases of TB are diagnosed a year, 1500 would require re-treatment. Depending on the existing economic and health care level, this figure is 50 to 500 times greater than the number of specialist physicians available in the country. This makes it practically impossible for each patient who is readmitted to the programme for re-treatment to be cared for by an expert in the field. Without great experience, the risk of incorrectly determining the cause for re-treatment (i.e., treatment abandonment, relapse, treatment failure, or poor patient compliance) is very high. This is a very important point, since only treatment failure indicates the existence of drug resistance (although poor adherence to therapy may also explain resistance). In the remaining cases (about 90% of patients), relapse or treatment abandonment is more likely the explanation, and the development of drug resistance is thus not likely to be expected.

Owing to the large numbers of patients admitted for re-treatment in countries with high TB endemic rates, it is necessary to treat these patients at the peripheral levels of the health care network, where it is not possible to establish which of the above four reasons is responsible for the indication of re-treatment. Moreover, it would be absolutely impossible, from the economic perspective, to provide individualised re-treatment schemes for all these patients. In effect, while these treatments cost US\$5000 to US\$7000, an initial treatment scheme, such as those recommended in the present Guide, costs only US\$10 to US\$20. In fact,

the cost of a single individualised (and less effective) re-treatment scheme would be equivalent to the cost of treating 250 to 350 patients with initial disease—the group of patients that constitutes the true epidemiological priority in TB endemic countries. Furthermore, most countries with a high prevalence of TB can only afford to spend less than US\$50 to US\$100 per inhabitant for all personal health care needs.

These operational and economic limitations led the International Union Against Tuberculosis and Lung Disease to consider the introduction of a standard re-treatment scheme with well-tolerated first-line drugs and easy management characteristics as the best approach to large-scale retreatment in NTPs. This 8-month re-treatment regimen is administered in three phases (2HRZES/1HRZE/5H₃R₃E₃), should be strictly supervised, and costs only about US\$30. The scheme is run in a similar way as that for initial TB patients. Under field conditions, the scheme will heal all relapse cases and cases of treatment abandonment (which is the cause of the great majority of re-treatments), along with 50% of patients reporting treatment failure. In this way, at the peripheral level, the proposed regimen will serve to identify the true cases of resistance (i.e., the patients who do not heal), which represents only 1% to 2% of the total number of patients. This small patient group can then be treated at higher levels within the health care system by specialist physicians, and with the possibility of using secondline medicines.

Re-treatment with second-line drugs in low-income countries: individualised or standardised?

It has already been explained why in all low- and middle-income countries the corresponding NTPs should implement a standard 8-month retreatment scheme involving first-line drugs for patients failing initial therapy. Such a scheme, conducted at the peripheral level and involving simple management, would enable the identification of true problem cases, i.e., patients with drug resistances. However, once these patients have been identified, what should be done with them? Is use of an expensive individualised regimen involving second-line drugs justified?

Two possibilities exist for the management of such patients: individualised re-treatment according to the resistance pattern observed, or re-treatment involving a standardised regimen of second-line drugs, without the need to perform sensitivity tests. There has been great controversy over which of the two is more appropriate. However, the fact is that second-line treatments are used infrequently in the great majority of countries with low-income levels; consequently, very few resistances to such drugs may be expected. Furthermore, such countries generally have very few chances of performing susceptibility tests with second-line drugs. Even if testing were possible, reliability is much more limited than in the case of first-line agents. For this reason, the most practical approach in these cases is to provide a standard re-treatment regimen involving secondline drugs that are known to be relatively well tolerated and easy to obtain, and that may offer important savings. Such a regimen may include pyrazinamide, a drug that is always administered together with other agents and to which there is rarely drug resistance. If the regimen does not include either isoniazid or rifampicin, the duration of re-treatment would be a minimum of 18 months. The most recommended option would be 3 months with pyrazinamide, ethionamide, ofloxacin (or ciprofloxacin), and an aminoglycoside other than streptomycin (although kanamycin is easier to acquire in most parts of the world), followed by another 15 months with the same drugs but withholding the aminoglycoside. This second-line drug regimen costs approximately US\$1000, which represents an important savings compared with the US\$5000 to US\$7000 associated with individualised re-treatment schemes.

Only when this third treatment step fails is it justified to move on to the fourth level of therapy—the most expensive and difficult in terms of management—which involves individualised re-treatment with second-line drugs lasting a minimum of 18 to 24 months.

Possibilities for re-treatment according to the available economic resources

It has already been emphasised that re-treatment is never an epidemiological priority. This is why, at least in theory, no resources should be spent on re-treatment until it is confirmed that all patients with initial disease have been granted free access to a short-duration treatment regimen. Unfortunately, this is not the case in many areas with serious TB problems; indeed, few resources are dedicated to sensitivity testing, second-line drugs, and other such concerns. This notion of maintaining cost-efficacy priorities is in conflict with the fact that caring for a patient with MDR-TB, without being able to offer the patient the necessary drugs, is almost the equivalent of a death sentence. This individualised clinical approach to the problem is the source of much controversy. As a result, the best approach may be to ensure treatment for all initial TB cases, under supervision, and to concurrently establish a minimum second-line drug bank that is to be managed only by personnel with extensive experience.

Once the above considerations have been applied, the implementation of the indicated re-treatment protocol becomes a matter of available economic and health care resources. All countries, including the poorest nations with the greatest TB endemic problems, should attempt to offer at least one standard 8-month re-treatment regimen involving first-line drugs (2HRZES/1HRZE/5H₃R₃E₃). As has been commented, this scheme is not particularly expensive and can be administered to a good proportion of patients (15% of the total); as a result, it can be expected to have epidemiological repercussions. This regimen should at least be included in the respective NTP manuals. Perhaps the results afforded by such treatment can provide the basis for future cohort studies.

Most of the low-income countries should not spend more resources on re-treatment apart from those already mentioned. However, countries with middle-level incomes (and also some low-income countries), which should have already moved beyond these two steps of treatment (initial patients and standard re-treatment with first-line drugs), may be able to establish a second-line drug bank so as to be able to offer a standard re-treatment scheme with these agents. However, the great risk posed by acquiring these drugs is possible indiscriminate use, which could lead to the amplification of drug resistance and to possible treatment inefficacy of second-line drugs in the future. For this reason, when an NTP decides that it possesses the necessary resources for, and considers acquiring, a secondline drug bank, careful assessment should be made of the number of annual re-treatments that may be required. Once acquired, this drug bank should always be managed by the NTP's Central Unit, which should not authorise any re-treatment without first confirming that sufficient amounts of medication exist for completion of the required 18 months of treatment. Moreover, before authorising such costly re-treatments, the prescribing specialist physician and the specialist physician working part- or full-time in the Central Unit should agree on the appropriateness of the course of therapy. This way, adequate management is guaranteed, resources are not wasted, and the validity (efficacy) of the drug is not compromised.

The last therapeutic step, which involves individualised re-treatment according to the sensitivity results obtained, is only advisable in richer countries and, as a last resort, in some middle-income countries.

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Chapter 17. Extrapulmonary tuberculosis

Chapter summary

From the initial phases of invasion of the human lung, *M. tuberculosis* can disseminate through the lymph vessels or bloodstream to any organ or tissue in the body. Extrapulmonary tuberculosis (TB) as a whole represents between 10% and 20% of all forms of TB in immunocompetent patients, although this rate is notably higher in those with some degree of immunodeficiency. The most common types of extrapulmonary TB, in order of frequency, are pleural, lymphatic, genitourinary, and joint; however, the disease does not spread from these sites.

In almost all cases, there is a primary pulmonary focus from which the disease may disseminate to adjacent sites, or via the lymph vessels or bloodstream to any part of the organism. Clinical symptoms will depend on the area affected by extrapulmonary TB. Symptoms are very non-specific and similar regardless of the location. This, along with the lower performance of diagnostic techniques when dealing with paucibacillary forms, means that greater importance is given to imaging techniques, even though there are no pathognomonic radiographic signs regardless of where the disease is located. Microbiological performance will depend on the quality of the samples taken; thus, if the diagnosis is uncertain, it will be necessary to perform a biopsy. Biopsy specimens must always be cultured in media for mycobacteria.

Treatment is the same as for pulmonary TB, although prolonged treatment in meningeal and joint TB, and in TB of the lymph nodes, is recommended by some. Under National Tuberculosis Control Programme conditions, the same initial treatment should be recommended for all patients.

From the initial phases of invasion of the human lung, *M. tuberculosis* can disseminate through the lymph vessels or bloodstream to any organ or tissue in the body. The epidemiology, frequency of presentation, pathogenesis, and performance of diagnostic methods in extrapulmonary tuberculosis (TB) differ from those of pulmonary TB. Only the treatment is the same, with the exception of a few specific details. The first part of this chapter will analyse the epidemiology, pathogenesis, diagnosis, and treatment of extrapulmonary TB, focusing on the aspects that differentiate this form of TB from pulmonary TB. The second part of the chapter will

be a brief summary of the most common forms of presentation of extrapulmonary TB.

A global perspective of extrapulmonary tuberculosis

Epidemiology

Extrapulmonary TB as a whole represents between 10% and 20% of all forms of TB in immunocompetent patients, although this rate is notably higher in those with some degree of immunodeficiency. Indeed, 60% of severely immunosuppressed HIV-infected patients affected by TB may have extrapulmonary forms of the disease.

The most frequent location is the pleura, which, as will be explained later, is the site predominantly involved in young patients. This is followed by the lymph nodes, which also mostly affect those during the early stages of life. The next two most commonly affected sites are the urinary tract and joints. There are other much less common locations of extrapulmonary TB; these may affect any patient.

Almost all cases of extrapulmonary TB are associated with negative smear microscopy, and as such it is accepted that the ability to infect others is practically nil. For this reason, extrapulmonary TB is not considered an epidemiological priority for National Tuberculosis Control Programmes (NTPs). Only the individual stands to benefit, while the community receives very little (if any) advantage. Consequently, when a contact study is required in such cases, the sole objective is to discover the initial source that infected the patient with extrapulmonary TB. Nevertheless, all cases should be reported to the NTP.

Pathogenesis

In almost all cases of extrapulmonary TB, there is a primary focus in the lungs, which may or may not be visible on a chest radiograph. This primary pulmonary focus may disseminate via means of proximity (e.g., pleural TB), through the lymph vessels (e.g., lymphatic TB), or through the bloodstream (to any location). When the bacillus reaches these areas, there is a local immunizing action that, together with the metabolic conditions of the area (pH and oxygen tension), will determine whether or not the disease will develop.

With the exception of pleural and lymphatic TB, in the vast majority of cases of extrapulmonary TB disease, dissemination occurs via the bloodstream. This may occur at any moment the organism is under attack by *M. tuberculosis*, even from the very first moments of primary infection. When TB is present in more than two locations, it is known as disseminated TB, which is an extremely serious condition since it indicates that the host defences are unable to control the infection. The probability of developing disseminated TB increases in persons with a greater level of immunodeficiency

Clinical manifestations

The clinical manifestations will depend on the area affected by the microorganism—the most common sites will be discussed below. All areas affected by the disease have a common denominator, i.e., the surprising non-specificity of the symptoms produced. Thus, there is no pathognomic symptom or sign characteristic of TB in any location. In each of the organs or tissues affected, the clinical manifestations are generally insidious and similar to those caused by any other disease. For this reason, TB can frequently be included in the differential diagnosis of any clinical condition.

Diagnosis

The methods used to diagnose each of the possible forms of extrapulmonary TB are the same as those used in pulmonary TB, although it can be difficult to obtain valid samples in these patients. Furthermore, as these forms of the disease are paucibacillary, the performance of the different techniques is very low. These limitations, in addition to the fact that the clinical manifestations are generally very non-specific, means that imaging techniques take on greater importance and that it is often necessary to obtain biopsy samples for histological and microbiological study. All biopsy samples should be cultured in media for mycobacteria, and great care should be taken to ensure that the best possible samples are obtained.

It is necessary to culture biopsy samples since the mere discovery of caseating granulomas is insufficient to confirm a diagnosis of TB, although it can be accepted if the clinical manifestations and radiology are compatible. Other environmental mycobacteria may cause similar histological lesions. It should also be noted that granulomas might not be present in biopsy samples taken from patients with advanced HIV disease, since this typical lesion cannot be produced in patients with pronounced immunodeficiency. If the sample is directly manipulated (e.g., involving abscess punctures, cerebrospinal fluid, biopsies), aseptic measures must be taken and the sample should be transported in a sterile container. The sample that is to be sent to the microbiological laboratory must not be fixed in place, but instead should contain some drops of distilled water to prevent it from drying out. In the case of a patient with TB and HIV co-infection, it is important to send as many samples as possible (e.g., from sputum, urine, cerebrospinal fluid), as well as three haemocultures if the patient is severely immunosuppressed and has a fever of unknown origin.

Although radiology takes on greater importance in extrapulmonary TB, no location of TB presents a pathognomonic radiological sign of the disease, and as such the disease can never be diagnosed solely on the basis of a radiographic image.

Treatment

Although there are no in-depth studies describing the length of time needed to treat extrapulmonary forms of TB, there are also no microbiological bases contraindicating the use of the same treatment pattern that is recommended for pulmonary TB. The only difference is that the vast majority of extrapulmonary TB locations are associated with a reduced number of bacilli, meaning that it will be more difficult to select naturally resistant mutants. Consequently, some experts recommend prolonging treatment in patients with meningeal or joint TB, as well as in those with TB of the lymph nodes, while others have disputed this recommendation. For this reason, in an NTP setting the same initial treatment should be recommended for all patients.

Pleural tuberculosis

Section summary

The main way that *M. tuberculosis* reaches the pleural cavity is through the rupture of a subpleural caseous focus, in this case involving an immunological mechanism. The organism can also reach this area by dissemination through the bloodstream. The acute form is the clinical manifestation that is most often observed, and in the majority of cases this begins with non-productive cough, chest pain, and a high temperature. The chronic form is found predominantly in the elderly. A chest radiograph will generally show a small-to-moderate unilateral pleural effusion. One third of patients will also have radiographically visible parenchymatous disease. On the other hand, the tuberculin test is negative in one third of pleural TB cases, although the test will yield a positive result in a maximum of 8 weeks. A positive tuberculin test in a country where the disease is not endemic strongly points to a diagnosis of pleural TB, especially if the effusion involves a young person.

Tuberculous pleural fluid is serous and rarely haematic; it meets the criteria for exudate, has high protein levels, has glucose levels generally over 3.3 mmol/l (60 mg/dl), and the pH is always 7.3 or lower. The total number of leucocytes is generally less the 5000/l, with a predominance of lymphocytes, and the mesothelial cell count is reduced (<1 per 100 leucocytes).

For a definite diagnosis, it is necessary to demonstrate the presence of M. tuberculosis in sputum, gastric lavage, pleural fluid, or a pleural biopsy sample. The performance of microbiological studies involving pleural fluid and biopsy specimens is very low, which means that for the majority of patients it is necessary to carry out a pleural biopsy for both microbiological and anatomic-pathological study. The combination of microbiological and anatomic-pathological studies can lead to the diagnosis of pleural TB in 85% to 95% of cases.

Determination of adenosine deaminase (ADA) in pleural fluid and other ADA serosal fluids is important in the diagnosis of pleural TB. The test is highly sensitive and specific, and has a specificity and sensitivity of over 95% in countries where the disease is highly endemic, with a certain number of false positives detected in metapneumonic effusions, empyemata, rheumatoid arthritis, lupus erythematous, and lymphomata.

Treatment is the same as that used for pulmonary TB. However, it is worth noting that two thirds of pleural TB cases will spontaneously evolve towards a cure, although these patients will have a higher probability of developing another form of TB in the years to come. Neither steroids nor repeated thoracocentesis have any effect on the development of possible complications.

A pleural condition caused by TB is relatively common. Its presentation is linked to the endemic rate in the area; as such, it is fairly common in poor countries but extremely rare in industrialised nations. As there are differences between this form of TB and pulmonary TB, it is necessary to define its pathogenesis, clinical manifestations, diagnosis, and treatment.

Pathogenesis

The principal way that *M. tuberculosis* reaches the pleural cavity is through a rupture of the subpleural caseous focus, in this case involving an immunological mechanism. It seems likely that with the arrival of the bacillus with its antigen protein components, a delayed hypersensitive reaction occurs, stimulating the T lymphocytes. The T lymphocytes, which can be found in the pleural fluid, release specific lymphokines, which may alter the permeability of the pleural vascularisation and induce the formation of granulomas.

The pleura can also be affected by other mechanisms, above all if the disease is disseminated through the bloodstream. It is also possible for the primary focus to be reactivated; this is particularly common in older, immunocompromised patients. In children and young adults, pleural involvement is more likely to be the first manifestation of TB, occurring in the months after the primary infection.

Pleural TB is more common among young people, although up to 40% of some series are found in people over the age of 40 years.

Diagnosis

Clinical manifestations

The acute form is the most common clinical presentation, and in the majority of cases early symptoms include a non-productive cough, chest pain, and a high temperature. The chronic form is found predominantly in the elderly, in whom it is more common to find systemic symptoms such as asthenia, anorexia, and weight loss, accompanied by a slight fever, cough, and chest pain.

Radiology

A small-to-moderate unilateral pleural effusion is generally seen on chest radiograph (Figure 20). One third of patients will also have radiographically visible parenchymatous disease, which will be located in the same hemithorax as the effusion in the majority of patients. The amount of fluid is rarely significant, although in some series up to 4% of the massive effusions are caused by TB.

The other imaging techniques are of very little use when diagnosing pleural TB. Occasionally, computed tomography may reveal whether atelectasis or parenchymatous disease exists in the case of massive effusions.

Tuberculin skin test

The tuberculin test is negative in one third of patients with pleural TB, although the test will usually yield positive results if repeated within a period of no more than 8 weeks. One explanation for this phenomenon is that there are circulating monocytes during the acute phase of the disease, and these monocytes are known to be suppressors as they interfere with the sensitivisation of the T lymphocytes in the peripheral blood and skin but not in the pleural fluid. Other authors believe that this tuberculin anergy is a result of the sequestration of specifically reactive lymphocytes in the tuberculous tissues.

A positive tuberculin test in a country with low endemic rates strongly suggests a diagnosis of pleural TB, especially if the effusion affects young people.

Biochemical study of pleural fluid

Tuberculous pleural fluid has serofibrinous characteristics. It can sometimes be serosanguinolent (10%), but rarely haematic. It meets the criteria for exudate, with a high total protein concentration (a level >50 g/L [5 g/dl] is indicative of disease) and glucose levels generally over 3.3 mmol/l (60 mg/dl), although this figure may be lower in approximately 20% of cases. Low glucose concentrations are frequently associated with concomitant empyema, a delayed diagnosis, or advanced disease with pleural fibrosis. It is not unusual for glucose concentrations to be lower than 1.1 mmol/l (20 mg/dl). The pH, which is generally 7.3 or lower

(similar to the neoplastic effusions), is very variable and is of little help in diagnosis. It generally ranges from 7.00 and 7.29. However, a pH of more than 7.40 is not compatible with this aetiology.

Pleural fluid cellularity

The total number of leucocytes is generally less than 5000/l. When a differential recount is carried out, more than 50% of the total leucocytes correspond to mature lymphocytes, although a percentage of more than 80% is highly suggestive of TB. Polymorphonuclear cells may predominate during the first phase of infection, in response to the entry of bacilli in the pleural cavity, but these cells will be rapidly replaced by mononuclear cells. A diagnosis of TB is certain when it is observed in a short period of time that the polymorphonuclear cells are substituted by mononuclear cells in the cellularity of the pleural fluid. Eosinophils are rare, with numbers generally lower than 10%.

The number of mesothelial cells is reduced in a pleural TB effusion. Generally, there is less than one of these cells for every 100 leucocytes, while with other pleural aetiologies this number is generally more than 5 for every 100 leucocytes. This absence of mesothelial cells has been attributed to the fibrinogenous layer covering the pleural surface, which prevents the cells from shedding.

Microbiology

To diagnose pleural TB with certainty, it is necessary to demonstrate the presence of *M. tuberculosis* in the sputum, gastric lavage, pleural fluid, or pleural biopsy specimen. The smears and the sputum and gastric lavage cultures may help confirm the diagnosis, although these rarely yield positive results unless there are parenchymatous lesions that can be seen on a radiograph.

The performance of microbiological studies on pleural fluid increases with the amount of fluid studied, and it is generally accepted that ideally 1 litre should be processed. A direct smear microscopy of this sample will be positive in less than 10% of patients, and the culture, depending on the series, will show evidence of the growth of M. *tuberculosis* in 11% to 70% of these cases. The low sensitivity of the smear and the excessive delay associated with culture means that it is necessary to carry out a blind pleural biopsy for many patients, a simple technique that is associated with low morbidity when using needles that
are currently available. This procedure is especially necessary if it is not possible to determine the presence of adenosine deaminase (ADA) in the fluid. In addition, since paucibacillary forms of TB are involved, genetic amplification techniques (e.g., polymerase chain reaction [PCR]) may be very useful in the diagnosis (see Chapter 8).

Direct microscopy of the pleural biopsy specimen shows acidalcohol–resistant bacilli in 14% to 34% of cases; in comparison, the culture is positive in 44% to 80%. Performance improves with a second and third biopsy, with regards to both the microbiological and anatomic-pathological study.

Histopathology

A diagnosis of pleural TB can also be accepted if granulomas are discovered in the pleural biopsy sample. These may appear in 50% to 87% of patients who have undergone a blind pleural biopsy. Nonetheless, pleural granulomatosis is not exclusive to TB, as other diseases such as sarcoidosis, rheumatoid arthritis, fungal infections, other mycobacteriosis, and even infections caused by *Francisella tularensis* may sometimes be the cause.

Fifteen percent of patients with pulmonary parenchymatous disease caused by M. *intracellulare* may present with pleural effusion, an occurrence that also applies to 3% of the mycobacteriosis caused by M. *kansasii*, and to 5% of the other diseases caused by other mycobacteria. These pleural afflictions may be confused with TB because pleural granulomatous inflammation is also observed, and because these mycobacteria are also shown as acid-alcohol–resistant bacilli on direct smears. Only cultures of this material can yield a definite diagnosis, although it is important to note that these mycobacteria do not lead to pleural involvement without also causing a pulmonary parenchymatous disease, and as such can also be diagnosed by processing the samples from the respiratory system.

Despite the above, more than 95% to 98% of cases in which granulomas are observed in the anatomic-pathological study of the pleural biopsy specimen involve TB, and this percentage is even higher if the granulomas have caseating necrosis.

By combining the microbiological studies of the fluid, pleural biopsy, and anatomic-pathological study of the biopsy specimen, a diagnosis of pleural TB can be established in 85% to 95% of cases. In the remaining 5% to 15%, this diagnosis can often be assumed. Nevertheless,

if these data do not suggest TB, or if there is any doubt about the diagnosis, a videothoracoscopy and biopsy of the suspicious areas will be indicated. If these samples are negative in the histological and microbiological studies, a diagnosis of pleural TB can be ruled out.

Adenosine deaminase and other determinants in pleural tuberculosis

Over the last two decades, a series of diagnostic tests involving pleural fluid has been developed. The determination of ADA in pleural fluid and other serosal fluids (see Chapter 8) has been shown to be particularly useful. This enzyme, which intervenes in the catabolism of purines and whose main physiological activity takes place in lymph tissue, has high levels of sensitivity and specificity (over 95% in countries where the disease is highly endemic), with a certain number of false positives detected in metapneumonic effusions, empyemata, rheumatoid arthritis, erythematous lupus, and lymphomata. Concentrations of ADA in the pleural fluid of over 43 to 45 U/l are associated with a sensitivity of 100% and a specificity of 95%.

The use of ADA has been sufficiently validated in several studies and can now be accepted as a routine technique in the diagnostic algorithm for pleural TB. It is important to bear in mind that the sensitivity of microbiological techniques in TB of serosal fluids is very low; therefore, when faced with a suggestive clinical manifestation and radiograph and a positive ADA test, a diagnosis of TB can be accepted. This is especially true in regions where it is not possible to perform a pleural biopsy. Measurement of ADA is not difficult to perform nor is it very expensive, so having reference centres in some low- and middle-income countries (depending on the population) that can perform determinations of this enzyme should be considered. ADA measurement is not indicated in poorer countries where there are limited resources and high TB endemic rates. In such countries, when there are clinical and radiological manifestations suggestive of TB, empirical treatment should instead be started.

Another test that has proved to be useful in the study of pleural TB is the determination of lysosyme (muramidase) in the fluid and, above all, the coefficient of this with the level of lysosyme in serum. Thus, a coefficient of more than 1.2 has excellent sensitivity (100%) and specificity (95%), with some false positives caused by empyemata and rheumatoid arthritis. Nevertheless, more studies are needed to validate this technique before it can be recommended as a routine procedure.

As the proportion and absolute number of T lymphocytes in tuberculous pleural fluid increase with respect to those found in the blood, the number of lymphokines produced also increases. One of these lymphokines, interferon γ , has also been shown to be a useful parameter in the diagnosis of pleural TB.

Lastly, there has been evidence that some tumour markers, such as immunosuppressive acidic protein and α_1 -acid glycoprotein, have considerably higher levels in tuberculous pleural fluid in comparison with neoplastic pleurisies. The determination of any of these parameters is not indicated in low- and middle-income countries.

Diagnosis of complications

Occasionally, infection of the pleural space by *M. tuberculosis* may lead to empyema. This may at times appear to be a complication of the parenchymatous disease, especially in the case of cavitary forms located in the upper lobes. This empyema is often associated with a bronchopleural fistula, as it is unusual for the empyema to drain, thus forming a fistula of the thoracic wall (*empyema necessitatis*). It is more common to find other infecting bacteria besides *M. tuberculosis*, in particular all gram-negative bacilli and *Staphylococcus aureus*, growing in the cultures of the pleural fluid.

A diagnosis of bronchopleural fistula is suggested by the development of water-air levels in the pleural cavity which can be seen on the chest radiograph. Injecting methylene blue or radioopaque material into the cavity and recovering it in the expectoration can confirm this.

Approach to the diagnosis of pleural tuberculosis

When the microbiological and anatomic-pathological studies of the pleural fluid and biopsy are negative, empirical treatment can begin in certain patients while awaiting the cultures, even if these also prove negative. Thus, in patients under the age of 40 years who have a positive tuberculin test and whose pleural fluid corresponds to an exudate with a predominance of lymphocytic cells, antituberculous therapy may well be indicated. This indication is even more justified in low- and middle-income countries, or if the presence of ADA has been determined. On the other hand, if TB is suspected in an effusion that appears in a patient older than 40 years with risk factors for bronchogenic carcinoma and in whom

studies of pleural fluid and blind pleural biopsy are negative, a pleuroscopy before starting empirical treatment is indicated.

Treatment

Two thirds of pleural TB cases will spontaneously evolve towards a cure. Nevertheless, in the case of these patients who are cured without receiving treatment, there is a high probability that pulmonary parenchymatous disease will appear during the following 6 to 12 months. Likewise, it has been found that 50% to 70% of patients who do not adhere to their treatment correctly will develop pulmonary TB or TB in another location in less than 5 years.

The treatment used for pleural TB is no different than that for TB of other locations, with the exception that, since it is a form of TB that has very few bacilli, it is difficult to select resistant mutants. This is why it may be advisable to omit a fourth drug during the first phase. Under NTP conditions, the same treatment as that for pulmonary TB should be recommended so as to facilitate the indications and follow-up. In addition, as has been explained earlier, the patient may also have a pulmonary parenchymatous condition, although this may not be visible.

Once treatment has begun, fever will generally disappear within the first 2 weeks, although this may not occur until 6 to 8 weeks later. The effusion may take up to 3 or 4 months to clear up; however, in the majority of cases it will do so in less than 6 weeks. In any event, this does not mean that the treatment should be changed in any way.

Some controversy surrounds the possible beneficial effect of corticosteroids in the treatment of pleural TB. It seems that corticosteroids merely cause symptoms to improve earlier and resolve the effusion more quickly, but they do not reduce the possibility of long-term sequelae. These agents have been described to decrease pleural thickening in the first 2 to 6 months of the disease. Therefore, corticosteroids are only indicated in the short-term if the symptoms are intense or the patient is gravely ill. They should never be administered in the long-term to reduce the sequelae of the disease. Still, it is important to remember that these sequelae are uncommon and negligible if correct treatment is initiated.

Therapeutic thoracocentesis or repeat aspiration of fluid offers no advantages regarding the decrease or resolution of the disease, nor does either technique have any impact on the sequelae on pulmonary functions. Nevertheless, in the case of massive effusions, thoracocentesis may be considered. As with other infectious pleurisies, it is necessary to wait a minimum of 6 months before evaluating the sequelae and their possible surgical treatment.

In the case of a bronchopleural fistula and/or empyema, apart from antituberculous chemotherapy, it is necessary to insert a chest drainage tube. In patients in whom pharmacological therapeutics have made the sputum cultures negative, decortication is required if the fistula remains. In such cases, intervention can be extremely serious and the operation is associated with high mortality. Pulmonary expansion will not be possible in certain patients due to the severity of the parenchymatous affection; thus, it is frequently necessary to perform a thoracoplasty to fill the pleural space.

A patient suffering from pleural TB does not need to be isolated from others. Isolation is only necessary in the case of parenchymatous disease with a positive result to direct smear microscopy of the sputum. Even in this event, the patient may remain at home unless the severity of the process indicates otherwise.

Lymph node tuberculosis

Section summary

Lymphatic TB can be divided into two main groups: the first affects the peripheral lymph nodes (scrofula), and the second affects the internal adenopathies. TB of the peripheral lymph nodes affects the adenopathies in the head and neck principally, although it can affect any other area. The most important differential diagnosis should consider lymphadenitis caused by other mycobacteria. In children, *M. tuberculosis* is isolated in only 10% to 20% of peripheral lymphadenitis cases, increasing to 90% in adults. This entails different therapeutic routes and underlines the importance of culturing the biopsy samples or aspirates obtained from these locations. TB of the internal lymph nodes is generally a complication of primary TB. When it affects the mediastinum, the most common location, large adenopathic masses may be formed, which may compress and even perforate the tracheobronchial tree.

The treatment of lymphatic TB is the same as that for pulmonary TB, although some experts advocate prolonging the treatment for up to 9 to 12 months. One problem is that the size of the adenopathies decreases very slowly (over weeks or months), and in 5% to 10% of cases they are still the same size after the treatment has ended. This, however, does not mean that the treatment was unsuccessful. Surgery may be indicated in cases of

lymph node TB caused by other mycobacteria, and when the mediastinum has been affected and is extremely compressive.

Owing to the high frequency with which *M. tuberculosis* disseminates through the lymph vessels, lymphatic TB is one of the most common forms of extrapulmonary TB presentation. Lymphatic TB can be divided into two main groups: the first affects the peripheral lymph nodes (scrofula), and the second affects mainly the internal adenopathies.

TB of the peripheral lymph nodes mainly affects the adenopathies in the head and neck, although it can affect any other area. The most important differential diagnosis must consider lymphadenitis caused by environmental mycobacteria. M. avium complex is isolated in 70% to 80% of cases of lymphadenitis. In Australia and the United States, the second most common cause is *M. scrofulaceum*, while in Northern Europe it is *M.* malmoense. In children, M. tuberculosis is isolated in only 10% to 20% of cases of peripheral lymphadenitis caused by mycobacteria, with M. avium and M. scrofulaceum isolated in the remaining instances. When an environmental mycobacterium is isolated, it can be argued that the form of entry and dissemination was not via the respiratory system, but via foci in the mouth, with the mycobacteria disseminating to the regional lymph nodes from there. *M. tuberculosis* is isolated in 90% of adults with this clinical manifestation. Awareness of the epidemiological differences between adults and children is of great importance since the vast majority of environmental mycobacteria that cause lymphadenitis in children are very resistant to antituberculous drugs. Furthermore, because this is a localised disease, surgical excision is indicated. However, in both adults and children in whom *M. tuberculosis* is isolated, this surgery is contraindicated and medical treatment is preferred. This evidence underlines the importance of culturing the samples obtained from a biopsy or by aspiration using a thin needle (the only way to obtain a definite diagnosis), rather than just sending these specimens to the laboratory for anatomic-pathological analysis.

For quite some time TB has been known to affect the lymph nodes. It was during the 1950s that involvement of the mediastinal lymph nodes in TB and its complications was first described, and that the earliest studies regarding the indication of surgery when the disease presented in this form were published. Involvement of the lymph nodes is usually a complication of primary TB. Perhaps for this reason and because there would seem to be greater lymph involvement in younger TB patients, this condition has been traditionally described in children (Figure 16). At present, with the HIV

epidemic, it is also quite common to find adults affected by this syndrome and with a pronounced cellular immunological deficit (Figure 31). Lymph node TB involving other sites such as the abdomen has been described in these patients. Large adenopathic masses may be produced in the mediastinum, which can compress and sometimes perforate the tracheobronchial tree. This process was considered relatively common in the past, especially before the advent of chemotherapy, with compressive symptoms reported in 67.8% of patients studied and bronchial perforations in 27.8%. Currently, with the availability of bactericidal treatments, this form of TB presentation and its complications is considered to be rare. Nonetheless, not everyone is in agreement with this belief, and certain series have been reported quite frequently. In these cases, early diagnosis is important, since aggressive treatment approaches will often be necessary to avoid complications.

Involvement of the abdominal lymph nodes by TB is common, and adenopathies can normally be found in various sites. These vary in size and the pathology can be studied with computed tomography, which will provide information regarding the size, location, and density of the adenopathies. Computed tomography will frequently be the method chosen in the case of HIV-infected patients as it provides information on all the regions affected. In patients with intestinal symptoms, it can help to discriminate among alimentary, mesenteric, and extraintestinal involvement. Findings are non-specific and a biopsy should be carried out, which can be complemented with use of imaging (an ultrasound scan or tomography). The adenopathies may obstruct the alimentary canal, urinary tract, or biliary tract (when their location is periportal or peripancreatic).

Biopsy samples must be obtained from the affected areas and then sent to the microbiological laboratory where a smear microscopy and culture can be carried out, as well as to the anatomic-pathological laboratory. Study of aspirate obtained using a thin needle to puncture the peripheral areas, or using a bronchoscope to obtain samples from the mediastinal lymph nodes, has also been shown to be useful.

Lymphatic TB should be treated in the same way as pulmonary TB, although some experts recommend prolonging treatment for up to 9 to 12 months, while others are in favour of maintaining treatment for the same length of time. One problem is that it is difficult for antibiotics to reach the lymphatic area, and the size of the adenopathy is largely due to a local immunological reaction. Consequently, adenopathies decrease in size extremely slowly (over weeks or months), and in 5% to 10% of cases they are still the same size after the treatment has finished, although this does

not mean that treatment was not successful. At the end, 5% to 10% will be considered cured, and the residual adenopathies will remain. Even after appropriate treatment, these residual adenopathies may increase in size, leading to apparent reactivations. If the adenopathies fistulate during treatment that is adequate and correctly followed, this does not indicate that treatment is not working.

Surgery is not only indicated in cases of lymph node TB caused by environmental mycobacteria, but also in mediastinal involvement that is compressive as a result of these adenopathies, as well as in cases where the lymph node mass finally perforates the tracheobronchial tree. The predominantly endobronchial lesions may be tributaries of the endoscopic treatment (granuloma resection). From a technical point of view, the most important surgical manoeuvres are the opening and curetting of the adenopathies. Attempts to dissect or extirpate are not justified when there are no important inflammatory adherences, as this may cause serious vascular accidents. Likewise, pulmonary resections are not indicated unless there is irreversible parenchymatous damage. In any event, surgical treatment of mediastinal lymph node TB must always be evaluated when complications exist, as this will solve the serious compression of the tracheobronchial tree. In these cases, surgery may prevent residual endobronchial lesions, which invariably lead to secondary evolutive complications. The morbidity and mortality associated with this type of surgery is almost nil.

Urinary tract tuberculosis

Section summary

Urinary tract TB is essentially a renal parenchymatous disease. It is produced as a result of dissemination through the bloodstream from a distant focus, which is generally pulmonary. It is therefore a bilateral disease, although it manifests as a localised one. In almost all cases there is a cured or active pulmonary lesion. It manifests when the lesion ulcerates a calyx or the renal pelvis, producing bacteriuria, pyuria, and abnormalities that can be detected radiographically; it is in this way that the rest of the urinary tract is most often affected.

This disease can be diagnosed with certainty only when the presence of *M. tuberculosis* is demonstrated in urine cultures. Diagnosis cannot be certain with the detection of the typical lesions in the intravenous urography, or with the detection of acid-alcohol-resistant bacilli in the urine—these merely suggest the disease. Radiological findings may be very useful in diagnosis, especially those provided by intravenous urography. Treatment is the same as that for pulmonary TB, and surgical intervention should be evaluated in the case of complications or sequelae.

Urinary tract TB is essentially a renal parenchymatous disease. It manifests when the lesion ulcerates a calyx or the renal pelvis, producing bacteriuria, pyuria, and abnormalities that can be detected radiographically. The absolute requirement for diagnosis is demonstration of urine cultures, and not the detection of the typical lesions in the intravenous urography, nor the detection of acid-alcohol–resistant bacilli in the urine—these merely suggest the diagnosis.

The presence of *M. tuberculosis* in renal TB is the result of dissemination through the bloodstream from a distant focus, which is generally pulmonary. It is therefore a bilateral disease, although it is manifested as a localised one. In almost all cases, there is a cured or quiescent pulmonary lesion. The only visible lesion is often a small scar or a lesion that has been forgotten by the patient, and it is not unusual for long periods of time to elapse between the pulmonary infection and urinary manifestation. Dissemination to the kidney from a contiguous lumbar lesion is rare, and secondary involvement in various parts of the organism may coincide. Secondary to the release of emboli through the bloodstream, multiple foci in glomerular tubular capillaries appear, the majority of which will spontaneously lead to a cure, depending on the number and

virulence of the bacteria and host resistance. When cure is not spontaneous, necrosis occurs and the bacteria pass into the tubules and can be detected in urine.

The first radiographically visible lesion will be ulcerous cavitary papillitis resulting from the necrosis and coalescence of the tubercles. Found on the narrow part of the loop of Henle, they will affect the calvceal wall, normally at the end of the papilla. There may be one or several lesions that may be unilateral or bilateral and may progress to produce a calyceal deformity where the outline disappears and a cavity is formed. The calyxes become connected to each other through a progression of cavities or through fistulous trajectories. The disease may evolve from a mucus involvement of the other calyxes, urethra, or bladder. The multiple granulomatous lesions are accompanied by ulcerations, fibrosis, and stenosis. In areas where there is physiological narrowing of the urinary tract, such as the calvceal infundibula and the ureteropyelic and ureterovesical junction, these stenoses will produce caliectasis, hydrophronesis, and urethrohydronephrosis, with secondary destruction of the renal parenchyma. Curing these lesions will further aggravate the fibrosis and stenosis, possibly amputating part of a calyceal group or the whole kidney, which will not be visible with intravenous urography (partial and/or total autoamputation, or autonephrectomy). Apart from fibrosis and stenosis, the cure is characterised by the existence of calcic deposits. Calcifications may vary in density and extension.

For diagnosis, it is necessary to take three urine samples over 3 consecutive days, preferably early in the morning when the patient wakes up. Because other environmental mycobacteria are present in the urethra and glans and thus may also be isolated, smear microscopy is non-specific. For this reason, a culture of the urine sample is essential for a definite diagnosis. However, this does not mean that it is *not* necessary to carry out smear microscopy of the sample. Despite its non-specificity, the fact remains that a request for this technique indicates a suspicion of TB of the genitourinary tract. If this is the case, a positive smear microscopy can be of great value as it increases the positive predictive value of the technique. It will be of less value if the positive smear microscopy is obtained from an asymptomatic patient or a patient with symptoms that are not suggestive of TB; in such cases, a request for microscopic study is not indicated.

It is necessary to highlight the value of imaging techniques in guiding the diagnosis of genitourinary TB, especially when the lesions that suggest this disease are shown on intravenous urography. A normal finding with intravenous urography does not rule out the diagnosis. Further, renal TB causes no radiographic pathognomonic changes. Although it frequently produces very characteristic radiographic abnormalities, it is important to remember that all chronic inflammatory lesions may conceal nontuberculous pyelonephritis. The imaging study should begin with a simple abdominal radiograph, which may show: 1) associated skeletal lesions; 2) calcifications in other areas—such as hepatic or splenic granulomas, or calcified mesenteric adenopathies; or 3) renal or genitourinary calcifications. Tuberculous calcifications are more tenuous and more poorly defined than lithiasic calcifications, and may differ in size. They are also more cortical in location, although they may coexist with calculi. When these are generalised, it is known as nephrocalcinosis (Figure 54). Apart from the kidney, the urethra may calcify, although this is much less common, and calcifications may also be found in the prostate, seminal vesicles, and other such sites.

When there is reasonable doubt about whether the symptoms are caused by TB, intravenous urography is indicated. The earliest urographic sign is in the calyxes, and caliectasis is the most common early finding. Sometimes there is minimal erosion of the point of the calyx (ulcerous cavitary papillitis) or very slight calyceal irregularities, which is quite often very difficult to distinguish from pyelosinus reflux. As the infection progresses the caliectases increase, and it is possible to visualise cavities that are at times connected by fistulous trajectories (Figure 55). The process of cicatrisation and fibrosis is the most common finding, with infundibular stenosis (Figure 56) that may partly isolate part of the collecting system or a calyx. It is sometimes difficult to distinguish a cavity (Figure 56) from a dilated calyx.



Figure 54. Simple radiograph of the abdomen showing a small right kidney, with cavities and calcic-deposits (nephrocalci-nosis). Autonephrectomy.

Figure 55. Intravenous urography demonstrating a small cavity in the upper left calyceal group. Renal TB was confirmed by urine culture.





Figure 56. Intravenous urography showing infundibular stenosis of the upper calyceal group, as well as a large tuberculous cavity. Diagnosis of renal TB was made by smear microscopy and urine culture.

Retractions in the renal pelvis are accompanied by characteristic deformities (Figure 57) and, in the urethra, by areas of stenosis (Figure 58), which are more commonly found in areas that are physiologically narrowed (intersection of the bladder trigone iliac vessels and the ureteropyelic junction). Non-tuberculous pyelonephritis and non-specific stenosis may produce similar findings.



Figure 57. Intravenous urography showing a typical retraction of the renal pelvis, with caliectasis caused by chronic tuberculous pyelone-phritis. Diagnosis of renal TB was made by smear microscopy and urine culture.

The lesions in the urethra are due to renal or vesicle TB, and the earliest changes to take place are dilatations and irregularities of the wall, indicating the existence of ulcerations and oedemas. Afterwards, the urethra loses elasticity and evolves towards fibrosis and stenosis, which will be more pronounced in physiologically narrowed areas (Figure 58). There may be one or multiple stenoses, which may be long or short and may alternate with other areas of dilatation and stenosis, giving the urethra the appearance of a rosary.



Figure 58. Ascending pyelography showing the pronounced destruction and deformity the of collecting systems and renal pelvis, with dense shadowy areas of caseum and the contrast in cavities. Cortical atrophy and urethral stenosis are also seen. Advanced renal and urethral TB was confirmed by smear microscopy and urine culture.

Bladder involvement is most commonly manifested as retraction, a decrease in capacity, showing smaller bladders that sometimes lead to a vesicular urethral reflux and cause an ascending infection. The evolution of the disease towards fibrosis and stenosis, and the actual effect of chemotherapy, will further aggravate retraction (Figure 59).

Ultrasounds can be particularly useful for evaluating a kidney that has been deemed functionally annulled with intravenous urography, and may serve as a guide for aspiration puncture when the diagnosis is uncertain. Computed tomography may help to delimit the extension of the renal or extrarenal abscesses; however, its use is not indicated in uncomplicated cases as intravenous urography provides sufficient support for a diagnosis of renal TB.



Figure 59. Intravenous urography showing

urography showing a vesicle retraction and deformity, with a trigone filling defect as a result of the affected urethra. TB of the bladder was confirmed by urine culture.

The genitals may also become affected, although this is extremely uncommon.

Any organ may be affected in male subjects, although perhaps the most common presentation is epididymis TB (Figure 60). Likewise, any organ may be affected in female subjects; the most commonly affected areas are the annexes (tuberculous annexitis), which causes an inflammation of the fallopian tubes and frequently causes sterility. One curious point worth mentioning is that genital TB can also be caused as a result of direct inoculation during sexual intercourse.

Treatment is the same as for pulmonary TB, and surgical intervention should be evaluated in the event of complications or sequelae.



Figure 60. Inflamed right testicle with internal suppuration. Diagnosis of epididymis TB was made by culture of a sample obtained through an aspiration puncture using a thin needle. The patient had advanced pulmonary TB.

Bone and joint tuberculosis

Section summary

Bone TB is a secondary condition that results when a pulmonary focus disseminates through the bloodstream. However, evidence of an active pulmonary lesion is 30% to 50%. The bone lesions result when the bacilli reach the bone marrow, which represents approximately 20% of all cases of extrapulmonary TB.

Bone TB more commonly involves the vertebrae, especially the lower part of the dorsal and lumbar column, in 50% of cases. Bone TB may affect the epiphysis, metaphysis, and diaphysis, although it more commonly affects the first two, secondary to affecting the joints. The lesion is typically destructive. It evolves slowly and is eccentric, and there is a lack of reactivity of the adjacent bone, although it may sometimes be accompanied by a periostic reaction and soft tissue mass (cold abscess).

The presence of multiple lytic lesions in an oligo-asymptomatic patient, with no demonstrable reactive changes, should lead to a suspicion of TB. Depending on the site affected, there are various types of vertebral TB: disc, paradiscal, somatic, ligamentous, and atypical. Joint lesions are the most common after vertebral ones, and the larger joints are more likely to be affected. Involvement is usually monoarticular, affecting only one joint and, in descending order of frequency, involving the hip, knee, ankle, shoulder, wrist, and elbow. The earliest radiographic sign of joint TB is severe periarticular demineralisation. Joint TB can only be diagnosed with certainty through a culture, which makes it necessary to obtain biopsy samples from the area affected. Data supporting the diagnosis can also be obtained from radiographic studies (simple radiology, techniques using isotopes, computed tomography, and magnetic resonance imaging). Treatment is the same as that used in pulmonary TB, although some groups recommend that treatment be prolonged for up to 9 to 12 months.

Bone TB is a secondary condition that results when a pulmonary focus disseminates through the bloodstream. There is evidence of an active pulmonary lesion in 30% to 50% of cases. The bone lesions result when the bacilli reach the bone marrow, which represents approximately 20% of cases of extrapulmonary TB. It more commonly (>50%) involves the vertebrae (Figure 61). Bone TB may affect the epiphysis, metaphysis, and diaphysis (Figures 62 and 63), although it more commonly affects the first two, secondary to affecting the joints. The lesion is typically destructive, evolves slowly, and is eccentric. There is a lack of reactivity of the adjacent bone, although it may sometimes be accompanied by a periostic

reaction and soft tissue mass (cold abscess). The lesion may sometimes be diaphyseal, with a very characteristic appearance when it affects the short bones of the hands and feet, widening the medullary channel of the affected bone, a lesion known as *spina ventosa*. When bone TB is disseminated and destructive, and accompanied by little reactive sclerosis, it is known as TB of the cystic bone (Figures 63 and 64). The presence of multiple lytic lesions in an oligo-asymptomatic patient with no demonstrable reactive changes should lead to suspicion of TB. Just one lytic lesion (Figures 63 and 64) may be similar in appearance to an osteosarcoma. In fact, the lesions caused by bone TB are similar to those caused by pyogenic osteomyelitis, but with less destruction and reactivity of the adjacent bone.

As explained earlier, vertebral TB is the most common form of extrapulmonary TB, and more frequently involves the lower part of the dorsal and lumbar columns (Figure 61). This condition has been known as Pott's disease for over 200 years. Depending on the site involved, there are various types of vertebral TB: disc, paradiscal, somatic, ligamentous, and atypical. The purely disc form possibly results from dissemination by contiguity from a vertebral focus that is not visible on a radiograph. The paradiscal form is the most common and is manifested by involvement of the discs, vertebral plates, and paravertebral soft tissue mass (Figure 61). Its evolution is characterised by a loss of bone density, which leads to early hyperaemia, followed by marginal erosion in the phase of granuloma formation and a slow progression towards final bone destruction (Figure 61). The intervertebral space is maintained for a long time in the case of TB, in contrast with pyogenic infection. This difference can be of great importance in a differential diagnosis, although it is almost always better to differentiate between these two processes using clinical as opposed to radiographic data. The somatic form, which is more common among children, is presented as a vertebral osteomyelitis that leads to collapse of the body, occasionally accompanied by neurological lesions. The atypical forms are those that affect the neural arch and that lead to serious complications if the canal is infected. In these instances, computed tomography and nuclear magnetic resonance imaging allow a more complete evaluation, both with regards to the extension of the lesions and the study of other complications such as the migration of cold abscesses.

Vertebral TB is accompanied by soft tissue mass, normally symmetrically arranged in the paravertebral region. These are the so-called "cold abscesses", which later end up calcifying. Computed tomography and nuclear magnetic resonance imaging can be valuable methods for



studying these lesions. They allow early detection and better evaluation of their characteristics, and also serve as a guide for percutaneous puncture in cases where diagnosis is uncertain.

When the vertebrae are affected, it may not be certain if vertebral collapse was due to a neoplastic or an infectious cause. The combination of loss of height in the disc and badly defined contiguous vertebrae are very important signs of infectious actiology, since a reduction in height of a disc is an exceptional occurrence in neoplastic lesions. The presence of paravertebral mass, in the phase when it has not yet calcified, is a finding reflecting both a neoplastic and an infectious process.

Joint lesions are the most common after vertebral ones, and the larger joints are more likely to be affected.

Figure 61. Lateral radiograph of the lumbar column showing discitis of L4–L5. Diagnosis o TB was through anatomic pathological study and culture of a biopsy specimen from the area. Usually only one joint is affected. In descending order of frequency, the joints involved are the hip, knee, ankle, shoulder, wrist, and elbow.



Figure 62. Simple shoulder radiograph showing severe joint involvement after evolving over 2 years, with destruction of the humerus head. Diagnosis of TB was through anatomic-pathological study and culture of a biopsy specimen from the area.



Figure 63. Simple tomographic scan of the right shoulder. Erosive epiphyseal involvement with cavitation was observed. TB was confirmed by anatomic-pathological study and culture of a biopsy specimen from the area.



Figure 64. Simple radiograph of the tibia. One lytic image. Osteosarcoma was initially suspected. Diagnosis of TB was by anatomic-pathological study and culture of a biopsy specimen from the area.

Joint TB has characteristics that differentiate it from the pyogenic conditions. In pyogenic arthritis the inflammatory exudate has many more proteolytic enzymes, which cause significant destruction, unlike what happens in tuberculous arthritis. The location of the cartilaginous destruction is also different, given that pyogen occurs in cartilage in apposition, which supports weight, whereas TB generally affects free surfaces. The exceptions to this are the hip, ankle, and metacarpophalangeal joints, which generally show profuse damage as they have little free surface. Another difference is progression time, which is quick in pyogenic arthritis but slow in the case of TB (Figure 62).

The earliest radiographic sign of joint TB is severe periarticular demineralisation. This is believed to be related to local hyperaemia, disuse, and the action of the bacterial toxins, although its origin is not vet understood. Initially, periarticular blurring may also occur, which is why it is important to compare radiographs so that this sign can be detected early on. Tumefaction of the soft tissues and changes in the bone are observed later, appearing in the articular margins that do not support weight (Figure 62). Joint involvement may present as synovitis or as osteoarthritis, with periarticular tumefaction in cases of synovitis with osteoporosis (cold tumour). The formation of granulomas in the synovial membrane will progress to osseous invasion with marginal erosions, which, in the case of the knee, may occur on both sides of the joint (kissing lesions). This type of lesion may lead to destruction of the joint (Figure 62) and calcification of the soft tissue; differential diagnosis will concern algodystrophy or other granulomatous involvement. In children, any cause of chronic synovitis that can also produce juxtaarticular atrophy or synovial hypertrophy, and an increase in epiphysias, should be noted. In adults, joint TB must be differentiated from primary rheumatoid arthritis, although joint TB is generally polyarticular and affects the small joints. Differentiation can be difficult when the condition begins as monoarticular, affecting a mediumsized joint. In such cases it is important to evaluate the critical and analytical criteria.

In order to diagnose joint TB with certainty, it is necessary to isolate colonies of *M. tuberculosis* in culture. Thus, biopsy samples should be obtained from the affected area and processed for microbiological and anatomic-pathological study. Obtaining valid biopsy samples may determine whether major surgical intervention is needed, since the suspected diagnosis, radiological findings, and endemic rate in the region may not point to the need for surgery. If other possible diseases can be reasonably ruled out, especially those of neoplastic origin, antituberculous treatment may be indicated in low- and middle-income countries and the disease can be followed to see how it evolves. For this reason, imaging techniques can play a very important role in these types of conditions. These methods may include:

1. *Simple radiology*. This is useful for detecting alterations at an early stage and to evaluate the effects of the therapy. The radiographic signs, which have already been covered, include tumefaction or blurring of

the soft tissue, juxtaarticular osteoporosis, marginal erosions in the free surfaces, a decrease in space, and, at times, joint destruction (Figure 62) with calcification of the soft tissue.

- 2. *Techniques using isotopes.* This estimates the physiological activity in the bones and joints, detecting small increases and decreases. Findings are non-specific and thus must be complemented with other methods in order to characterise the abnormal areas. Scanning with technetium will yield signs of involvement early on in the initial phases with a normal radiograph. In addition, it provides information on the whole skeleton. Scanning with gallium, which is sensitive for the detection of inflammation, is useful in detecting early cellulitis. A decrease in the activity of gallium is a good indicator of follow-up with regards to response to therapy, as well as of follow-up to discern the presence of chronic osteomyelitis or its reactivation.
- 3. *Computed tomography*. This makes it possible to discriminate between contiguous structures based on slight differences in density. The method provides information on the extension of the process, the characteristics of the lesion, and the identification and extension of the extraarticular abscesses. It also guides percutaneous puncture when diagnosis is uncertain.
- 4. *Magnetic resonance imaging*. Like computed tomography, this method provides spatial resolution, showing better resolution in the contrast of the soft tissue. No contrast injection is required and it is more sensitive than computed tomography when detecting abnormalities, although this does not mean that it has higher specificity. It provides a more precise anatomic delineation of all the structures in the column.

Treatment is the same as that used in pulmonary TB, although some groups recommend that treatment be prolonged for up to 9 to 12 months—a recommendation that, however, has not been shown to be associated with greater benefit. Nevertheless, depending on the site affected, surgical interventions are quite often necessary, especially to correct deformities or other sequelae.

Peritoneal and digestive tract tuberculosis

Section summary

Digestive tract TB can be caused via four mechanisms: 1) by swallowing bacilli when drinking milk from infected cows, or (if infected) by swallowing one's own sputum, thus affecting the mucous membranes and mesenteric lymph nodes; 2) by dissemination through the bloodstream; 3) by dissemination through the lymph vessels; and 4) by contiguity.

Intestinal TB can appear in many forms: ulcerative, hypertrophic, or with ileal colic involvement (the ileum and colon are affected in 70% to 90% of cases). During the initial phases, there may be spasms and accelerated transit, followed by thickening of the ileocaecal valve, which appears as a mass in the caecum. The valve is distorted as a result of the ulceration and fibrosis and the ileum narrows.

A gastric or duodenal location is extremely rare and has no specific radiological characteristics. It may appear as an ulcer, or like a carcinoma if the hypertrophic form predominates. Oesophageal involvement is even more rare and also has no specific radiological signs, appearing as an ulceration, area of spasm or adherences, or mediastinal lymph nodes.

Peritoneal TB constitutes two thirds of cases of abdominal TB, with the peritoneum as the primary site in 35% to 58% of patients. Secondary involvement may occur through direct extension (rupture of a lymph node or perforation of the intestinal tract), by dissemination through the bloodstream, or through the lymph vessels. There are three types of tuberculous peritonitis: wet, dry, and fibrotic. The symptoms are nonspecific and the most common finding is ascites, with a high protein content and leucocytosis with a predominance of lymphocytes. The culture proves positive in 50% of cases. The determination of ADA in the peritoneal fluid can be very helpful in this case.

Diagnosis should be confirmed by a culture, although this will frequently entail aggressive diagnostic attitudes, which may not be justified in low- and middle-income countries. Treatment is the same as for pulmonary TB.

Owing to the pasteurisation of milk and an improved control of pulmonary TB in developed countries, the incidence of peritoneal and digestive tract TB has decreased considerably, representing 11% to 13% all cases of extrapulmonary TB. Before this, 50% to 90% of patients with pulmonary

TB suffered a gastrointestinal infection. The incidence in poorer countries is not known. The microorganism that is frequently involved is *M. bovis*.

This form of extrapulmonary TB can be caused through four mechanisms: 1) by swallowing bacilli when drinking milk from infected cows, or (if infected) by swallowing one's own sputum, thus affecting the mucous membranes and mesenteric lymph nodes; 2) by dissemination through the bloodstream; 3) by dissemination through the lymph vessels; and 4) by contiguity. The presence of the bacilli at a mucous membrane will cause acute localised inflammation of the lymph tissue of the submucosa. After 2 to 3 weeks, tubercles are formed with epithelial cells and lymphocytes, after which the tubercles undergo gas necrosis and fibrous cicatrisation.

There are several types of intestinal TB:

- 1. Ulcerative form. This is characterised by elongated ulcers perpendicular to the large intestinal axis, which may have irregular edges and have a pronounced spasm of the loop affected during the acute phase, leading to functional stenosis (Figure 65).
- 2. Hypertrophic form. This is characterised by a pronounced inflammatory fibroblastic reaction. An abdominal mass can frequently be felt, with severe associated mesenteric involvement. The surface of the mucous membrane may have a multi-nodular pattern or be a large mass that cannot be distinguished from a tumour. There may be mixed ulcer-hypertrophic forms with cobbled markings or a large abdominal mass. The areas most frequently affected are the proximal and distal colon, and the ileocaecal region is the most typical location (Figure 66). Involvement of the oesophagus (Figure 67), stomach, duodenum, and rectum is extremely rare. The most frequent complication is obstruction (12-69%). Fistulisation and haemorrhage are less common. Perforation is also very infrequent (<10%), probably due to thickening of the intestinal wall and the mesentery, which produces the disease.
- 3. *Ileal-colic involvement.* The ileocaecal location is the most frequent involved, constituting 70% to 90% of cases (Figure 66). In the early phases, spasms and accelerated transit are observed, followed by thickening of the ileocaecal valve, with the appearance of a mass in the caecum. The valve is distorted as a result of the ulceration and fibrosis, and the ileum narrows (Figure 66). More advanced studies may show retraction with a pronounced shortening of the caecum. A fibrotic ileum terminus ending in a retracted caecum with an incompetent ileocaecal valve is classically known as the Stierlin's sign. Other

intestinal diseases may be similar in appearance, and at times differentiation can be difficult, especially in the case of Crohn's disease and amebiasis. In the colon the disease is manifested as an ulcerated segmented lesion, with spasms and rigidity of the wall, which may be accompanied by a mesenteric mass and, at times, fistulisation (Figure 65).

A gastric or duodenal location is extremely rare and has no specific radiological characteristics. It may appear as an ulcer, or as a carcinoma, if the hypertrophic form predominates.

Oesophageal involvement (Figure 67) is even more rare and also has no specific radiological signs. It sometimes appears as an ulceration, area of spasm or adherences, or mediastinal lymph nodes. Stenosis and fistulous tracts may be produced as a result of the penetration of the contents of a mediastinal adenopathy in the oesophageal lumen. This is a very unusual location. The differential diagnosis should include bronchogenic or metastasic carcinoma with secondary infiltration of the oesophagus; neoplasia of the oesophagus; and, rarely, Crohn's disease. In cases where the oesophagus is involved, computed tomography will be useful for observing the extraluminal extension.



Figure 65. Opaque enema. TB of the colon, ulcerative type, with functional stenosis. Diagnosis was made by anatomic-pathological study and culture of a biopsy specimen of the area, obtained using a colonoscope.



Figure 66. Opaque enema. Ileocaecal TB. Ileal stenosis with mesenteric thickening and retraction of the caecum. Diagnosis was made by anatomic-pathological study and culture of a biopsy specimen of the area, obtained using a colonoscope.

Peritoneal TB constitutes two thirds of cases of abdominal TB, with the peritoneum as the primary site in 35% to 58% of patients. Secondary involvement may occur through direct extension (rupture of a lymph node or perforation of the intestinal tract), by dissemination through the bloodstream, or through the lymph vessels. There are three types of tuberculous peritonitis: 1) *the wet type*, with free or encapsulated ascites; 2) *the dry type*, with caseous nodules (plastic peritonitis); and 3) *the fibrotic type*, fixed with an abdominal mass.

The symptoms are non-specific and the most common finding is ascites, with a high protein content and leucocytosis with a predominance of lymphocytes. The culture proves positive in 50% of cases. Ascites may be detected through clinical exploration, using ultrasound or computed tomography, where highly attenuated peritoneal fluid can generally be seen. Likewise, involvement of the mesentery and epiploon may be observed, appearing to have a higher density, or as a solid mass replacing the normal epiploon (omental cake). The differential diagnosis should include bacterial peritonitis, peritoneal carcinomatosis, and mesothelioma.



Figure 67. Oesophageal transit. Large mass in the oesophagus. Diagnosis of TB through an anatomic-pathological study and culture of a biopsy sample of the area, obtained using an oesophagoscope.

To diagnose peritoneal TB and TB of the digestive system with certainty, it is necessary to isolate colonies of *M. tuberculosis* in a culture. This means that it is frequently necessary to obtain biopsy samples from the affected area, which must be processed for microbiological and anatomic-pathological study. Obtaining suitable biopsy samples may determine whether or not major surgical intervention is needed, since surgery may or may not be indicated by the suspected diagnosis, the radiological findings, and the endemic rate in the region. If other possible diseases can be reasonably ruled out, especially those of neoplastic origin, in low- and middle-income countries it may be advisable to start antituberculous treatment and see how this evolves. For this reason,

imaging techniques can play a very important role in these types of extrapulmonary involvement. The determination of ADA in the peritoneal fluid can be very helpful, since detection of ADA very often obviates the need for more aggressive explorations. In addition, as these extrapulmonary types of TB are paucibacillary forms, genetic amplification techniques (e.g., PCR) may be very useful in the diagnosis (see Chapter 8).

Treatment is the same as that used in pulmonary TB, although some groups recommend that treatment be prolonged for up to 9 to 12 months. This recommendation, however, has not been shown to be any more beneficial than the standard regimen. Depending on the site involved, surgical intervention may be necessary, especially in the case of intestinal obstruction. It is necessary to evaluate the rest of the cases carefully as this type of surgery may lead to even more adherences.

Tracheobronchial and upper airways tuberculosis

Section summary

TB of the trachea and large bronchi in children is frequently a complication of the primary disease. It may be associated with mediastinal adenopathies. Endobronchial involvement is the result of rupturing of caseous material in the bronchial wall, or of dissemination through the lymph vessels throughout the bronchial tree producing ulcerations in the mucous membrane. This form is known as gangliobronchial TB. Endobronchial involvement occurring via the direct implantation of tuberculous bacilli from active parenchymatous lesions, which are transmitted through the air, is most common in adults. The most common symptoms, apart from the general TB symptoms, are a persistent cough and possible stridor. The epiglottis, larynx, and pharynx are frequently affected and are usually an extension of pulmonary TB. Clinical manifestations include hoarseness, earache, pain on swallowing, and ulcerations on the tongue.

It is difficult to know how prevalent these types of TB are since a smear microscopy and/or positive culture are the principal diagnostic tests used in all cases. Nevertheless, in the case of pulmonary TB, these results will aid in the diagnosis of the disease in the vast majority of cases, considering that the upper airways, trachea, or large bronchi may also be affected.

The prevalence of tuberculous involvement of the trachea and large bronchi is currently unknown. Before chemotherapy, it was traditionally considered a complication of cavitary TB and was a fairly common presentation. Since the arrival of effective antituberculous drug treatment, these forms of extrapulmonary TB are more likely to be considered a rarity, although there is some disagreement regarding this point. Various mechanisms are involved in these forms of TB.

In children, this is frequently a complication of primary TB. It may be associated with large mediastinal adenopathies, and endobronchial involvement is the result of rupturing of caseous material in the bronchial wall, or of dissemination through the lymph vessels throughout the bronchial tree, which produces ulcerations in the mucous membrane. This form is known as gangliobronchial TB. These adenopathies do not only perforate the bronchus but may also compress it. For various reasons, residual stenotic lesions may form in the airway, possibly leading to future repeated infections. In these cases, early diagnosis is of critical importance as a high percentage of cases will need aggressive treatment if complications are to be avoided.

Endobronchial involvement via direct implantation of tuberculous bacilli from active parenchymatous lesions, which are transmitted through the air, is the most common cause of infection in adults, and less so in children. The most common symptoms, apart from the general TB symptoms, are a persistent cough and possible stridor.

The epiglottis, larynx, and pharynx are frequently affected, usually as an extension of pulmonary TB. Clinical manifestations include hoarseness, earache, pain on swallowing, and ulcerations on the tongue. This type of TB must be differentiated from cancer of the larynx, although the latter condition is rarely painful.

It is difficult to know how prevalent these forms of TB are as smear microscopy and/or positive culture are the principal diagnostic tests used in all of these cases. It is generally accepted that the incidence of bronchial TB is increasing in the HIV-infected population, which may explain some cases in which a smear-positive result and a normal chest radiograph have been observed. In the case of pulmonary TB, these results will be true in the vast majority of cases and may lead to a diagnosis of the disease, while considering the possibility that the upper airways, trachea, or large bronchi may also be affected. For this reason, in order to determine if there is extrapulmonary involvement, it is necessary to use endoscopic techniques, such as laryngoscopy and bronchoscopy, which are capable of locating the lesions and serving as a guide for a biopsy to confirm the diagnosis. In any event, treatment and cure are the same as with pulmonary TB, although surgical intervention is sometimes necessary in the case of gangliobronchial TB in children.

Cerebral and meningeal tuberculosis

Section summary

This type of TB is one of the most serious forms; therefore, early diagnosis and treatment are essential. The bacilli may enter the subarachnoidal space, causing inflammation of the meninges, formation of a mass at the base of the brain, or inflammation and reduction of the diameter of the arteries, which can lead to brain damage. General manifestations include a decline that takes place over the course of 2 to 8 weeks, malaise, irritability, change in behaviour, anorexia, weight loss, and a slight increase in temperature. A lumbar puncture is necessary for diagnosis. The sensitivity of smear microscopy (<10%) and culture (<50%) in cerebrospinal fluid is very low, since cerebrospinal fluid generally has very few cells, low glucose levels (which is a prognostic factor), a predominance of lymphocytes, and ADA levels over 9 international units.

Treatment is the same as that used for pulmonary TB, although some scientific societies advocate prolonging the treatment for up to 9 to 12 months, owing to the difficult diffusion of drugs in the meninges.

This type of TB is one of the most serious; therefore, early diagnosis and treatment are essential. In fact, it is one of the few times that it is imperative to take urgent action and provide guidelines for the treatment when there is the slightest suspicion, chiefly because of the low performance of the available diagnostic techniques. The bacilli may enter the subarachnoidal space, causing inflammation of the meninges, formation of a mass or tuberculoma at the base of the brain (Figure 68), or inflammation and reduction of the diameter of the arteries, which can lead to brain damage.



Figure 68. Computed tomographic scan of the cranium showing a large cerebral mass on the right. Cerebral tuberculoma was diagnosed by anatomic-pathological study of a biopsy sample.

General manifestations include a general decline occurring over the course of 2 to 8 weeks, malaise, irritability, change in behaviour, anorexia, weight loss, and a slight increase in temperature. Symptoms then progress to include headache, vomiting, and cervical tension, quickly leading to a loss of consciousness. It is important to be able to recognise the different stages of this progressive clinical manifestation, since the prognosis is different for each stage. Thus, increased awareness of the clinical symptoms is absolutely essential in order to obtain a diagnosis during the earliest phases of the disease. To this end, the following three stages have been described:

Stage I.	Manifestations are mostly systemic. The patient is conscious and lucid. There are meningeal symptoms but no neurological signs.
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Stage II. The patient has time-space disorientation. Confusion is observed.

There are neurological signs of endocranial hypertension.

Stage III.The patient finds it very difficult to remain conscious.Profound stupor, delirium, or coma may occur.Hemiplegia or paraplegia may occur.

To diagnose the disease, it is necessary to carry out a lumbar puncture and obtain cerebrospinal fluid. However, cerebrospinal fluid contains very few bacilli, and as such the sensitivity of smear microscopy (<10%) and the culture (<50%) is very low. The disease can be suspected if the clinical manifestation includes cerebrospinal fluid with very few cells, high protein levels, and low glucose levels, which is a prognostic factor, and a predominance of lymphocytes. In addition, the determination of ADA in cerebrospinal fluid can be very helpful as it is highly sensitive and specific (>90%). The cut-off point to achieve these high levels of sensitivity and specificity will be established by the laboratory carrying out the test, but it is generally accepted that more than 9 international units is highly suggestive of TB. In addition, as they are paucibacillary forms of TB, genetic amplification techniques such as PCR may be of great help in the diagnosis (see Chapter 6).

The patient will die if treatment is not started immediately. With a correct diagnosis and effective treatment, there is a higher chance of recovering without incurring any permanent, serious brain damage. A delay in diagnosis will severely affect prognosis, with the possibility of hydrocephalus. Treatment is the same as for pulmonary TB, although some scientific societies advocate prolonging the treatment to 9 to 12 months because of the difficult diffusion of drugs in the meninges. The drug that best passes through the blood-brain barrier is pyrazinamide, although isoniazid, rifampicin, ethambutol, and streptomycin also have good capacity, especially if the meninges are inflamed.

Other locations of tuberculosis

Section summary

As already explained, TB can affect any organ or tissue. Nevertheless, many locations of extrapulmonary TB are extremely uncommon, even anecdotal.

M. tuberculosis can affect both the female and male *genital tracts* if disseminated through the blood, or through the *urinary tract*. In women, it is frequently manifested as abdominal or pelvic pain, an abdominal mass, infertility, or ectopic pregnancy. Men with genital TB will often present with a mass in the testicular region.

Adrenal TB will always enter in the differential diagnosis of adrenal insufficiency. General manifestations include weakness, fatigue, nausea, vomiting, and pigmentation of the skin.

The clinical presentation of *cutaneous TB* is very variable, but there will always be non-pathognomonic lesions, which may range from small papules and erythemas to large tuberculomas. TB should always be suspected when chronic, painless cutaneous lesions are observed.

TB of the skeletal muscle is exceptionally rare and is almost always a result of dissemination through the bloodstream. *TB of the diaphragm* is even more rare, which, despite being a well-vascularised muscle, is more frequently affected via invasion by contiguity from the pleura and subpleural pulmonary foci.

Genital tuberculosis

M. tuberculosis may also reach the female and male genital tracts if disseminated through the blood or through the urinary tract. In women, it frequently manifests as abdominal or pelvic pain, an abdominal mass, infertility, or ectopic pregnancy. Men with genital TB will often present with a mass in the testicular region (Figure 60).

To diagnose this disease, it is frequently necessary to use invasive techniques to obtain biopsy specimens or to perform thin needle aspiration. Ultrasounds can often provide valuable data. Differential diagnoses should include this form of the disease. Treatment is the same as for pulmonary TB.

Adrenal tuberculosis

In countries where the disease is highly endemic, TB is the most frequent cause of adrenal insufficiency or Addison's disease. This frequency is notably reduced in developed countries. In any event, the disease should be included in the differential diagnosis of adrenal insufficiency. General manifestations include weakness, fatigue, nausea, vomiting, and pigmentation of the skin. One important radiological finding is calcifications in the adrenal region in 20% of cases. The laboratory test, if available, will show low serum sodium levels and high potassium levels. Diagnosis is almost always reached after a biopsy or puncture of the area, and treatment is the same as for pulmonary TB.

Cutaneous tuberculosis

As the location is very unusual, this form of TB is frequently not diagnosed. Its clinical presentation is very variable, although there will always be non-pathognomonic lesions, which may range from small papules and erythemas to large tuberculomas. It is necessary to distinguish between nodular erythema—an expression of delayed hypersensitivity produced after a primary infection generally yielding a non-specific biopsy result—and Bazin's erythema induratum, tuberculids, and other types of cutaneous TB—where the biopsy sample generally does reveal typical lesions, with calcified granuloma. Nodular erythema frequently provides no characteristic data, not even in the biopsy sample, suggesting that diagnosis is made by exclusion. An antituberculous treatment will cure the disease.

TB should always be suspected when chronic, painless cutaneous lesions are observed. Diagnosis will be based on results from a biopsy of the area that is cultured for mycobacteria. Treatment is the same as that for pulmonary TB, although it is important to note that the lesions may evolve very slowly, even when the treatment is correct.

Tuberculosis of the skeletal muscle and diaphragm

Skeletal muscle is extremely well irrigated; thus, it is thought to become infected as a result of dissemination through the bloodstream. In some cases though, it may be due to contiguity. Before the availability of chemotherapy, contiguity was fairly common in cases of TB, especially as a result of local dissemination from empyemata necessitatis. At present,
TB of the skeletal muscle is exceptionally rare. TB of the diaphragm is even more rare. The diaphragm, despite being a well-vascularised muscle, is more frequently affected through invasion by contiguity from the pleura and subpleural pulmonary foci. It can also occur as a result of dissemination through the bloodstream, although this is less likely. Tuberculous involvement has also been described in the breast, subcutaneous tissue, pericardium, oesophagus, chest wall, retroperitoneum, flank, and even in the groin, hip, and thigh as a result of contiguity and always related to empyema necessitatis. The infrequent involvement of the chest wall by TB, as is the case for the other skeletal muscle locations, is more often related to dissemination through the bloodstream than to direct extension, despite the fact that periostitis in the ribs subadjacent to the areas where pulmonary lesions are caused by TB has been frequently described.

In diagnoses involving these extrapulmonary sites, it is necessary to obtain biopsy samples. In some cases, the anatomic-pathological or microbiological laboratory reports may indicate that it is TB. In any event, treatment and cure are the same as for pulmonary TB.

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Chapter 11. Resistance of *M. tuberculosis* to antituberculous drugs

Chapter summary

The prevention of drug resistance must be the first and most important premise of tuberculosis (TB) management. A TB patient with multidrug resistance (MDR) is much more difficult and costly to cure. Consequently, all efforts should be made to prevent the development of drug resistance by adopting a series of basic measures that are easy to implement under control programme conditions. These measures can be summarised as follows:

- 1. Implementation of a good National Tuberculosis Control Programme for the entire country.
- 2. Directly Observed Therapy, Short Course (DOTS) for all initial TB cases.
- 3. Recommendation of directly observed treatment for all patients.
- 4. Administration of antituberculous drugs combined in the same tablet.
- 5. Minimisation of the influence of the private health sector on the management of TB.
- 6. Treatment without any financial cost to the patient.

It is necessary to clearly distinguish between the concept of initial or primary resistance and secondary or acquired resistance. The truth is that barely 50 years after the introduction of the first antituberculous drugs, the indiscriminate use of such agents has led to the appearance of many MDR-TB cases in many parts of the world. It is thus important to know the future extent of this problem. Current estimates suggest that the situation may reach catastrophic proportions if MDR bacilli are as capable of causing disease as drug-sensitive bacilli.

Fortunately, *M. tuberculosis* only acquires resistance through genomic alterations, and the mutation, which most frequently phenotypically expresses resistance to isoniazid, is linked to a vital gene (katG) that also encodes for enzyme activities (catalase and peroxidase), which in turn are integral for the survival and virulence of the microorganism. For this reason, the development of resistance is often associated with a fitness cost to the bacilli. Thus, transmission of MDR strains in the community probably will only have clinical importance in the future for severely

immunosuppressed individuals, particularly those infected with HIV. In contrast, in immunocompetent individuals, only isolated cases of such resistance will continue to appear, as has occurred in the past decades with the contacts of chronic TB patients.

There is abundant evidence of the capacity of *M. tuberculosis* to adapt to adverse environmental conditions. One example, of a microbiological nature, has only become apparent in the last three to four decades, when the selective pressure exerted by antituberculous drugs revealed one of the many mechanisms used by *M. tuberculosis* to defend itself against treatment. In effect, only after the introduction of drugs for the treatment of tuberculosis (TB) was it realised that within the enormous population of microorganisms present in the diseased individual, many bacteria present genetic mutations that make them resistant to such drugs. Resistance of *M. tuberculosis* to antituberculous drugs was emerging—becoming in the last decade one of the most serious problems identified by the international organisations attempting to control this endemic illness. Fortunately, *M. tuberculosis* only acquires resistance through genomic alteration, a fact that has greatly facilitated the struggle against this problem.

Basic concepts and definitions

Section summary

It is necessary to distinguish among three different concepts in relation to the development of drug resistance. Natural resistance is resistance found in wild strains as a result of their continuous multiplication, which does not constitute a significant population. Such wild-type resistance must be selected by an antituberculous agent in order for that natural resistance to be expressed phenotypically. When this happens as a consequence of deficient therapy (i.e., genuine or masked monotherapy), so-called acquired or secondary resistance develops. If a patient with acquired resistance from prior therapy infects an individual who has not used antituberculous treatment in the past, the second subject may develop TB; in this case, the type of resistance is referred to as initial or primary resistance (i.e., patients not previously treated). When a patient shows concomitant resistance at least to isoniazid and rifampicin, he or she is said to have multidrug resistance.

Thus, resistances in the context of TB always reflect substandard treatment of the disease. Acquired resistance is a direct consequence of poor therapeutic practice, whereas primary resistance constitutes evidence of the transmission of resistant strains found within the community.

The natural resistance of *M. tuberculosis* to antituberculous drugs is a characteristic of the genetic plasticity of the microorganism, although it did not become apparent until antituberculous drugs were introduced. At present, and as has been commented before (Table 5), spontaneous natural mutation in a wild-type TB culture yields one mycobacterium resistant to isoniazid for every 10^5 to 10^7 bacilli, and one strain resistant to rifampicin for every 10^7 to 10^9 microorganisms. The frequency for the rest of drugs is one mutant per 10^5 to 10^7 bacilli, with the exception of pyrazinamide, for which the ratio is one mutant bacterium per 10^2 to 10^4 bacteria. This rate of genetic alteration is different for each anti-TB drug. Consequently, the probability that resistance to two drugs may develop is equal to the product of their respective mutation rates. The bacillary population present in a host with cavitary lesions is 10^8 to 10^9 , versus 10^3 to 10^5 in the case of caseum or nodular disease (Table 6). Therefore, if treatment is started with a single drug in a case of cavitary TB, the patient experiences a first phase in which most of the bacteria are eliminated and the symptoms improve. However, this treatment selects the resistant bacilli, which in a short time become the dominant microbial population. In addition, the drug in question will have lost its efficacy for that patient, since TB resistance is chromosomal, definitive, and irreversible. Thus, all monotherapeutic regimens (real or masked by combination with drugs to which resistance has been developed, or which prove ineffective) inevitably lead to treatment failure and to the appearance of drug resistance. Accordingly, the need to combine drugs that have never been used by the patient (or which have been correctly associated in the past) must be the first guiding principle in TB therapy. This principle is relatively simple to follow in the case of an initial patient, but can become difficult when the patient has already been subjected to various treatment schemes. On administering two or more antituberculous agents, the risk of developing resistance is practically zero, since the bacillary load required to select a wild-type resistant strain would be too large for the human body: 10¹⁴ for isoniazid plus rifampicin, 10¹⁹ for isoniazid plus rifampicin plus ethambutol, and 10^{10-12} when three second-line drugs are combined.

Based on the above considerations, three completely different situations can be identified within the concept of resistance. The first situation is represented by natural resistance, which is found in wild-type strains as a result of their continual multiplication. In effect, on reaching a certain number of bacilli, a genetic alteration takes place in one mycobacterium; this mutation by chance may specifically affect the target site of some antituberculous drugs. However, such resistance must be selected by the drug in question in order for natural resistance to be expressed phenotypically. When this happens as a consequence of deficient therapy (i.e., genuine or masked monotherapy), so-called acquired or secondary resistance develops. In all such cases, the underlying cause is human error, owing to incorrect treatment prescription by the physician, or to patient selection of the drug being taken. If a patient with acquired resistance infects an individual who has not used antituberculous treatment in the past, this second subject may develop TB—resistance in this case is referred to as initial or primary resistance (involving patients not previously treated). The best term in microbiological terms is "primary" resistance, which is advocated by the World Health Organization (WHO) and by the International Union Against Tuberculosis and Lung Disease (IUATLD). In contrast, in operational terms, the designation "initial" resistance is preferred, because it encompasses genuine primary resistances and also patients who claim to have never used anti-TB medication in the past, but who have indeed used such drugs and hide the fact (either out of ignorance or intentionally to gain access to treatment). In many countries, patients who have received TB therapy in the past no longer have access to treatment regimens financed by the state, since the limited resources make it necessary to restrict treatment to initial disease cases only. The time limit for previously administered treatment to differentiate between primary and acquired resistance is 1 month, for it is acknowledged that although monotherapy may have been provided during this time, it would not have been sufficient to have allowed the selection of naturally resistant mutants in the initial bacterial population.

Finally, when a patient shows concomitant resistance at least to isoniazid and to rifampicin (a very serious situation), he or she is said to have multidrug-resistant (MDR) TB.

Thus, resistances in the context of TB reflect substandard treatment of the disease. Acquired resistance is a direct consequence of poor therapeutic practice, whereas primary resistance constitutes evidence of the transmission of resistant strains found within the community.

Evolution and present status of drug resistance worldwide

Section summary

Depending on the more or less appropriate management of TB in the world during the past decades, regions can be found with high, medium, or low primary resistance rates (data for 1997-2000). The worst situation is found in the Baltic republics of the ex–Soviet Union (Latonia: 14.4%; Estonia: 10.2%), the Dominican Republic (6.6%), the Ivory Coast (5.3%), Russia (4%), Thailand (3.8%), Romania (2.8%), and Peru (2.5%). However, in terms of magnitude of the TB burden, the most worrisome situations are found in India (13.3%) and China (11.3%).

At the opposite extreme, there are countries with practically no cases of primary multidrug resistance. This situation is attributable to the existence of good National Tuberculosis Control Programmes (Kenya: 0%; Botswana: 0.2%; Benin: 0.3%; Scotland: 0.3%; France: 0.5%; New Zealand: 0.7%; Cuba: 0.7%; Lesotho: 0.9%; the Czech Republic: 1%; Nepal: 1.1%; Chile: <0.5%; and Uruguay: <0.2%), the infrequent use of rifampicin in some areas (Botswana and Lesotho), or the fact that rifampicin has always been used in combination with other drugs, especially in countries where the combination of drugs in the same tablet has become standard (Spain: 0.5%; Brazil: 0.9%).

In the 1960s and 1970s, the flawed management of TB led to a great increase in resistances to streptomycin and isoniazid, i.e., the drugs which, at that time, constituted the basis of TB therapy in many parts of the world where good National Tuberculosis Control Programmes (NTPs) had not been instituted. Extensive regions, particularly in Asia, even reported primary resistance rates to isoniazid of over 25% to 30%, all due to the indiscriminate use of the drug.

In 1967, rifampicin was introduced. Rifampicin, along with isoniazid, is considered the best antituberculous agent for TB treatment. From the start, rifampicin has always been combined with isoniazid, and in parts of the world that have good NTPs it has been used in combination with other drugs. As has been commented, the appearance of a single mutant resistant to rifampicin requires the existence of a large bacillary population $(10^7-10^9 \text{ microorganisms})$, as a result of which this is the antituberculous drug requiring the greatest mutation rate for the selection of resistances. Consequently, if rifampicin is administered under conditions of real or masked monotherapy, it is the drug with the least tendency to lead to

naturally resistant mutant selection. This, and the fact that its higher cost has not facilitated its use in many parts of the world until only very recently, caused resistances to the drug to take longer time in emerging. Nevertheless, in the last decade, rifampicin has been used increasingly throughout the world, and, unfortunately, in a manner that is not according to the guidelines of a good NTP. Consequently, resistance to rifampicin is beginning to emerge as a genuine public health problem, particularly because this resistance almost always appears associated to resistance to isoniazid (i.e., multidrug resistance [MDR]).

The WHO estimates that in the year 2000, there were 50 million people globally who were infected with MDR-TB. However, this is considered to be only the tip of the iceberg, with unpredictable consequences for the future, since in the coming years this important reservoir may give rise to a potentially incurable TB epidemic in most parts of the world. The future concerns are closely tied to the question of whether MDR-TB is as transmissible and virulent as drug-sensitive bacilli.

An established fact is that the present situation of TB resistance, and particularly of MDR, is extremely varied in different parts of the world, with the phenomenon being associated with the past implementation of effective or ineffective NTPs. Thus, based on WHO data for the period 1997 to 2000, a series of so-called hot zones can be identified, with a significant presence of primary MDR (the most serious situation) found in the Baltic republics of the ex-Soviet Union (Latonia: 14.4%; Estonia: 10.2%), the Dominican Republic (6.6%), and other countries where TB drugs have been administered indiscriminately and with very poor control during the last decades (Ivory Coast: 5.3%; Russia: 4%; Thailand: 3.8%; Romania: 2.8%; Peru: 2.5%). More recent reports indicate that some regions in India and China-the two countries with the largest TB burdens in the world (in absolute numbers)—have MDR rates of 13.3% and 11.3%, respectively. These data, extended to the incidence of new cases estimated for both of these countries and considering their large populations, yield a figure of 238,806 possible new cases of MDR-TB in India and 158,813 in China. This would imply that 94% of all cases of MDR are developing in the 35 countries that correspond to the so-called MDR hot zones.

At the opposite end are countries with practically no cases of primary MDR. This situation is attributable to the following: 1) the existence of a good NTP (Kenya: 0%; Botswana: 0.2%; Benin: 0.3%; Scotland: 0.3%; France: 0.5%; New Zealand: 0.7%; Cuba: 0.7%; Lesotho: 0.9%; the Czech Republic: 1%; Nepal: 1.1%; Chile: <0.5%; Uruguay: <0.2%), 2) the still infrequent use of rifampicin in some areas (Botswana and Lesotho), or 3)

the fact that rifampicin has always been used in combination with other drugs, especially in countries where the combination of drugs in the same tablet has become standard (Spain: 0.5%; Brazil: 0.9%), where although good NTPs have not been implemented, such combination dosing in the same tablet has afforded low primary resistance and MDR rates. Similar considerations may also apply to places such as Lesotho, New Zealand, and Scotland.

The future of multidrug-resistant tuberculosis in the world: infectivity, pathogenesis, and virulence

Section summary

There is considerable uncertainty about the potential magnitude of the epidemic of MDR-TB. The severity of the spread of resistant bacilli depends on the capacity of patients infected with MDR-TB to actually develop the disease. The transmission capacity and infectivity of these MDR strains is very similar to that of sensitive bacteria, and tuberculin skin test conversion in individuals recently exposed to MDR bacilli is associated with an equal prevalence of infection. However, it is very possible that the virulence of these bacteria is comparatively less, i.e., MDR-TB may be much less able to result in the development of active disease, as has been seen in the contacts of chronic infectious patients. There are molecular mechanisms to justify these observations.

In order to define the future state of MDR-TB in the world, it is necessary to study the transmissibility and infectivity of MDR bacteria. If it is assumed that these resistant microorganisms have the same capacity to produce contagion and cause disease, then the situation will truly become critical, since of the 50 million people infected with MDR bacilli, an estimated 10% (5 million) could develop active TB. However, as early as the 1950s, Middlebrook and colleagues demonstrated that guinea pigs inoculated with bacilli resistant to isoniazid produced much fewer lesions and had lower mortality than did animals infected with sensitive bacilli. These observations, which have been corroborated by subsequent investigators, were attributed to a lack of catalase and peroxidase production capacity on the part of the resistant bacteria—indicating that the synthesis of these bacterial enzymes was linked to virulence. It was thus assumed that bacteria resistant to isoniazid effectively lose their capacity to produce catalase and peroxidase, and are therefore less virulent than sensitive bacteria and are practically non-pathogenic.

This theory had good epidemiological support, as seen, for example, by the lack of increase in the primary resistance rates among American soldiers who were infected in Vietnam or Korea. However, this hypothesis generated controversy in the 1980s, when attempts were made to show that the resistant bacilli were as pathogenic as the rest. As a result, the alarm was raised in the United States during the late 1980s and early 1990s, when there were reported cases of important foci of nosocomial MDR-TB transmission, which led to a true panic situation that justified the implementation of a national plan to combat this so-called third TB epidemic (the first being the original epidemic and the second being the TB epidemic linked with the emergence of HIV).

Although over 80% of the cases of disease developing as a result of MDR-TB transmission were in severely immunocompromised patients (particularly HIV-infected subjects), it was the observation that a substantial percentage of health care personnel (22-50%) who were caring for these patients also showed tuberculin conversion which caused concern in the medical profession. This suggested that a reservoir of persons infected with MDR bacteria was being created, with the potential capacity of causing refractory TB disease in the future, when, theoretically, the chemoprophylactic regimens involving isoniazid would be ineffective. This concern led several prestigious scientific societies to recommend chemoprophylaxis (in many cases of a truly questionable nature) involving second-line drugs for which no preventive efficacy had been demonstrated.

The above considerations are a consequence of frequent confusion among the terms "infectiveness", "pathogenesis", and "virulence". *Infectiveness* refers to the capacity of a microorganism to infect or colonise a given host. *Pathogenesis* is the capacity of that microorganism to cause clinically manifest disease, and depends on the conflict between the microorganism and the host defence mechanisms. *Virulence* refers to the aggressiveness of the microorganism and its capacity to cause damage or death. Concepts such as infectiveness and transmissibility are indeed intimately related, although the possibility of infection is also dependent on the initial response capacity of the non-activated alveolar macrophage population. Infectivity can be measured by the proportion of people showing tuberculin skin test conversion from negative to positive after exposure to an infectious case. Pathogenesis and virulence are intimately related terms. For many years there has been discussion about the different transmissibility of bacilli resistant to isoniazid, even though it would seem logical for both resistant and sensitive bacteria to be equally transmissible, since transmission is dependent on physical factors such as proximity and the duration of contact, and on the capacity of the contagious individual to cough and produce aerosol droplets loaded with bacteria. Theoretically, both individuals with sensitive bacilli and those with MDR-TB have the same capacity to cough and generate aerosols, as a result of which transmissibility should be similar in both cases. Indeed, this has been shown to be the case in some studies. If a close contact inhales bacteria from a patient, the microorganisms will trigger a macrophage- and T lymphocyte–mediated immune response on reaching the alveoli. This reaction should be very similar, regardless of whether the penetrating organism is resistant or sensitive, unless the less virulent bacteria can be destroyed more easily by the non-specific host defences.

This reaction occurs in the context of tuberculin intradermoreaction conversion, i.e., the production of a cellular immune reaction to the aggression of these mycobacteria. For this reason, the fact that a given susceptible population is seen to undergo tuberculin conversion upon exposure to MDR-TB only suggests that transmission and consequent infection have taken place. Whether or not actual clinical disease subsequently develops will depend not only on the host immune system but also on the virulence of the bacteria involved. This is why the observation of new tuberculous infections among health care personnel caring for MDR-TB patients does not indicate that they will have the same probability of suffering TB disease as if they had been infected with sensitive mycobacteria.

Professionals caring for chronic TB patients have observed that the great majority of the relatives of infected patients yield positive tuberculin test results, but very few of these relatives go on to develop the disease (clearly less than the estimated 10% to be expected). Similarly, a great number of publications during the 1980s and early 1990s reported a substantial number of tuberculin conversions among health care personnel caring for patients with MDR-TB, but there has been no evidence to date of these individuals developing active TB (when in theory up to 10% should have developed the disease). These epidemiological observations suggest that patients with MDR-TB are as transmissible and infective as individuals with sensitive strains, although patients infected with resistant mycobacteria are much less likely to develop active disease—probably

because the resistant bacteria present attenuated virulence, as demonstrated by Middlebrook and Canetti during the period from 1950 to 1970.

The contribution of molecular biology in predicting the future of multidrug resistance

Section summary

Fortunately, *M. tuberculosis* only acquires resistance through mutation. The mutation that most frequently phenotypically expresses resistance to isoniazid is linked to a gene (*katG*) that also encodes for enzyme activities (catalase and peroxidase), which in turn are essential for the survival and virulence of the microorganism. For this reason, the transmission of MDR strains in the community probably will only have clinical importance in the future for severely immunosuppressed individuals, particularly those infected with HIV. In contrast, in immunocompetent individuals, only isolated cases of such resistance will continue to appear, probably linked to other genomic alterations that also affect resistance to isoniazid, as has occurred in past decades with the contacts of patients with chronic TB.

At present, with molecular biological techniques already incorporated into the diagnosis of TB, it has been shown that the gene altered in 90% to 98% of all cases of TB resistant to rifampicin is the *rpoB* gene, which does not encode for any activity vital to bacterial virulence or survival. Therefore, resistance to rifampicin does not imply a reduction in bacterial virulence, and these resistant microorganisms will not only be as transmissible and infective as sensitive bacteria, but will also cause disease in the infected individual. The fact that this particular gene is altered in the great majority of cases of TB resistant to rifampicin, and that such resistance is frequently also associated with resistance to isoniazid, has led to the development of innovative techniques to detect this altered gene in clinical samples. Thus, if this mutation is identified, MDR status can be presumed at the time of actual TB diagnosis, and the appropriate measures can be taken.

Unlike in the case of resistance to rifampicin, which involves a single mutation, resistance to isoniazid is governed by several genetic mutations. The most frequently identified alteration affects the katG gene. This alteration is present in 22% to 64% of all cases in which isoniazid resistance is phenotypically identified. It is the katG gene that affects bacterial catalase and peroxidase activity. Therefore, when this gene is either mutated or missing, the resulting bacilli not only show resistance to

high doses of isoniazid, but also are also unable to produce these two enzymes that are essential for the life of the microorganisms, and particularly for maintaining the intracellular status in infected individuals. Thus, in MDR-TB, where isoniazid resistance is conditioned by an altered *katG* gene, the theories of Middlebrook and Canetti that pertain to a clearly lesser virulence of these bacteria would be applicable. In fact, this is the situation that is most often found in patients with acquired resistance. A different situation is presented in HIV-infected patients, in whom the existing immune deficiency makes the host vulnerable to disease produced even by microorganisms as mildly pathogenic as *Pneumocystis carinii* or the *M. avium* complex. Thus, in such individuals infected by much less virulent bacilli, the lack of an adequate immune response makes it much more likely for them to develop MDR-TB.

Only in those cases where resistance to isoniazid is conditioned by other genes (*inhA*, *ahpC*, *kasA*) are the resulting *M*. *tuberculosis* strains equally transmissible, infective, and pathogenic. This is what was probably found in the 1980s, when the theory of lesser virulence was questioned. However, in almost all cases of *ahpC* gene mutation, and in 50% of *kasA* gene alterations, a *katG* mutation is also present. The same occurs in a substantial proportion of patients in whom mutation affects the *inhA* gene.

In short, the future of MDR in the world will indeed constitute a very important problem for seriously immunosuppressed HIV-infected individuals. In contrast, the impact will be much less in the immunocompetent population, in which reports of MDR will almost always be linked to alterations of a gene other than katG producing resistance to isoniazid or, in exceptional cases, to over-expression of the *ahpC* gene, which could restore full virulence to these bacteria with deficient *katG* activities.

Basic measures for successfully combating multidrugresistant tuberculosis

Section summary

The treatment of MDR-TB is associated with high morbidity and mortality, and is extremely costly and difficult to treat in the great majority of poor countries. Therefore, the best first option is to work with simple and specific measures that have been shown to reduce the emergence of MDR-TB. These measures, which should be implemented under the conditions of an NTP, can be summarised as follows:

- 1. Implementation of a functioning NTP for the entire country.
- 2. DOTS for all initial TB cases.
- 3. Recommendation of directly observed treatment for all patients.
- 4. Administration of antituberculous drugs combined in the same tablet.
- 5. Minimisation of the influence of the private health sector in the management of TB.
- 6. Treatment without any financial cost to the patient.

Only by strictly implementing these measures can it become possible to prevent the emergence of drug resistance, since "even in countries with unlimited resources, it takes less time to generate 10, 50 or 100 cases of MDR-TB than to cure a single one of them."

Based on the above considerations, it is likely that the bleak future consequences of MDR-TB have been overestimated. Nevertheless, it remains true that treating MDR-TB is extremely difficult and very expensive (possibly costing more than US\$10,000)—a situation that greatly limits the use of second-line drugs in low- and middle-income countries, which are most affected by this problem. Two directions should be taken in an attempt to reduce this problem worldwide. First, measures should be implemented under NTP conditions to prevent new cases of MDR-TB from developing. Second, efforts should be made to establish a basic second-line drug bank to offer treatment to the existing cases with MDR-TB. The best measures are those aimed at reducing the number of future MDR cases, since "even in countries with unlimited resources, it takes less time to generate 10, 50 or 100 cases of MDR-TB than to cure a single one of them." A series of basic measures have therefore been designed, to be implemented under NTP conditions. If implemented in

their entirety, these measures will offer the best solution to the emergence of drug-resistant cases:

- 1. Implementation of a good NTP for the entire country, with demonstration of its efficacy over the years. A good control plan should include a series of actions, including the five measures indicated below, to gradually minimise the problem of MDR-TB in the world. A clear example of the importance of this measure is afforded by the experiences of countries such as the Czech Republic, Algeria, some parts of the United States, Lesotho, Botswana, Zimbabwe, Nepal, Korea, New Zealand, Scotland, and some Latin American countries (e.g., Nicaragua, Uruguay, Venezuela, Chile, and Cuba).
- 2. *DOTS for all initial TB cases.* The efficacy of this measure has been demonstrated in many countries and cities. Improvisation in a field as thoroughly studied as TB treatment only encourages the appearance of drug resistance.
- 3. *Recommendation of directly observed treatment for all patients.* When this measure is implemented under NTP conditions, it has been shown to reduce the resistance rates in both countries with limited resources (e.g., Cuba, Botswana) and those with middle-income levels (e.g., Czech Republic, Chile, Uruguay), as well as in some wealthy areas such as the United States (Baltimore, Maryland; New York; Texas).
- 4. Administration of the antituberculous drugs combined in the same tablet. This measure ensures that in the event that the patient abandons treatment, he or she does so completely, thereby avoiding the risk of selecting naturally resistant mutants. This is the measure recommended by the WHO and the IUATLD since 1988, and its efficacy in reducing the appearance of resistances in the community has been demonstrated in different studies. The low rates of resistance in Spain, Brazil, Lesotho, New Zealand, and Scotland clearly illustrate the efficacy of this measure. However, this measure does not facilitate adherence to treatment, as a result of which these drug combinations must be subjected to good quality control of both the associated drugs and their doses. The combinations of isoniazid + rifampicin, or isoniazid + rifampicin + pyrazinamide, are particularly recommended.
- 5. *Minimisation of the influence of the private health sector on the management of TB*. This is one of the major points of concern in highly endemic regions, such as Southeast Asia and the Western Pacific. Many of the countries in these areas present the most serious

TB problems in the world, with physicians in private practice usually employing a therapeutic course that does not meet the specifications of the respective NTPs. As a rule, physicians in private health care tend not to follow NTP guidelines and do not participate in the training plans designed by these programmes. Moreover, private practice often does not recommend treatments in a standardised fashion, and these treatments also tend to be longer, expensive, and inadequate. The detrimental management effects by this influential sector in countries such as India has had much influence on the high resistance rates found in these regions. This impact is difficult to quantify, however, since the cases treated in private care often are not reported to the respective NTPs. The best way to counter this negative influence is to promote the NTPs, and to generate patient confidence and encourage the public to actively seek medical care for TB in the public sector. Another option would be to educate physicians in the private sector, as well as to offer incentives to encourage patient referral to public health care.

6. Treatment without any financial cost to the patient. In order to ensure success, this measure is one of the premises to be demanded before implementing an NTP. If the patient must personally pay for the medication, he or she will probably buy it and use it only for as long as the symptoms persist, particularly if the economic resources are limited, as is very often the case. When the symptoms improve, the natural tendency for the patient is to stop taking the medication—a behaviour that is reinforced when the patient must personally pay for the treatment and has little money to do so. At this point, the patient may decide to either completely abandon treatment, which has detrimental effects on the community but does not affect the problem of drug resistance, or selectively stop taking the most expensive drug. This second situation is understandable, especially when the patient must pay 10 times more for rifampicin and pyrazinamide than for isoniazid. However, this also represents the worst possible situation, since the patient will not only continue to be infectious to others in the community, but will also generate conditions ideal for the selection of drug-resistant mutants.

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Chapter 12. Control of tuberculosis

Chapter summary

Tuberculosis (TB) is a transmissible, preventable, and curable disease. Consequently, the cost-benefit ratios of adequate control measures are among the best known. Since no completely effective vaccine is yet available, the measures that have been shown to be most efficacious in controlling the disease have been early detection and treatment. However, the lack of resources for covering the costs of the treatments, and the fact that therapy is very prolonged, is causing these simple measures to fail in many of the poorer parts of the world.

In order to facilitate the large-scale treatment of TB cases, even under the most adverse conditions, it is necessary to implement the Directly Observed Therapy, Short Course (DOTS) strategy, which comprises five major variables: 1) political will; 2) establishment of a minimum network of laboratories capable of performing smear microscopy; 3) administration of short course and directly observed (supervised) treatment to all patients; 4) guarantee of drug and material resources; and 5) implementation of a registry and information system allowing periodic evaluation of the National Tuberculosis Control Programme.

At present, there are only three possibilities for intervention in the community in an attempt to control TB: 1) administering adequate chemotherapy to diseased patients; 2) providing preventive treatment or chemoprophylaxis for infected individuals at high risk of developing TB disease; and 3) mass vaccination of the population. Undoubtedly, the most important intervention strategy is chemotherapy, with chemoprophylaxis ranking a distant second, followed by bacille Calmette-Guérin vaccination. Efficacy, the duration of the intervention, the capacity to eliminate the sources of infection, and the possible benefits for the community are the bases of these priorities.

It is difficult to understand how tuberculosis (TB) continues to be the most important infectious disease affecting humans today, even though effective treatments have been available for over 40 years and the scientific bases for achieving disease control in the community have been known for more than three decades. This situation demonstrates the serious failure to control this old disease, despite the fact that we now have all the elements needed to combat the disease effectively.

History of the control of tuberculosis

Section summary

The lack of knowledge in the course of history concerning the aetiology of TB led to the inability to implement specific measures for controlling the disease. The means of controlling the disease first became possible when it was realised that TB was a disease transmitted through the sputum of ill patients. This led to the recommendation of isolating infected individuals to prevent further transmission. However, the sanatorium-reclusion era of TB did not likely have any epidemiological impact on the evolution of this endemic disease. Similar considerations apply to surgical treatment attempts. In truth, TB rates did not begin to decline in the developed world until the socioeconomic situation of the various populations began to improve, with a reduction in crowded living conditions and poverty. Apart from these social improvements, only the introduction of chemotherapy, with its promise of curing TB, has had a genuine impact on the prevalence of the disease. The remaining measures adopted over time have had practically no influence.

From ancient times to the discovery of the causal agent of tuberculosis

Despite the fact that TB has existed since antiquity (see Chapter 3) and that it is probably one of the illnesses most often studied and covered in the literature, there has been a surprising lack of sound knowledge about the disease throughout the course of history. This explains why humanity has been unable to defend itself from this terrible illness for the most part through history. Only very recently has it become possible to introduce measures for controlling the disease in the community. Koch was the first to mention the possibility of TB control, when he showed in 1882 that TB was an infectious disease. He not only isolated the bacterium, which was later named after him (Koch's bacillus), from the sputum of infected patients, but he also suggested that the principal measure for controlling the disease in the community would be to isolate affected patients. This was the definitive step that paved the way for the "sanatorium era of TB", which alleged that the prolonged reclusion of affected patients in sanatoriums was the only effective way to try to cure TB and control its spread within the community.

Improvement in socioeconomic conditions

However, as was explained in Chapters 3 and 4, even before these scientific reasonings and advances took place, the disease had begun to come under control in richer countries, without the adoption of any specific control measures. The improvements in the socioeconomic conditions of the populations in developed countries from the mideighteenth century had already started to exert a slight influence over the disease, with a sustained decrease in the associated mortality and morbidity. It is now accepted that once an optimum level of development has been achieved in a given country, the resulting reduction in crowding and poverty has an important effect on the disease. By reducing the number of crowded living conditions, each source of M. tuberculosis transmission is no longer able to generate sufficient new cases of contagion to ensure a new diseased patient (an estimated 20 individuals would have to be infected for this to occur). In this way, the disease would tend to undergo self-elimination, with a sustained 4% to 5% annual drop in infection risk. However, these improvements in living conditions that have occurred in developed countries have still not been achieved in many of the poorer parts of the world-as a result of which spontaneous selfelimination of the disease seems unlikely in these countries.

Isolation in sanatoriums and surgery for the treatment of tuberculosis

It has not been possible to demonstrate whether patient isolation in sanatoriums had an impact on TB control or whether it contributed to the annual reduction in disease mortality attributed to improved living conditions. Although there were probably benefits in certain cases, the prolonged duration of contagion at diagnosis, the fact that only few patients had access to such centres, and the lack of truly effective therapy suggest that sanatoriums did not exert an important epidemiological effect on the evolution of TB. Similar considerations apply to the various surgical procedures developed to treat TB in the first half of the twentieth century. Although such treatments afforded individual benefits, patients continued to spread the disease in the community for a long time. Thus, although quantification of the impact of these control measures is difficult, it is likely that such procedures afforded little more than the benefits brought about by the improvement in socioeconomic conditions.

Modern chemotherapy

Beginning in the late 1950s, the cure of TB cases based on chemotherapy became the main option for shortening the epidemiological chain of the disease. It finally became possible to quickly detect contagious cases and provide treatment to shorten the period of infectivity. However, such measures to establish cure, which seemed straightforward and promised an optimistic future for the control of TB, are in fact very difficult to implement because of the high rate of default associated with the lengthy treatment required for the eradication of the disease.

The cost-effectiveness and difficulty of controlling tuberculosis

Section summary

TB is a transmissible, preventable, and curable disease. Consequently, the cost-effectiveness of adequate control measures is among the best known. Since no completely effective vaccine is yet available, the measures that have been shown to be most effective in controlling TB have been the early detection and curing of cases. However, the lack of resources to cover the costs of these treatments, and the fact that therapy is very prolonged (which leads to a high rate of treatment default), is causing these simple measures to fail in many of the poorer parts of the world. These challenges, and the bleak situation of TB will remain an elusive goal for many decades to come.

As is well known, TB is a transmissible, preventable, and curable disease. Consequently, the cost-benefit ratios of adequate control measures are among the best known. It has been calculated that if good disease control measures (treatment and cure) were implemented, the cost per year of life saved would be less than US\$10. This has led the World Bank to regard adequate antituberculous treatment—involving short duration or course, with the inclusion of rifampicin—as the most cost-effective public health intervention today, offering performance only comparable to vaccination against measles or oral hydration formulations. The problem is that while these interventions are clearly profitable, they are associated with a cost that many poor countries with high TB rates cannot afford; in fact the amount dedicated to TB management alone would exceed their total budget for all health problems. These considerations should encourage wealthier countries to dedicate more resources towards helping less fortunate nations, with the dual aims of controlling TB in these regions and ensuring that the massive migratory movements from such economically depressed areas to industrialised countries do not constitute a step backward in the struggle against the epidemic. TB not only fuels poverty but is also fuelled by poverty. Consequently, failure to control TB in the community is of great importance, not only in terms of the resulting high morbidity and mortality, but also in terms of the economic repercussions of the increase in the number of ill patients, owing to the failure to control the infection.

The best way to eradicate an infectious disease is to use an effective vaccine against it. Since it is well known that the only available TB vaccine has failed to yield the desired efficacy-offering no epidemiological impact on the course of the disease—it is necessary to correctly implement those intervention strategies that have demonstrated efficacy in controlling the disease. However, while these measures, which are based on the early detection and healing of TB cases, are able to adequately reduce the rates of this endemic disease, the reduction is slow (10-14% annually in the best of cases) and can easily be affected. This aspect and the current bleak world situation of the disease suggest that the eradication of TB will remain an elusive goal for many decades to come. Perhaps only a truly effective vaccine will be able to change this disheartening prognosis. TB will only enter the "elimination phase" when the annual rate of new ill patients (incidence for all forms of TB) becomes less than 1 per 100,000 inhabitants. Styblo defined the "elimination threshold" as fewer than 20 cases per 100,000, and the "advanced elimination phase" as fewer than 10 cases per 100,000. Theoretically, "elimination" will be accepted when the rate of smear-positive cases drops to less than 1 per million inhabitants, or when the prevalence of infected individuals in the general population drops to less than 1%. In relation to these estimated figures, which will take 40 to 50 years to reach in countries that have been most effective in their struggle against TB, the term "lowincidence countries" is accepted in reference to nations where the annual rate of affected patients is less than 10 per 100,000 inhabitants. This status has already been achieved by some developed countries, but remains well out of reach in the great majority of regions.

The need to supervise therapy in order to achieve treatment success

Section summary

Despite the great expectations raised by the introduction of chemotherapy and its disease-curing capacity, it soon became apparent that treatment with effective drugs is not synonymous with treatment success in the case of TB, since therapy must be continued for many months, even after symptoms disappear. For this reason, starting in 1958, it became clear that the administration of treatment must be supervised (i.e., directly observed) in order to be effective. This requires special organisation of the treatment services—a very difficult goal to achieve, particularly in the poorest countries. As a result, in most countries, especially those with fewer economic resources, the impact of chemotherapy on TB control proved limited or even negligible for more than three decades. Thus, although individual successes were recorded, an analysis of the cure rates globally showed the figure to be no better than 50% in most cases.

Despite the great expectations raised by the introduction of chemotherapy with its disease-curing potential, it soon became apparent that treatment with effective drugs is not synonymous with treatment success in the case of TB, since therapy must be continued for many months, even well after symptoms have disappeared. This necessity was realised during the 1950s, and gave rise to two major challenges vital to the future of TB control: 1) the quest for new drugs capable of shortening treatment time; and 2) the development of strategies to ensure increased patient compliance with therapy.

The introduction of rifampicin and pyrazinamide in antituberculous treatment shortened the duration of therapy to the 6 to 8 months recommended at present. Unfortunately, there have been no other significant advances in TB treatment for more than 25 years—a situation that reflects the fact that the disease fundamentally affects the less favoured sectors of the population, which have fewer economic and political influences. It is universally accepted that if a 2- to 3-week treatment regimen were available, it would be possible to heal almost all patients, and the impact on TB control in the community would indeed be substantial.

Since the mid-1950s, the possible options for ensuring successful completion of a TB treatment scheme have been evaluated, especially since compliance is a crucial factor for TB control at both the individual and collective level. Initially, it was proposed to admit TB patients to specific sanatoriums for prolonged stays. However, the studies made by Madrás in the late 1950s showed treatment efficacy to be similar between interned patients and individuals who received TB treatment on a supervised ambulatory basis. In 1958, Wallace Fox, supervisor and promoter of other studies, drew attention to the need for direct observation or supervision of the administration of medication in order to ensure efficacy—although this measure requires special organisation of treatment services, particularly in the rural setting. At that time, it was becoming apparent that the basis for TB control in most of the world would be what is now called the Directly Observed Therapy, Short Course (DOTS) strategy.

Over the following decades, it became clear that it was very difficult to organise such services in countries with low-income levels and poor health care infrastructures. In most countries, especially those with fewer economic resources, the impact of chemotherapy on TB control proved to be limited or even negligible for more than three decades. Although individual treatment successes were recorded, an analysis of the cure rates globally showed the figure to be no better than 50% in most cases. A number of studies have demonstrated that while the large-scale administration of drugs to the tuberculous population (without control or individualised follow-up) increases cure rates and reduces mortality, the percentage of patients remaining smear positive for years in the community is greater than when no chemotherapeutic intervention is carried out (i.e., when no treatment is provided and TB is allowed to continue its natural course). So while the provision of self-administered treatments, without effective control, can afford individual benefits (i.e., cure and reduced mortality) from the community perspective, this practice is in effect worse than no treatment, since in the latter case affected patients die and can no longer spread the disease in the community. These same studies also showed that the best intervention at the individual and community level involved directly observed treatments, with individualised control of each case.

The DOTS strategy

Section summary

In the late 1970s, the International Union Against Tuberculosis and Lung Disease (IUATLD) considered it a priority to ensure the adequate establishment of National Tuberculosis Control Programmes (NTPs), particularly with the purpose of facilitating the treatment success of large numbers of cases even under the most adverse conditions. The IUATLD designed a practical intervention model that included collaboration between the clinical services and laboratories, the performance of smear microscopy on a routine basis, the development of an effective and practical information system, and multiple variables for administering treatment on a directly observed (supervised) basis. The model showed that it was possible to achieve high cure rates. These studies constituted the definitive stimulus for elaborating the currently acknowledged basic strategies for the control of TB in the community, which reflect the fundamentals of the DOTS strategy, designed in 1994 by the World Health Organization (WHO) with the purpose of implementing the strategy in the largest number of countries possible. The DOTS strategy comprises five major variables: 1) political will; 2) the establishment of a minimum network of laboratories capable of performing smear microscopy; 3) administration of short course and directly observed (supervised) treatment in all patients; 4) the guarantee of drug and material reserves; and 5) elaboration and implementation of a registry and information system that allows periodic evaluation of the corresponding NTP.

In the late 1970s, the IUATLD considered it a priority to ensure the adequate implementation of NTPs, particularly with the purpose of facilitating the large-scale treatment success of cases even under the most adverse conditions. The IUATLD designed a practical intervention model that included collaboration between the clinical services and laboratories, the performance of smear microscopy on a routine basis, the development of an effective and practical information system, and multiple variables for administering treatment on a directly observed (supervised) basis. The model was tested in five countries (Tanzania, Malawi, Mozambique, Benin, and Nicaragua), with the support of international bodies and the dedication of much effort, and showed that it was possible to achieve high patient cure rates, even under the worst circumstances.

These studies constituted the definitive stimulus for elaborating the currently acknowledged basic strategies for the control of TB in the

community. However, there have been two major limitations. First, the extent of the HIV epidemic throughout most of sub-Saharan Africa and the extreme poverty in these countries have led to such a tremendous increase in the number of TB cases that the success of these strategies is being compromised. Tragically, the number of new cases is increasing yearly in sub-Saharan Africa, even with good NTPs in operation—clear evidence that HIV infection and extreme poverty are exacerbating the problem of TB. Second, NTPs have not been as effectively established in other parts of the world, as a result of which global success has proved elusive and the TB endemic has remained out of control.

In view of the above, and considering the global epidemiological situation, the WHO in 1994 declared the disease a global health emergency and established the DOTS strategy as the basis for intervention in as many countries as possible. This strategy, based on the work of the IUATLD, comprises five major components (and not only directly observed treatment):

- 1. The *political will* of governments to solve the problem of TB in their respective countries. This is the first requirement, since without the necessary political commitment, the rest of efforts will be of little help. It is very common to find countries where the authorities speak of their commitment to the struggle against TB, when in fact they fail even to secure the funds required for minimal maintenance of the personnel in charge of implementing management, or to guarantee the provision of drugs.
- The establishment of a minimum laboratory network for performing 2. smear microscopy. Smear microscopy is used to establish diagnosis among symptomatic cases. Culture and radiology cannot serve this function, although they may be useful in specific cases. Attempts must be made to ensure that the entire population has access to smear microscopy, with adequate quality control of the studies made. Until this is achieved, it is not advisable to introduce other diagnostic methods, such as culture, identification, or sensitivity testing. A laboratory network should ultimately be established in each country, structured in the following way: level 1 (capacity to perform smear microscopy); level 2 (smear microscopy, quality control for level 1 laboratories, culture and identification of *M. tuberculosis*); and level 3 (central level, with all the diagnostic capacities needed for the country in question). It is also advisable to have an extensive distribution of centres at the most peripheral level for the collection of samples.

- 3. The administration of *DOTS* to all smear-positive cases, at least during the intensive management phase (2 months). The importance of direct observation will be discussed further.
- 4. The establishment and maintenance of a system of *regular supply of the necessary drugs and materials* for functioning of the programme. This is one of the main inconveniences in the poorest countries. If this supply cannot be guaranteed, it is best to postpone initiation of the NTP until the required drugs can be obtained. The irregularity of medicine supplies is an important cause of NTP failure. In some cases, in order to begin operating such a programme, contributions may have to be sought to ensure the availability of the needed drugs. However, in subsequent years, the cost must be assumed by the governmental authorities of the country, since this is the only way to ensure that the programme will be sustainable.
- 5. The implementation of an adequate registry and information system to allow periodic evaluation of the NTP. This system will also serve as the basis for requesting material and drugs. It should include at least the following instruments:
 - An individualised case declaration form.
 - A report of the results of treatment (studying the patients in 3-month, 6-month, or annual cohorts).
 - A General Case Registry.
 - The following items, depending on the organisation and resources available in each country:
 - Microbiological study request form
 - Laboratory registry
 - Registry of symptomatic respiratory and suspected TB cases

In the past 5 years, major resources have been dedicated and significant efforts have been made to implement this strategy in as many countries as possible, with the aim (for the year 2000) of healing at least 85% of the cases diagnosed on the grounds of positive smear microscopy, and of detecting 70% of the existing smear-positive cases. Although it is now clear that these objectives have not been reached, it is also true that there has been considerable progress in the last 3 to 4 years in the implementation of the DOTS strategy. Indeed, if things continue in the same direction, the currently bleak world situation may begin to improve in the coming years.

On the other hand, many developed countries have been experiencing a different situation. While TB endemic rates in these parts of the world began to decrease more than a century ago as a result of improved socioeconomic conditions, in the past 40 to 50 years good NTPs have been instituted, leading to early detection and high cure rates. For this reason, the risk of infection in these countries has further declined throughout the last century, particularly with the introduction of chemotherapy. At present, these nations not only have low disease rates, but the remaining infected subjects tend to be adults or elderly individuals—thus paving the way for possible complete elimination of the disease within 30 to 40 years. Still, it should be emphasised that TB will not be completely eliminated in any country until it is brought under control globally.

Interventional strategies

Section summary

At present, there are only three possible interventions for controlling TB in the community: 1) administering adequate chemotherapy to ill patients; 2) providing preventive treatment or chemoprophylaxis to infected individuals at high risk of developing the disease; and 3) large-scale vaccination of the population. Undoubtedly, the intervention strategy on which all efforts should focus is chemotherapy, with chemoprophylaxis ranking a distant second, followed by bacille Calmette-Guérin (BCG) vaccination. Reasons such as efficacy, action, rapidity, duration of the intervention effects, the capacity to eliminate the sources of infection, and the possible benefits for the community all justify these priorities.

There are presently only three possible interventions for controlling TB in the community: 1) administering adequate chemotherapy to ill patients; 2) providing preventive treatment or chemoprophylaxis to infected individuals at high risk of developing the disease; and 3) large-scale vaccination of the population. Each of these three possibilities have been, or will be, analysed in detail elsewhere in this Guide, according to their level of importance, with emphasis on the conditions affecting the significance of each possibility in TB control.

Undoubtedly, the intervention strategy that should earn most attention is chemotherapy, with chemoprophylaxis ranking a distant second, followed by BCG vaccination. Different reasons account for this order of priorities:

- 1. *Efficacy*. While the efficacy of an adequate treatment regimen approaches 100%, the best studies on chemoprophylaxis report rates of no more than 75% to 90% (in terms of capacity to prevent disease). In turn, and despite considerable controversy, recent meta-analyses indicate that the BCG vaccine affords a mean efficacy of only 50%.
- 2. Action. Chemotherapy exerts very rapid action, starting from the first drug dose, thus decreasing the risk of death and contagion in the community. Chemoprophylaxis also exerts rapid action, since from the first moment of administration it also reduces the risk of TB. In contrast, the BCG vaccine is very slow acting, and months or even years are needed to evaluate its effects.
- 3. *Duration*. Chemotherapy protects the cured patient for life (except in the infrequent cases of relapse or exogenous reinfection). The duration of protection afforded by chemoprophylaxis is not known. However, in immunocompetent individuals, chemoprophylaxis has been shown to protect for at least 20 years, although little is known about its protective effects in immunocompromised subjects. In contrast, the effect of BCG vaccination is both transient and inconstant.
- 4. *Infection sources.* Chemotherapy eliminates sources of infection (this being the best option), whereas chemoprophylaxis merely avoids such sources (only a small proportion of treated subjects go on to become sources of infection), and BCG vaccination has no effect on infection sources.
- 5. *Benefit for the community.* Chemotherapy benefits both patients (because they get cured and do not die) and the rest of the population (since the risk of contagion is eliminated). With chemoprophylaxis, only some infected subjects stand to possibly benefit (i.e., the few who could go on to develop the disease), and not the rest of the community (because only patients with active disease transmit the bacillus). BCG vaccination only benefits those few cases who, at very young ages, may have become ill with serious forms of the disease.

Basic measures for controlling tuberculosis

Section summary

The first aim of all NTPs must be to maximise the cure rates among patients subjected to treatment—for which it is essential not only to use short-course treatment regimens (6-8 months), but also to ensure patient compliance. In countries with low- or middle-income levels, all patients should undergo directly observed treatments, the aim being to achieve cure rates of over 85% among smear-positive cases as well as a treatment default rate of less than 6%. In order to evaluate performance, systematic cohort studies must be conducted of the treatment outcomes.

A second aim is to maximise passive case detection, i.e., localisation of TB cases among the population that is using the health care system. In order to improve case detection and shorten the time to diagnosis, the education of physicians, health care professionals, patients, and public is important, particularly to make them aware that any respiratory symptom (cough and expectoration for more than 2-3 weeks) can constitute a case requiring the evaluation of possible TB. It is also essential to facilitate access to health care.

Only when the process of ensuring effective detection and treatment of cases has been achieved can active detection of cases and infected individuals among high-risk groups in the population (i.e., sectors with disease rates of over 100 cases per 100,000 inhabitants) be undertaken. This action is most effective in individuals who have contact or live with TB cases and who are smear positive, as well as in HIV-infected subjects—these individuals should receive treatment (if they constitute disease cases) or chemoprophylaxis (if infected). Still, even adequate implementation of chemoprophylaxis has not been shown to have an epidemiological impact, since the action is carried out on a population that is not diseased. The epidemiological impact of BCG vaccination on the community has been demonstrated to be practically zero.

The implementation of other control measures is only warranted in the industrialised world, especially with regards to immigrants from highly endemic regions, the homeless, and subgroups in which the incidence of TB is high.

Cure of cases

The first aim of all NTPs must be to maximise the cure rates among patients undergoing treatment. This is the most effective means of disrupting the chain of transmission of *M. tuberculosis*, since it eliminates the sources of infection in the community. In order to achieve this aim, two equally important points must be taken into account: 1) the selection of a good chemotherapeutic regimen (with preference going to short-course regimens lasting 6-8 months); and 2) strict confirmation that the patient takes the medicine correctly until the end of treatment. If the patient is correctly treated but follow-up is deficient (an operational problem), then antituberculous action in the community can fail and the personal efforts of many health care workers can be rendered ineffective.

TB is a disease that can be cured in practically all cases; however, it requires strict adherence to prolonged treatment lasting at least 6 months. Incorrect treatment, or treatment lasting less than that required, will give rise to treatment failure and relapses-this in turn ensures the persistence of sources of contagion in the community and the risk of transmission of drug-resistant bacilli. It has been estimated that approximately half of all patients do not use the prescribed medication correctly, with the rates of poor compliance ranging from 20% to 80%, depending on the country, socioeconomic and cultural levels, and many other factors. This is why for the past 40 years, direct supervision of antituberculous treatment has been emphasised as the only sure way to ensure cure. In countries with low- or middle-income levels, all patients should receive treatment with direct supervision by health care personnel. It is debated whether these same measures should also apply to all TB patients in developed countries. In any case, directly supervised treatment means that the patient is directly seen to take the medication, or that trained personnel administer the dose.

The implementation of directly observed treatment constitutes one of the major challenges of NTPs, since it requires considerable organisational effort on the part of the health authorities. However, although it may be easy to recommend such measures, implementation can be an entirely different matter. Nevertheless, the example set by countries as poor as Tanzania, Peru, and Nicaragua, with their many limitations, shows that such measures can be implemented even in the worst settings.

Although the maximisation of cure rates must be the central concern of NTPs, evaluation of the work done in this area is equally important. Cohort studies are therefore needed (covering periods of 3, 6, or 12 months) of the results of treatment of patients diagnosed with TB. In this way, all patients enrolled in the programme must enter or leave the programme based on the following definitions:

- 1. *Cured.* This is a patient for whom there is evidence that treatment was correctly completed, and the microbiological results were negative at the time of treatment suspension. In developed countries, the acceptance of negative microbiological results should comprise at least a negative culture at the end of the fourth month of treatment and a smear-negative study after 6 months. In the case of longer treatment regimens, the requirement would be a negative culture 2 months before suspending therapy and a negative smear study at the end of suspension of therapy. In poorer countries, evaluation would be restricted to smear microscopy, although at the same treatment time points as described above. As has been commented in Chapter 7, follow-up of treatment should not be done radiologically.
- 2. *Completed treatment*. This is a patient for whom there is evidence that treatment was correctly completed, although no negative microbiological results were obtained at the time of treatment suspension. This definition mainly comprises patients who either do not expectorate or from whom samples cannot be obtained (as in the case of extrapulmonary TB).
- 3. *Treatment defaulted*. This is represented by a disease case in which directly observed treatment at the periodic controls indicates that the patient had abandoned or interrupted the antituberculous regimen for more than 1 month.
- 4. *Transferred out*. This is represented by a patient included in the cohort of a given centre and who in the course of treatment changes place of residency and from that point onwards is followed up at another centre. If possible, the final patient outcome at the destination centre (when known) should be recorded.
- 5. *Died*. This is a patient diagnosed with TB who dies in the course of antituberculous treatment. When possible, include either of the following classifications in this category: death due to TB or death due to other causes.
- 6. *Failure*. This is a patient who is known to have correctly adhered to treatment but who presents with positive cultures at the end of the fourth month of therapy (fifth month in the 8-month treatment scheme). In poor countries, a positive smear would suffice as treatment failure. All cases of treatment failure must be confirmed by culture.

In developed countries, the aim is to achieve cure rates of over 90% in all patients, with a treatment default rate of less than 6%. It is necessary to carefully analyse the cohort, since the desired cure rate may not be reached for many reasons, such as high mortality (common in countries with a high prevalence of HIV infection) and high transfer rates. However, in poorer countries, the goals are more modest, and a positive outcome will be considered with a cure rate of over 85% in relation to patients with smearpositive results (the only subjects to be evaluated in the cohort), with a default rate of less than 6%.

In countries with low- or middle-income levels, the follow-up of TB cases until cure is achieved should not be a specific responsibility of specialist physicians, whose main role instead should be the management of cases with diagnostic problems (cases with suspected TB but serial smear-negative results) and treatment difficulties (e.g., adverse effects, retreatments). Action should be limited to following the treatment guidelines recommended by the NTP and referring cases to the programme, where treatment supervision will be ensured and cohort studies will be performed based on the outcomes of treated patients. Since the great majority of TB cases diagnosed by specialist physicians are ultimately referred to the peripheral levels of health care, it is essential for the specialist physician to ensure that the transferred patient reaches his or her destination centre. This way, these specialists more selectively influence the interruption or default of treatment and transfer rates.

However, this important aspect analysed in the section above is different in developed countries, which tend to have few patients but many specialist physicians, who may then be involved in patient follow-up and performing cohort studies.

Passive case detection

The second objective—which should not be a priority concern until cure rates of over 85% are achieved with treatment default rates of under 6%—is represented by maximisation of passive case detection, i.e., localisation of TB cases among the population consulting the health care system. An extremely important consideration here is the definition of suspected tuberculous disease and TB case (these being the minimum criteria for initiating a study).

Since pulmonary TB is the most frequent presentation of the disease and the truly infectious form, most efforts should focus on identifying those individuals suspected of having this form of TB. TB should be suspected in any person presenting with cough and/or expectoration for more than 2 to 3 weeks (or other clinical manifestations suggestive of TB). These subjects are referred to as symptomatic respiratory patients, and require a chest radiograph and sputum sampling for smear microscopy and culture. In the poorest countries, serial smear microscopy studies will suffice as a diagnostic tool to identify TB disease. Despite the fact that a patient with such respiratory manifestations is unlikely to actually have TB (1-6%, depending on the prevalence of TB disease in the community), the resources and organisation of the NTP must focus only on case detection among symptomatic cases by smear microscopy.

In order to evaluate this important aspect relating to TB control, all elements of the NTP registration and information system must be strictly followed. Analysis of the population over the ages of 10 to 15 years, the number of symptomatic respiratory patients detected, the number of diagnostic sputum smear microscopies performed, the number of smearpositive cases, and the total number of cases entering the NTP will all provide very useful information for the evaluation of case detection status. In developing countries, where standardised information systems are often not used, it is essential to encourage case reporting by the diagnosing physicians, with the use of active epidemiological surveillance systems. One recommendation involves the control of microbiologically confirmed results (smear microscopy, culture), the control of hospital discharges with the diagnosis of TB, and the crossing of TB and HIV registries. Pharmacy registries relating to antituberculous drug use (particularly rifampicin) and death registries can also be useful.

Another important aspect in the detection of cases is the possibility of calculating the diagnostic delay, which should be based on three parameters: 1) total delay, comprising the period between the onset of symptoms and the start of treatment; 2) delay partly attributable to the patient, which will depend on the cultural level, the accessibility of health care, and the TB information available in the community (comprising the period from symptom onset to patient consultation of the health care service; and 3) delay attributable only to the health care system or NTP, comprising the period from patient consultation to the start of treatment. This diagnostic delay, which is little studied in most NTPs, is extremely important, since it represents the time during which the patient continues to infect the community.

In order to improve case detection and shorten the time to diagnosis, a critical consideration is the knowledge and education of the physicians,

health care professionals, patients, and public. In particular, they should be made aware that any respiratory symptom (cough and expectoration for more than 2-3 weeks) can constitute a case requiring the evaluation of possible TB. It is also essential to facilitate access to health care for all patients with such symptoms.

If the NTP functions properly, the specialist physician in countries with low- or middle-income levels will play a limited role in case detection, since he or she will only see those cases presenting with smearnegative results and a high suspicion of TB disease. However, since in many countries such physicians also end up seeing cases with smearpositive results (particularly in more developed areas), these professionals must also be familiar with the recommendations and applications covered in the above section. This role of specialist physicians changes as countries develop and as TB rates are reduced, since at this point specialist physicians begin to play a more important role in case detection.

Active detection of cases and infected individuals among at-risk populations

Only when the first two objectives described above have been reached (i.e., the detection and cure of cases), can attempts be made to actively detect cases and infected individuals among groups at high risk of TB (i.e., sectors with disease rates of over 100 cases per 100,000 inhabitants). This can only be done in countries with good economic resources, since poor countries have many problems (ranging from economic to organisational and logistical) in implementing such a strategy. One example is limited access to tuberculin testing and the questionable validity of results, owing to the widespread practice of BCG vaccination. Active detection is most useful in individuals who have contact or live with TB cases and who have smear-positive microscopy results, and in HIV-infected subjects. It may also be useful in patients with silicosis, cases of untreated inactive TB, intravenous drug abusers, prison populations, the homeless, the poor, immigrants from high TB endemic regions, and patients with some form of immune deficiency.

However, detection of cases and infected individuals has very little epidemiological impact, since with the exception of the contacts of patients with TB disease and HIV infection, only individual benefits can be expected in the rest of the groups (because of the very limited number of cases that will be detected). Even adequate intervention among the TB contacts and HIV cases—the great majority of whom will not have TB— will have much less impact than intervention among patients. This is why such measures should not be contemplated until the primary goals of healing and case detection have been satisfied.

In this section, particularly with regards to the active detection of cases among immunocompromised patients, specialist physicians will indeed play a very important role. Management of TB contacts should preferably be done by the NTPs.

Chemoprophylaxis and BCG vaccination

In relation to the active detection of cases and infected individuals, it must be stressed that it is not only essential to treat patients, but also to provide chemoprophylaxis for infected subjects at high risk of developing active TB and to implement a system to ensure compliance. Here, it is even more difficult to ensure patient adherence when chemoprophylaxis is provided (since treatment is being administered to individuals who are not clinically ill) than when diseased patients are treated. This should not lead to a loss of prioritisation regarding the use of resources, which should always centre on treatment. Such chemoprophylaxis, which will be extensively addressed in Chapter 13, is currently referred to as the treatment of latent tuberculous infection by the American Thoracic Society and the Centers for Disease Control and Prevention. In Chapter 13, it will be pointed out that the operative efficacy of chemoprophylaxis, under NTP conditions, will depend largely on three variables: 1) the efficacy of the treatment regimen chosen; 2) the risk population designated for intervention; and 3) patient adherence to therapy. In any case, the epidemiological impact of this strategy (even when chemoprophylaxis is correctly executed) is limited, since only some potential infectious sources will be avoided, which is in contrast to treatment of diseased patients, which effectively eliminates the infectious sources of TB.

Chapter 13 will also address the subject of BCG vaccination and the fact that while studies of the efficacy of the vaccine have yielded contradictory results (good protection vs. poor or non-existent protection), the standard use of the technique is advised in newborn infants in countries with high or moderate TB endemic rates. The epidemiological impact of extensive BCG vaccination in the community has been shown to be practically zero, although it does reduce the appearance of serious TB presentations in childhood, with a resulting reduction in childhood mortality in countries where the risk of infection remains high. It is due to
these individual benefits (not to the epidemiological impact) that BCG vaccination is advised.

The role of specialist physicians in this area is very limited, since such intervention should be based on the central and peripheral actions of the NTP.

Other measures

Other complementary measures can also be adopted in industrialised countries, such as the control of immigrants from highly endemic regions, the homeless, and regional population groups in which the incidence of TB is high.

Immigrants should be subjected to control measures upon entering the country. In countries with the best antituberculous efforts, such immigrants are not granted a residency permit unless a TB study has been performed, including radiographs and tuberculin testing. Still, the great majority of immigrants do not have TB on reaching their country of destination, but instead develop the disease years later as a result of poor living conditions.

In the case of the homeless, a social service programme may be developed to allow admission to supervised residencies for the completion of therapy, or at least to provide access to hospital centres with free medication and the provision of certain incentives, such as meals.

Measures for controlling tuberculosis transmission and their importance in the fight against the disease

Section summary

The control of *M. tuberculosis* transmission is of extreme importance for controlling TB. However, it is necessary to evaluate the cost and benefits associated with specific measures for achieving this goal. Early diagnosis and cure is clearly the priority concern, since only in this way can infectious sources be eliminated. In comparison with these measures, the rest of interventions are very secondary and could be summarised as follows:

- The best way to avoid the nosocomial transmission of TB is to avoid the need for patient hospitalisation.
- Despite the acknowledged usefulness of ensuring fresh environmental air and ultraviolet (UV) radiation, in countries with low- or middleincome levels, good room ventilation would only be indicated in those areas where TB patients are found, allowing the air to circulate freely, and with natural, solar UV exposure.
- Masks and oral protectors are most effective when used by the patient, since they prevent transmission of the aerosol form of particles that is loaded with bacilli.
- Chemoprophylaxis and BCG vaccination have very little impact on transmission, since they do not act on the sources of infection.

As was explained in Chapter 4, the most important mechanism for the transmission of TB which is responsible for almost all cases of infection is the airborne route. There are a series of conditioning factors that may clearly influence easier or more difficult disease transmission in the community (e.g., degree of spread of the disease, the severity and frequency of coughing, chemotherapy, the characteristics of exposure). The control of *M. tuberculosis* transmission may thus be of extreme importance for controlling the disease. However, it is necessary to evaluate the cost and benefits associated with the use of specific measures to achieve this goal. An analysis will be made below of the potential benefits and costs of such measures.

Early diagnosis and cure of tuberculosis cases

Early diagnosis and cure is undoubtedly the priority concern for controlling the transmission of *M. tuberculosis*, and has been covered at length in other parts of this Guide. Nevertheless, emphasis should be placed on the fact that only correct treatment and cure of patients can help to shorten the epidemiological chain of transmission of TB to others. Only with successful treatment can the sources of infection be eliminated from the community. Hence, it is important to detect possible infectious sources as quickly as possible, particularly those individuals with smear-positive results (who are the most infectious subjects), providing them with adequate treatment. In addition, patient follow-up throughout the duration of treatment until cure is achieved is essential, as is the adoption of measures to reduce the delay in diagnosis. Apart from these measures relating to early diagnosis and cure, the remaining interventions discussed below are of secondary importance.

Patient hospital admission

Hospital admission plays practically no role in the control of TB. On the contrary, the best way to prevent the nosocomial transmission of TB is to avoid the need for hospitalisation. Unfortunately, it is very common in all countries (regardless of income level) to hospitalise TB patients, even though the disease will heal equally well in an ambulatory or outpatient setting. Patients should stay at home, reporting to the hospital only to receive treatment and undergo periodic control follow-ups. When the patient is diagnosed, he or she has already been transmitting the disease for a number of weeks. Furthermore, from the first day of chemotherapy, this capacity for transmission is already reduced. Hence, the patient receiving treatment on an outpatient basis will not increase the possibility of infecting those living with him or her, since it can be safely assumed that all possible cases of transmission already occurred before the diagnosis was established. What must be avoided, however, is transferring this transmission potential to other environments and settings where the patient has not been before—which would be the case in the event of hospitalisation.

Hospital admission of a TB patient would only be warranted when the clinical situation is deemed to be extremely serious (see Chapter 9), for

social reasons (e.g., extreme distance between the home and centre), or because of other suspected diagnoses.

Room airflow renewal. Ultraviolet radiation

M. tuberculosis is highly sensitive to heat, sunlight, and UV radiation. In closed rooms where none of these elements are found, the bacterium can persist with infective potential for prolonged periods of time. One of the measures that may be adopted to control TB transmission is to keep environmental air fresh and to allow exposure to UV light.

In countries with low- or middle-income levels where the ideal objectives of TB detection and cure have not yet been reached, the sole indication would be to provide good room ventilation in areas where TB patients are hospitalised, allowing the air to circulate freely, and with exposure to natural UV light. Since many of these poorer countries are located in tropical or subtropical zones, room ventilation may be allowed for several hours a day, all year round. No further resources should be spent on ventilation and UV radiation, since all efforts must focus on diagnosis and treatment.

In industrialised countries, and provided there are adequate economic resources and levels in antituberculous management, other measures can be adopted to keep environmental air fresh and to provide exposure to UV radiation. Air renewal can be achieved with machines (which vary in size, function, and price) that generate negative pressure, thereby continuously renewing the air in the room or ward. UV lamps can also be installed; this measure is much less expensive than the former, but has a similar effect to that of renewing room air several times every hour. UV radiation may therefore be regarded as more effective than air renewal. The more developed countries, which have achieved high cure rates with early diagnosis of the affected cases, should assess the convenience of having these systems (environmental air renewal and UV light) in all rooms where patients with possible TB are being studied or treated, as well as in the emergency department wards where such patients may be admitted, and in the aerosol generator systems used for induced sputum. These practices are being adopted in the great majority of hospitals in the industrialised world, although their use is suboptimal in many instances.

Masks and oral protectors

Although there has been considerable controversy regarding the true benefits of these measures, their efficacy has been acknowledged for a long time. Nevertheless, several limitations should be mentioned. The efficacy of masks used in daily clinical practice is questionable, since over 50% of the potential mycobacteria present in the environment can permeate these barriers. For this reason, such masks are most effective when worn by the patient, since they prevent the formation of bacterialaden aerosol droplets. This same function can be achieved with handkerchiefs or oral protectors. The adoption of such measures by people living with or visiting the patient is of very limited value, even though the typical situation in hospitals corresponds to one of visitors wearing masks and the patients wearing none. The fact is that once the patient has produced these aerosol particles by coughing, others can inhale these droplets despite the wearing of masks. Hence, all patients in the contagious phases of TB (smear-positive microscopy results) should always wear masks or oral protectors.

If a mask is to be used by contacts or visitors, it must be effective in blocking the aerosol particles emitted by the affected patient. Such masks are much more expensive than the masks traditionally used in hospitals, but they are able to filter 99% of all bacilli. The best models cost more than US\$10; as such, general use in a hospital would constitute a considerable expense. Such measures are thus restricted to richer countries, and only once the priority concerns of case detection and healing have been satisfied. In low- and middle-income countries, only the patient should wear a mask or oral protector.

Chemoprophylaxis and BCG vaccination

Chemoprophylaxis with isoniazid has clearly been demonstrated to be effective in people infected with *M. tuberculosis* and in patients from certain TB risk groups. Chemoprophylaxis prevents infected patients from developing the disease, thus controlling transmission by preventing the appearance of infection sources in the community. However, as has been pointed out, its efficacy in controlling transmission is still far inferior to that of case detection and cure, since the latter measures act on the true sources of infection rather than on possible sources, as in the case of chemoprophylaxis.

On the other hand, although the efficacy of BCG vaccination has been contested, its use seems to be accepted in certain settings, such as in children of contagious patients who exhibit poor treatment compliance, and in health care personnel (with negative tuberculin test readings) who are in continuous contact with TB patients. Because of the possibility that some cases of TB can be avoided with BCG vaccination, these measures could also be considered for transmission control, although the corresponding cost-efficacy ratio is very high compared with the abovementioned alternatives. Still, it should be noted that such recommendations are not supported by scientific studies, hence the controversy and continued discussion surrounding the role of BCG vaccination.

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Chapter 13. Prevention of tuberculosis

Chapter summary

The best way to prevent tuberculosis (TB) is by providing appropriate treatment and curing all infectious cases. Unless high cure rates are achieved, no resources should be invested in the two other major areas of TB prevention: chemoprophylaxis and bacille Calmette-Guérin (BCG) vaccination. These two measures have very little epidemiological impact, since they do not act directly on the sources of infection. In any event, if a community has attained high cure rates, then the next focus should be on the endogenous reservoir, i.e., by offering chemoprophylaxis to infected persons who are at risk of developing TB. The BCG vaccination, on the other hand, with its zero impact on prevalence trends in the community, is recommended for reducing childhood mortality from TB.

Treatment and cure of cases

Although chapters on tuberculosis (TB) prevention traditionally include chemoprophylaxis and bacille Calmette-Guérin (BCG) vaccination, the best way of preventing TB is still by curing infectious cases, as stated previously in this Guide. As explained in Chapter 12, the most effective means of breaking the transmission chain, and thus preventing infection and possible disease in the rest of the community, is to provide appropriate treatment to cure existing cases. The treatment of TB has been reviewed at length in Chapter 9, and aspects related to the importance of healing have been covered in Chapter 12. For this reason, the importance of appropriate treatment and healing of cases will not be covered in this chapter, although it is necessary to reiterate that if these measures are not implemented correctly, there is no justification for investing any resources whatsoever in chemoprophylaxis or BCG vaccination. These last two forms of intervention, which have been the subject of much debate in the twentieth century, have scarcely had any epidemiological impact, even when implemented under the best possible conditions. Many countries have invested much money in these two measures, without even achieving minimum levels of case healing.

In any event, if a community overcomes problems of chemotherapy and achieves high cure rates, its focus should shift to the endogenous reservoir, which means offering chemoprophylaxis to infected persons at risk of developing TB. The BCG vaccination, on the other hand, with its zero impact on endemic trends in the community, is recommended under other circumstances, such as reducing childhood mortality from TB.

Chemoprophylaxis or treatment of latent tuberculous infection

Section summary

Despite the unanimous agreement regarding indications for the treatment of latent tuberculous infection (LTBI) in certain patient groups, there is still a great discrepancy between the United States and Europe concerning recommendations for other groups of patients.

In every case of individualised indication for LTBI, the benefits and risks of employing this treatment in a person without active tuberculous disease should be carefully weighed. There are only three groups in which the indication of treatment of LTBI is unquestionable: 1) persons co-infected with *M. tuberculosis* and HIV; 2) new cases of infection, particularly in children; and 3) patients with radiological lesions suggestive of residual TB. In all other risk groups, the indication is a matter of debate and is ultimately a decision that must be made by the treating physician.

When treatment of LTBI is approached as an intervention strategy in the community, it is necessary to evaluate the operational efficiency of the measure, which will depend on three major factors: 1) efficacy of the treatment regimen used (the best is achieved with 9 months of isoniazid); 2) risk of developing TB in the group in question (only justified in HIV coinfection, new infection, and residual TB); and 3) adherence to lengthy treatment, which requires much work in order for this measure to have a successful outcome.

A National Tuberculosis Control Programme (NTP) should not spend any resources on LTBI if not enough efforts have been spent on achieving high cure rates and detection of cases. Depending on the level of treatment success and detection, extent of prevalence of the disease, and available resources and infrastructure, treatment of LTBI should be indicated as follows—in low-income countries, it should only be indicated (without effecting a tuberculin test) in HIV-infected persons and in children under the age of 5 years who have been in contact with smearpositive cases; in middle-income countries, selective LTBI treatment may be considered in specific patients (e.g., those with HIV) and large-scale LTBI treatment could be administered in children who have been in contact with smear-positive cases (without effecting a tuberculin test), with the possibility of extending the age criterion up to 10 to 15 years; finally, in developed countries, treatment of LTBI is an important strategy in dealing with the endogenous reservoir for TB disease. Epidemiological observations during the pre-chemotherapy era of TB demonstrated that for persons who lived with infected patients, the greatest risk of developing the disease occurred during the initial weeks and months following infection, and that this risk clearly fell during the course of time. With the discovery of drugs that were shown to be effective in TB treatment, the aforementioned studies gave grounds to suggest that it was possible that if these drugs were used in this contact group, the risk could be reduced. Therefore, since the 1950s, attempts were made to reduce the disease rate in this recently infected group of persons, by administrating drug therapy despite good health. This type of therapy was referred to as preventive treatment of latent tuberculous infection (LTBI). Although the efficacy of this measure has been demonstrated in specific population groups, it has not been shown in other groups; hence, there is controversy regarding many of the indications for treatment of LTBI.

Controversy over the indications for treatment of LTBI

Among the first studies on this subject, the study by Ferebee in 1970 is of particular interest, since it not only demonstrated the increased risk among household contacts of TB patients in the initial weeks and months following the infection, but it also showed that if this contact group was administrated a long course of isoniazid, this increased risk of developing TB almost disappeared and became similar to the risk among those who had been infected more than 5 years ago. This study, together with studies of a similar group involving Eskimos, provide the best examples of the excellent efficacy of treatment of LTBI, demonstrating that it can also be highly effective if appropriately implemented as an intervention strategy in the community.

Subsequent research has since showed a notable reduction in the risk of infected persons developing the disease if they undergo treatment of LTBI. However, there are major differing opinions regarding the benefit of this form of intervention in certain risk groups. Guidelines from the United States—specifically that of the Centers for Disease Control and Prevention (CDC) and the American Thoracic Society—assert that treatment of LTBI plays a significant role in TB control; thus, they recommend a very broad use of this measure, including in almost all persons at risk of developing TB and (in the recent 2000 recommendations) even in the healthy population of a certain age. On the other hand, European societies believe

that the measure should not be used indiscriminately, as they consider that treatment of LTBI has very limited impact in TB control. The TB Working Group of the European Respiratory Society believes that treatment of LTBI should be limited to recent converters; those with untreated, inactive, or residual TB; and HIV-infected patients. The British Thoracic Society recommends limiting indications to contacts under the age of 16 years who have not received the BCG vaccination, recent converters, and immigrants.

From an objective standpoint, the impact of treatment of LTBI, implemented under NTP guidelines, should be greater in countries with low TB transmission rates (i.e., which have decades of good cure rates) and in countries where it can be presumed that the majority of new cases arise from endogenous reactivations.

In any event, there is great controversy over the subject of treatment of LTBI, when in fact, too much has been written in comparison with the number of well-designed efficacy studies that have been conducted. This may be the result of not considering when treatment of LTBI is recommended in a specific, individual case, versus when it is used in certain risk groups, as an intervention strategy that forms part of NTP actions. It is thus important to analyse the significant differences that arise from the evaluation of LTBI treatment from these two points of view.

Rationale for recommending treatment of LTBI

It is essential to assess treatment of LTBI from two very different points of view: when it is indicated in a specific patient to obtain individual benefit, and when recommendations are for general application, for example, as an intervention strategy in certain groups in the community.

LTBI treatment as an individualised clinical intervention

Risk factors for developing tuberculosis

From this point of view, it is necessary to thoroughly evaluate the individual benefits and risks of LTBI treatment in a healthy subject. The three minimum requirements provided in Table 15 should be fulfilled. In practice, this implies weighing the benefits of this intervention between patients with TB infection who are at the highest risk of developing active TB (e.g., recent infection; children under 5 years; HIV infection; untreated, inactive, or residual TB) and factors that increase the risk of

pharmacological toxicity (e.g., patients over the age of 35 years, alcohol intake, other diseases).

Table 15. Minimum requirements that should be met in order to consider the individualised clinical indication for treatment of LTBI.

- 1. Demonstrate that the person who is to be given LTBI treatment belongs to a group that is at an increased risk of progressing to TB disease.
- 2. Possess evidence that intervention in this group (receiving LTBI treatment) clearly reduces the risk of developing TB disease.
- 3. Demonstrate that the benefit of reducing the risk of progressing to TB disease outweighs the risk of pharmacological toxicity.

In the individualised clinical evaluation, there are only three groups of subjects in whom there are reliable data from studies demonstrating (Table 15) that the indication for LTBI treatment should be conclusive (Table 16), since in these subjects the benefits (reduced probability of progressing to TB disease) clearly outweigh the possible risks. These groups are patients with *M. tuberculosis* and HIV co-infection; those with recent infection, particularly children; and those with radiological lesions suggestive of residual TB. Furthermore, in most studies of treatment of LTBI involving the three patient groups described above, it has been shown that there is low toxicity associated with the pharmacological regimen administered, which is why the balance between benefits and risks clearly tips in favour of treatment. However, except in cases of HIV infection, in the other two groups the intervention occurs in a healthy population and it is therefore reasonable to expect minimal pharmacological toxicity levels.

Table 16. Unquestionable indications for treatment of LTBI.

3. Individuals with radiological lesions suggestive of untreated residual TB.

In the rest of the groups at risk of developing TB (Chapter 4, Table 1), the risk is variable, and many of these patients will experience increased pharmacological toxicity (with other underlying diseases). In the great majority of these groups, there have not yet been studies demonstrating the

^{1.} *M. tuberculosis* and HIV co-infection.

^{2.} Recent infection (tuberculin test converters), particularly in children.

efficacy of treatment of LTBI. Below is a critical review of the scientific evidence available on the different risk groups and the role of treatment of LTBI as an intervention.

Co-infection with M. tuberculosis and HIV

The risk group that has lately been the subject of the greatest number of studies concerning treatment of LTBI is patients with co-infection with HIV and *M. tuberculosis*, perhaps because in this group of patients it is not necessary to monitor the treatment for long periods of time in order to demonstrate treatment efficacy, unlike in other more immunocompetent groups. Most of these studies have demonstrated the efficacy of different treatment regimens used, with varying degrees of protection, depending on the treatment regimen, length, and place where the study was undertaken. Some of the studies have even shown different degrees of protection with other shorter alternative regimens that do not include isoniazid. Therefore, it has not only been demonstrated that this group of patients is at a greater risk of developing TB, but there are also sufficient studies to prove that treatment of LTBI is highly effective in these patients.

Individuals living with tuberculosis patients and recently infected persons

Most studies that were initially developed during the 1950s and 1970s with the objective of demonstrating the efficacy of treatment of LTBI included household contacts of TB patients and recently infected persons, the majority of whom had also been from the "household contact" group. These studies demonstrated a significant degree of protection in groups treated with isoniazid, and that the effectiveness of this intervention varied according to the length of treatment and the total quantity of drug taken. This is another group that has been shown to be at risk of developing TB and in which the indication of treatment of LTBI is unquestionable.

Inactive residual tuberculosis not previously treated

One of the best prospective studies that has been designed with the objective of demonstrating the utility of treatment of LTBI was the one performed by the International Union Against Tuberculosis and Lung Disease (IUATLD), which involved 27,830 healthy persons with residual TB. This excellent work (in which many other conclusions can be deduced and which will be analysed later), together with its subsequent study, clearly demonstrated the efficacy of isoniazid intervention in these subjects. This is the last group in which there is evidence showing that

these persons are at a high risk of developing TB disease and that treatment of LTBI is beneficial.

Silicosis

With regards to the remaining groups at risk of developing TB disease (Chapter 4, Table 1), the only one in which studies have been effected with the objective of demonstrating the efficacy of treatment of LTBI is the group involving patients with silicosis. Although there are studies that clearly show that this disease increases the risk of developing TB disease, only two well-conducted studies have been performed with the objective of assessing the efficacy of treatment of LTBI in these patients. While one of the studies found no evidence of protection with isoniazid + rifampicin + pyrazinamide for 3 months, the other only showed slight efficacy with the different treatment regimens studied for this intervention. Although the adverse effects did not increase, there was insufficient evidence to recommend treatment of LTBI in patients with silicosis, also especially since the risk of developing TB disease in these patients depends on the stage of the disease (silicosis stage) and the effects it has caused. For this reason, subgroups would have to be defined within this general group of silicosis patients for whom treatment of LTBI would be more justified. This, together with the fact that data from clinical studies are lacking, obscures the applicability of this intervention in this group of patients.

Prolonged corticotherapy

Patients who receive more than 15 mg of prednisolone for longer than 1 month are defined in recent CDC and American Thoracic Society recommendations as belonging to a high-risk group (where a positive tuberculin test is established at 5 mm), and treatment of LTBI is recommended in all such patients. Experimental studies have demonstrated that treatment with high-dose corticoids reduces natural resistance against *M. tuberculosis* in animals; however, the great majority of studies and articles state that this risk is minimal or almost non-existent. There are no studies demonstrating that LTBI treatment in this group reduces the risk of TB, and its possible toxicity has not been evaluated. In short, there is not enough evidence to definitively include corticotherapy amongst the groups at risk of developing TB disease, and even less evidence to justify the use of treatment of LTBI.

Diabetes mellitus

Another important risk group is patients with diabetes, because of the prevalence of the disease in the community. Although diabetic patients have for a long time been recognised as an at-risk group, there have been very few studies conducted that comprised sufficiently large patient samples. The risk of TB has been reported to be three to four times greater in diabetic patients than in healthy subjects, but the studies reporting these rates did not compare the prevalence of the infection in the community. This limitation does not render the results totally invalid; in fact, there are other studies that *do not* demonstrate an increased risk, whereas there have been no studies evaluating the efficacy or toxicity of LTBI treatment in this group of patients. This paucity of data, together with the relatively low risk of developing TB disease in diabetic patients, do not justify the treatment of LTBI in these patients.

Other groups at risk of developing tuberculosis

Treatment of LTBI in the remaining groups at increased risk of developing TB disease (Chapter 4, Table 1) is also highly questionable. In many instances, the classification of some groups as "at risk" was based on the results of a very small number of studies, many of which reported very low numbers of patients who progressed to TB disease. This is the case, for example, with intravenous drug users with HIV (-); persons suffering from malnutrition; those with chronic renal failure (undergoing haemodialysis) or head and neck carcinoma; or persons who have undergone gastrectomy, jejunoileal bypass, or organ transplantation. All these groups are included in the at-risk group of subjects in whom treatment of LTBI is recommended by the American Thoracic Society and CDC in their guidelines published in April 2000.

What is irrefutable is that there are no studies that demonstrate that treatment of LTBI offers any protection against TB in these possible risk groups, or that assess the possibility of increased toxicity.

Treatment of LTBI as an intervention strategy in the context of an NTP

Factors determining its efficacy

A completely different approach must be adopted if treatment of LTBI is to be implemented as an intervention strategy in the community. It will be necessary to evaluate the benefits for the community under NTP conditions if this measure is implemented in selected groups, and to estimate the associated cost. Cost does not only include the price of the drugs, but also the costs associated with maintaining a health care infrastructure, which will guarantee the application of the treatment regimen, and with setting up a training programme and educational campaign, which is essential if this therapy is to be implemented correctly. The evaluation of benefits to the community will be more complex, as it will depend on the number of infected persons who will have to be given treatment of LTBI in order to prevent one case of potentially infectious TB. In the end, the operational efficiency of administering treatment will depend on three major factors (Table 17): 1) the risk of developing TB disease in the group in question; 2) the pharmacological efficacy of the regimen employed; and 3) adherence to the prolonged treatment of LTBI.

Table 17. Factors that condition the operational efficiency of administering treatment for LTBI under NTP conditions.

Depending on these three factors, the operational effectiveness of treatment of LTBI will vary greatly (Table 18), and its use will be fully justified in some groups but not in others. Therefore, if the risk of progressing to TB disease in the infected group is low (5%) and a low adherence rate (30%) to treatment of LTBI is also expected, even if the efficacy of the pharmacological regimen is high (80%), the operational effectiveness will be low.

^{1.} Risk of developing TB disease in the intervention group.

^{2.} Pharmacological efficacy of the therapeutic regimen employed.

^{3.} Patient adherence to the prolonged treatment of LTBI.

Table 18. Operational efficiency of administering treatment for LTBI under NTP conditions. Number of LTBI treatments to be administered in order to prevent one case of TB.

TB risk (%)*	Efficacy of regimen (%) [†]	Adherence (%) [‡]	Global efficiency (%)	Prevention of one case [§]
5	80	30	1	100
10	80	30	2	50
10	80	60	5	20
30	80	30	7	14
30	80	50	12	8
30	90	70	19	5

*Estimated risk of developing TB disease in the group in which treatment of LTBI will be used.

[†]Assumed efficacy of the pharmacological regimen to be used for treatment of LTBI.

[‡]Estimated percentage of LTBI treatment candidates who will complete the therapy.

[§]Number of LTBI treatments to be administered in order to prevent one case of TB.

Therefore, to prevent one case of TB, it will be necessary to treat a large number of infected persons (over 100) in this risk group. This could be the case with the majority of groups that were defined earlier as having a "lower risk" of developing TB. At the other end of the scale are the groups at high risk of developing TB disease (30%), in whom if a better adherence rate is attained (50%), and there is the same treatment efficacy (80%), then operational efficiency increases and treatment of LTBI will only have to be administered to very few infected persons (less than 10) in order to prevent one TB case. These types of patients comprise the groups in which the indication for treatment of LTBI should be unquestionable, such as patients with HIV and M. tuberculosis co-infection, recent infection (tuberculin test converters), or previously untreated residual TB. Although the greatest risk of TB corresponds to the first of these groups (HIV infection), from a community perspective the largest number of cases can be prevented by managing the recent infection group (contacts). Thus, as an intervention strategy, treatment of LTBI in an NTP should be carried out in this group. The management of patients with HIV and M. tuberculosis co-infection or residual TB is more a matter of individualised clinical management, since they have less epidemiological impact than do TB contacts and those living with TB patients. The great majority of recent infections are from this latter group.

A thorough analysis will now be made of the factors that influence the operational efficiency of implementing LTBI treatment under programme conditions.

Efficacy of LTBI treatment as a conditioning factor for operational efficiency

Of the three factors that influence the operational efficiency of LTBI treatment, the one regarding the efficacy of the pharmacological regimen used will be a consistently critical factor. Until the onset of the HIV epidemic, most studies on LTBI treatment only included the use of isoniazid for different treatment lengths. However, in recent years, research on patients with HIV and M. tuberculosis co-infection has demonstrated that other drug regimens can be equally effective, with the important advantage that treatment time can be reduced, which helps to improve patient adherence. It has yet to be demonstrated, however, that these regimens are also effective in immunocompetent persons. It should not be forgotten than in subjects with relatively healthy immune systems, *M. tuberculosis* is usually in a latent state or has little metabolic activity, and under these circumstances these subjects will not be susceptible to antibiotic action. This explains why the best efficacy is observed with the longer regimens for isoniazid. It is likely that these circumstances also apply to other drugs, such as rifampicin and pyrazinamide (despite their greater sterilizing action), although it is yet to be demonstrated whether 2or 3-month regimens with these drugs are effective in immunocompetent persons (i.e., in household contacts of TB patients and healthy converters), or in other subjects in whom immunodeficiency is not as serious a problem as for HIV-infected persons. In HIV-infected patients, shorter regimens may be more appropriate because, considering the severe immunodeficiency, it can be assumed that the bacilli will have higher metabolic activity (thus facilitating antibiotic action, even over short periods), and a smaller proportion of bacilli will be found in a latent state. In any event, in consideration of the above and the high hepatic toxicity that has been demonstrated with the combination of rifampicin and pyrazinamide, this regimen cannot be recommended for routine use.

With regards to isoniazid, there has been much debate concerning the optimal length of time for treatment of LTBI in order to achieve maximum efficacy. Data from three major studies—the Ferebee study involving contacts, the Comstock studies on Eskimos, and the IUATLD multicentre study on residual TB—suggest that the best protection is perhaps provided with 9 to 10 months of isoniazid. Based on available data to date, LTBI treatment is most efficacious in the form of 9 months of isoniazid. The dose of isoniazid is the same as in treatment: 5 mg/kg body weight per day for adults and 10 mg/kg body weight per day for children, not to exceed 300 mg/day in either case (Chapter 9).

The risk of developing tuberculosis disease as a conditioning factor for LTBI treatment operational efficiency

Another factor influencing the operational efficiency of LTBI treatment is the risk of developing TB disease among different groups. As illustrated in Chapter 4 (Table 1), this risk can be estimated and used to define very high-risk groups in which intervention should be implemented automatically (Table 16), and to define lower-risk groups in which LTBI treatment is the subject of great debate and thus would be difficult to recommend in a control programme.

Of the three aforementioned groups in which intervention is unquestionable (Table 16), the most important group from an NTP management perspective pertains to persons living with TB cases and recent converters. The operational efficiency of LTBI treatment in this group will be influenced by the age of the contact person and the prevalence of the infection in the different age groups. For example, a child contact aged 5 to 10 years in a developed country where the prevalence of infection is less than 1% for this age group will not be managed in the same way as a 50-year old adult in a poor country with infection prevalence rates of over 75%. In the first case, the infected subject is almost always a recent converter and the efficacy of LTBI treatment is unquestionable. In the second case, however, it is highly likely that the infection has occurred before the present contact, and therefore LTBI treatment efficacy will be practically zero. This is why, depending on the prevalence of infection in the different age groups, an evaluation should be made of the age below which treatment of LTBI should be recommended to contacts. This issue has hardly been discussed by scientific societies.

Adherence to LTBI treatment as a conditioning factor for operational efficiency

This third factor is where most work can be done by an NTP. If treatment abandonment among TB patients is the biggest obstacle encountered by NTPs, it should be remembered that this rate would increase in infected subjects, because LTBI treatment in a healthy population has to be maintained for longer than for those with TB disease. Many studies have demonstrated minimum adherence to this treatment, although some studies have shown that adherence in itself is not as important as the fact that the patient must take the recommended number of tablets, albeit over a longer period. For this reason, the training and education of health personnel, together with other important measures, are fundamental if treatment of LTBI is to be used as a strategy by the NTP. Some programmes in places such as Cuba and New York have introduced directly observed LTBI treatment, albeit on different scales, which has been shown to improve patient compliance and increase the operational efficiency of this intervention. However, cost-efficacy studies are still needed to assess the public health value of this strategy. It is likely that too high an investment is required in comparison with the achievements that are obtained from a community perspective.

Other factors conditioning treatment of LTBI operational efficiency

Other factors, such as the characteristics of the health system and the individual being treated, are also important and may determine if LTBI treatment is suitable, particularly since such factors play a role in an early diagnosis of TB in the event that the disease develops. It is also important to consider the risk level of those who live with TB cases and of the community that is in contact with the possible future TB case (if LTBI treatment is not be administered). In short, it will be necessary to analyse the characteristics and circumstances of the susceptible individuals described above, since contacts such as children in nurseries, prison inmates, and elderly residents in nursing homes differ from adults without risk factors. This difference in community risk is that which persuaded the CDC and the American Thoracic Society to include the first group of contacts in their LTBI treatment recommendations.

Finally, another important factor is the necessity to demonstrate that the individual who is under consideration for LTBI treatment is infected by *M. tuberculosis*. It is well known that the only available method for demonstrating this infection is the tuberculin test, which is associated with significant limitations, particularly with regards to preservation of the tuberculin, application and reading of the test, false positives and false negatives, and the window period between onset of infection and positivity of the purified protein derivative in recently infected patients and contacts. This subject has been reviewed at length in Chapter 6 of this Guide and should be given careful consideration when deciding on an intervention in the community.

As it can be seen, the community perspective on treatment of LTBI as an intervention depends on a large number of complex factors that vary by country, region in the country, the achievements and motivation of the local NTP, and, above all, the risk of developing TB disease. Resources should only be spent on the management of contacts, those who live with TB patients, and recent converters.

Importance of LTBI treatment in NTP strategies

When LTBI treatment is analysed as an intervention strategy, under no circumstances can it be compared with appropriate chemotherapy and curing of TB cases. It must be remembered that there is almost 100% efficacy in correct TB treatment, and that its operational efficiency is much higher, particularly because this measure eliminates sources of infection, whereas LTBI treatment only prevents infection in certain cases. Therefore, when TB treatment is implemented appropriately, not only does the patient benefit (as in cure and prevention of possible death), but so does the entire community, since the source of infection is eliminated. In contrast, this benefit is highly uncertain with LTBI treatment. This is why a TB control programme should not waste any of its resources on treatment of LTBI without previously having worked actively on case detection and having attained high cure rates of above 85% to 90%. There are many areas in the world, particularly in medium- and high-income countries, where LTBI treatment has been adopted as a strategy in the last few decades, but where there is often little enthusiasm to achieve high cure rates.

If we divide the world according to the epidemiology of TB, available resources, and the appropriateness of anti-TB campaigns carried out in the past, we can clearly identify three major blocks:

- The first block consists of low-income countries, where the disease is highly prevalent and there has been little use of appropriate TB control measures. There is no doubt that in this group of countries, which includes most countries in Africa and Asia and certain zones of Latin America (accounting for an estimated 80% of TB burden in the world), all resources must be invested in achieving appropriate treatment and in guaranteeing a health infrastructure that permits implementation of directly observed treatment and the detection of cases through smear microscopy. Furthermore, in this block, since there is mass BCG

vaccination in infants (many schoolchildren are also revaccinated), and peripheral health centres do not have the facilities to conserve tuberculin, the use of the tuberculin test to detect infected cases is not valid. Thus, extensive use of LTBI treatment should only be indicated (without having to perform the tuberculin test) in contacts of smearpositive cases under the age of 5 years, in order to lower TB-related mortality. Cases of HIV and *M. tuberculosis* co-infection are also indicated.

- The second block comprises middle-income countries, where TB rates are high but not as high as in poorer countries, and where despite having had NTPs for many years, there has been limited impact on the decline in TB rates. In this group, which includes the large majority of Latin America and Eastern Europe, and some countries in Asia, mass BCG vaccination is widespread, and there is a lack of facilities to guarantee proper conservation of tuberculin in peripheral health centres. Still, these countries have more resources and a better health infrastructure than do the poorer countries, and their priority is to invest in case detection and attain high cure rates. Only when progress is made with respect to case detection and high cure rates should LTBI treatment be considered in specific risk groups. LTBI treatment should be offered to those patients with HIV infection and contacts of smearpositive cases (without performing tuberculin tests), with consideration of extending the age range in these contacts by up to 10 to 15 years.
- Finally, the last block comprises developed countries, which are characterised by low TB rates and good NTPs that have implemented effective TB control for many decades. Most of these countries have been attaining the priority objectives with regard to case detection and high cure rates for many years now. Furthermore, many do not vaccinate and all possess facilities to conserve tuberculin in all health centres. For these reasons, LTBI treatment is indeed an important intervention strategy in these countries when dealing with the endogenous reservoir for TB disease. Thus, the inclusion of LTBI treatment as an intervention strategy in the NTPs of these countries should be evaluated. However, the epidemiological impact of this measure is very low, and its use implies spending large amounts of resources. A thorough evaluation must be made to select the risk groups that are to receive LTBI treatment, and to determine how such intervention should be performed, either on an individualised basis or universally to achieve a community-wide impact.

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BCG vaccination

Chapter summary

In 1919, after 13 years and 230 consecutive passages, Albert Calmette and Camille Guérin obtained a live *M. bovis* strain with attenuated virulence. At present, there are four varieties of these strains that are distributed by the United Nations Children's Fund (UNICEF): French (Pasteur) 1173 P2, Danish 1331, Glaxo 1077, and Japanese 172.

Some of the most important negative aspects of this vaccine are that it interferes with the tuberculin test result, thus complicating the indication for the treatment of LTBI; it is associated with complications; and its costbenefit ratio is uncertain. Furthermore, many aspects of the immunological response to the BCG vaccine are unknown, although it is clear that the vaccine offers limited immunity against certain *M. tuberculosis* antigens.

The best means of evaluating the efficacy of the vaccine is through controlled clinical trials and case-control studies. However, both have yielded markedly different rates of protection by the vaccine, ranging from 0% to 80%. Many factors have been proposed to explain these differences. Meta-analyses have reported high vaccination efficacy (75-86%) against TB meningitis and miliary TB, but only moderate overall efficacy (50%). There is no evidence or theoretical rationale to justify re-vaccination in schoolchildren.

The BCG vaccination does not prevent *M. tuberculosis* infection or protect previously infected cases; its protective effects are inconstant, temporary, and time limited. Perhaps its best action is the protection it offers against disseminated TB and severe forms of TB that can occur after primary infection, especially in children. Even if it is implemented under the best conditions, and presuming maximum efficacy, it has very little impact on the endemic rate and does not help to reduce the annual rate of infection.

Considering the above, wide-scale BCG vaccination at birth should be employed as an intervention strategy in all countries with high or moderate TB prevalence rates, not because of its epidemiological impact, but so as to reduce infant mortality. In countries with low TB prevalence rates, where infant mortality from TB was overcome decades ago, BCG vaccination is not indicated.

A brief history

When Robert Koch demonstrated in 1890 that TB was an infectious disease, work commenced in different parts of the world to develop a vaccine. However, the only anti-TB vaccine that has been widely used in the world is the one obtained from *M. bovis* by Albert Calmette and Camille Guérin at the Pasteur Institute in Lille. In recognition of their discovery, it became known as the bacille Calmette-Guérin (BCG) vaccine. Calmette and Guérin believed that it was necessary to obtain live avirulent bacilli, capable of producing systemic and local immunity in the digestive tract, which at the time was considered to be the disease's principal route of access. They commenced their studies in 1906 with a bovine bacillus strain isolated in the milk of a cow with tuberculous mastitis, and observed that consecutive passages in this culture medium progressively reduced the virulence of the bacillus. After 13 years and 230 consecutive passages, it was believed that characteristics of the bovine bacillus could no longer be modified as they were hereditarily fixed. They had obtained a new strain of bovine bacillus, which they named the bile bacillus of Calmette and Guérin, or BCG.

In 1921, the first experiments involving vaccinations in humans began, and on June 25, 1924, Albert Calmette reported the results to the Medical Academy of Paris. From then on, mass vaccination was commenced in children in France. BCG strains were distributed to different countries, which also began vaccinations. The end of the Second World War brought about mass use of BCG vaccination as a preventive measure against TB. It was initiated by the Red Cross in Denmark in 1947, and Sweden and Norway soon joined the International Tuberculosis Campaign, which grew rapidly. By 1948, approximately 5 million persons had been vaccinated in 35 countries, and BCG vaccination was considered the only method that achieved effective immunity against TB. By the 1970s, the vaccination was provided in 169 countries and an estimated 2000 million persons had received it. In 1988, the World Health Organization (WHO), as part of its Health for All in the Year 2000 Programme, included the BCG vaccination in the Expanded Programme on Immunisation.

Immunogenic differences of BCG attributable to the production process

When the use of the vaccine became widespread, the original BCG strain was distributed to different laboratories worldwide, in light of keeping the strain alive and preparing BCG vaccinations for subsequent use. In the 1950s, it was confirmed that there were marked differences in the immunogenicity of vaccines prepared by the different laboratories, since the techniques in culture maintenance and vaccine preparation had been adapted to the individual characteristics of each laboratory. Even the vaccines prepared in the same laboratory revealed significant differences. It was therefore decided that it was necessary to standardise the vaccine, taking into consideration the purity of the strain, its viability, the pathology caused in animals, local lesions caused by vaccination in humans, tuberculin test conversion, and immunity provided in animals and humans. In 1959, a study was begun, coordinated by the WHO Research Office in Copenhagen, with the participation of laboratories from seven countries, to analyse each laboratory's methods of evaluating the vaccine. Participating laboratories were given the same vaccines: the freeze-dried Japanese vaccine (strain 10-034 A) and the liquid Danish 1343. The findings were startling; the live unit count for the same vaccine, assessed with a strict and standardised technique, varied not only from one laboratory to another, but also within the same laboratory, with much wider margins than expected.

The use of BCG vaccination in mass worldwide campaigns revealed other problems, such as the importance of transportation and conservation of the vaccine until its use. The allergenic potency of the live BCG vaccine is reduced considerably when stored above 30°C, even for a short period of time. In order to prevent unforeseen and undetected modifications in the biological properties of the BCG strain, it was recommended to use a seed-lot system in vaccine production. A seed-lot comprises a certain amount of BCG strain, of uniform composition, that is used as a seed in the preparation of BCG cultures. The colonies are harvested from the primary seed-lot for successive passages, up to a maximum of 12 passages. Nowadays, the preparation method has been standardised and it is always used in vaccine production.

However, although all vaccines come from the same master strain created by Calmette and Guérin, each laboratory that produces the BCG vaccine maintains the corresponding substrains by means of successive passages and different methods, which has brought about morphological, physical, and biological changes in the initial bacillus. It was suspected that these mutations were also of a genetic nature, a hypothesis that was subsequently confirmed with molecular biological techniques. This may at least explain in part the differences observed in the immunogenicity and efficacy of the different vaccines.

Recommended vaccines and vaccination techniques

Since 1960, the WHO has recommended that cultures be stabilised through lyophilisation and freezing in order to reduce the possible immunogenic differences observed in different vaccine strains.

At present, there are four strain varieties distributed by UNICEF:

- French (Pasteur) 1173 P2
- Danish 1331
- Glaxo 1077; this strain, which was obtained in Copenhagen in the 1950s and was derived from the Danish 1331 strain, is somewhat different. (Merieux in France and Evans in the United Kingdom use this strain.)
- Japanese 172, selected because of its high resistance to lyophilisation and because it is more stable to heat.

In addition, there are other vaccine strains that are widely used in the world, such as Moreau (Brazil), Montreal (Canada Connaught), Russian (Russia), and Tice (United States).

A live vaccine contains approximately 10^8 bacilli per mg of BCG, although it will only provide between 5 x 10^6 and 45 x 20^6 colony-forming units (CFU). The proportion of viable BCG bacilli may be halved after drying and freezing.

Although Calmette and Guérin commenced their studies employing the oral route for vaccination, the method of choice to perform BCG vaccination is by intradermal injection, which was recommended at the First International BCG Congress in 1948, and subsequently by the WHO Expert Committee. This route of administration was introduced in Sweden in 1928 and it is used because it permits correct dosage.

A special BCG is required for intradermal injection and it is administered at a dose of 1/20 mg of BCG diluted in 0.1 ml, although in children under 1 year, half of this amount is recommended with the same concentration. This dose is injected intradermically in the deltoid region, in order to produce an oedematous papule with a diameter of 8 to 10 mm. After 2 to 3 weeks, there will be central necrosis at the injection site, developing into a pustule or small blister that will secrete a thick serous liquid; this will resolve spontaneously in 3 to 4 weeks, leaving a scab for a further 6 to 12 weeks that will then fall off and leave a depressed, round, blanched scar.

Prior considerations and deficiencies in the BCG vaccine that may affect its indication

Before analysing the efficacy and possible indications of the BCG vaccine, it is necessary to consider a series of extremely important practical factors regarding its limitations, which may affect its indication in different regions. The most important factors are:

- BCG vaccination interferes with tuberculin test results, as explained in Chapter 6. This will hinder the interpretation of the only technique available for the diagnosis of tuberculous infection.
- It will also hinder possible indication for treatment of LTBI, a TB control strategy that, as has already been analysed, is much more effective than BCG vaccination.
- The vaccine is not harmless. There are potential complications, particularly in immunosuppressed individuals, because the vaccine involves a strain with live bacilli. This is a common source of controversy, particularly because of its wide-scale use in countries with a high prevalence of HIV.
- The cost-benefit ratio has not been sufficiently studied despite the amount of research conducted on the subject. The truth is that these efficacy studies are very difficult to perform and compare. The uncertainty about the cost-benefit ratio is one of the biggest limitations in the possible recommendation of the vaccination, and it explains, in part, the enormous differences encountered from one zone to another. This point will be discussed in further detail later in this Guide.
- The influence of the complexity of the TB immune response (covered in Chapter 5). Many aspects of the immunological response to BCG vaccination are still unknown. In any event, the BCG vaccination affords a limited immunity to certain important antigens to *M*. *tuberculosis*—e.g., secretion proteins, Ag 30-32 kD.
- The presence of cell hyperactivity (positive tuberculin reaction) is not synonymous with protection.
- There are many factors that influence the vaccine quality, such as the percentage of viable bacilli present in each vaccine dose, culture dispersion, and lyophilisation method.
- Despite the intradermal route being the recommended route of administration, other routes have also been used, such as the oral route. Of late, the inhalatory route is being tested, which would permit a specific local response.

- The fact that a third of the world population is infected makes this vaccine useless in this segment of the global population. One possibility of improving the BCG vaccine would be to develop one that destroys the bacilli inside the macrophages.

Assessment of BCG vaccination efficacy

Since the very beginning of the mass vaccination campaigns, it was clear that there were great differences in the protection afforded by the vaccine depending on the different regions where it was used. Protection rates were over 80% in certain areas, and yet in others vaccinated individuals were more likely to develop TB than those who were not vaccinated. From the start, it was observed that it was very difficult to assess the efficacy of this vaccine, and comparing results was even more difficult. Attempts to assess the efficacy of the vaccine have been based on: *BCG scar*

The presence of a vaccination scar is one of the objective indicators of vaccination. However, the size of the local reaction and the scar are related to the dose and the level of BCG bacilli (either dead or alive) in the vaccine, and to the type of vaccine used. Usually, the vaccination scar is permanent, but small scars and those that result from low vaccine doses may disappear in the course of time. The vaccination scar is not synonymous with protection, although it is believed that if no reaction or scar occurs, no type of protection is possible.

Tuberculin testing

Although the BCG vaccine clearly interferes with tuberculin test results, it is not known to what extent this is true. Therefore, although it is possible that in some individuals it causes significant interference for many years, in others there is hardly any interference. All this is difficult to estimate or predict. In any event, a positive tuberculin test after vaccination is not synonymous with protection, although it is reasonable to assume that if the vaccine is not even capable of converting the tuberculin test, it will not be able to offer any level of protection. The relation between skin test reaction and the BCG vaccine is closely connected to the number of viable bacilli in the vaccine (which may range from 5×10^6 to 45×10^6).

Inoculation in animals

The few animal studies that have attempted to quantify the efficacy of the BCG vaccine have been unsuccessful.

Controlled clinical studies

Almost all controlled clinical studies of BCG vaccination have significant methodological limitations, although the controlled trial is the least ambiguous method to evaluate vaccination efficiency. In this type of study, vaccinated subjects are monitored for a certain period of time, with the objective of determining the rate of onset of disease in the presence of a certain factor or exposure, which, in this case, is BCG vaccination.

The results of the major controlled clinical studies of the efficacy of BCG vaccination are highly discordant (Table 19). In a study involving Native American Indians, the vaccination efficacy observed over a 20-year period was 80%. In contrast, the vaccination efficacy among schoolchildren in Puerto Rico, after 7 years of observation, was 31%. The Medical Research Council studied intradermal vaccination in 14,100 schoolchildren aged 14 to 15 years, comparing results from this group with that from a control group of 13,200 individuals. Findings, which were published every 5 years, revealed high and consistent vaccination efficacy over the 20 years of observation (81% vs. 76%). However, in two studies conducted in India, the first study (in Madanapalle; 1950-1955) revealed a vaccination efficacy of 57%, whereas the second study (in the district of Chingleput; 1968-1971), which included 115,000 tuberculin-negative individuals over the age of 1 month, revealed very low levels of protection: 17% in children aged 0 to 14 years and 0% in those over the age of 14 years. These results were somewhat surprising as this study was one of the best-designed studies on vaccination efficacy.

Vaccination in infants at birth has also been analysed in different controlled studies, again with highly discordant results. Aronson and colleagues reported an efficacy of 59% in Indian babies vaccinated at birth. However, Sergent and colleagues observed a vaccination efficacy of 6% to 36% after 7 years among 20,174 vaccinated newborns; whereas Curtis observed a 75% vaccination efficacy rate in newborns in Manchester, England.

Table 19. Major controlled clinical studies evaluating the efficacy of BCG vaccination.

Place	Age at vaccination (years)	Vaccinated population	Monitoring (years)	Vaccination efficacy (%)
United States	0-20	North American Indians	9-11	82
Puerto Rico	1-18	Schoolchildren	18-20	29
Georgia	5-17	Schoolchildren	20	-57
Georgia and Alabama	>5	General population	14	14
Chicago	Newborn infants	Contacts with TB patients	12-24	75
England	14-15	Schoolchildren	15	77
France	6-14	Schoolchildren	20	74
Madanapalle (India)	All ages	Rural	20	19.5
Chingleput (India)	All ages	Rural	15	0
New York (US)	Newborn infants		2-13	6.6
Saskatchewan (Canada)	Newborn infants	North American Indians	15	81

Case-control studies

In these studies, subjects are selected precisely because of the presence (cases) or absence (controls) of disease. These studies require a strict design and appropriate statistical analysis, as they may present significant bias. Since they are retrospective studies, verification of vaccination history is a problem. Furthermore, it is not sufficient to check for the presence of a scar, which would overestimate vaccination efficacy.

Results from case-control studies of vaccination efficacy have also been highly discordant (Tables 20 and 21), with a maximum efficacy of 84% and a minimum of 0%. Differences in the methodology of these studies have been detected, which may have influenced the efficacy rates obtained. In Yaounde, Cameroon, the BCG vaccination was administered to individuals aged 0 to 18 years without previous tuberculin reaction, in a population with a 25% prevalence of tuberculous infection in this age group. TB was ruled out in the control group only on the basis of the absence of symptoms.

Table 20. Major case-control and contact studies evaluating the efficacy of BCG vaccination.

e)									
Case-control studies									

However, these studies demonstrated that vaccination efficacy was greater in patients with disseminated or meningeal forms of TB (Table 21), and that there was much inconsistency in the relation between vaccination efficacy and the time elapsing after vaccination. **Table 21**. BCG vaccination efficacy (as a percentage) in the prevention of different clinical forms of tuberculosis, according to the major case-control studies.

City/region (country)	Meningitis	Miliary TB	Pulmonary TP	Pleural TB	All forms	
Sao Paulo (Brazil)	86.8		ID			
Sao Faulo (Blazil)	02.0					
	20.5					
	29.5					
Bangkok (Thailand)	100	100	64		96	
Buenos Aires (Argentina)	100	78	64	57	96	
Madras (India)	77					
Manitoba (Canada)					79	
Yaounde (Cameroon)			66*			
England	70		76		45	
Santiago (Chile)			9			
Rangoon (Burma)	52	80	26			
Papua (New Guinea)	58	70	25			
Jakarta (Indonesia)	75		27			
Barcelona (Spain)		-40 [†]	12*	38	32	
			21 [‡]			
[*] Bacilliferous post-primary pulmonary TB.						
[†] Extrapulmonary TB.						
[*] Primary pulmonary TB.						

Studies of contacts

These studies have been conducted in countries where BCG vaccination is mandatory for newborns. The effect of the vaccination was analysed in children who were studied as contacts with an index case; comparisons of results depended on whether there was a vaccination history. In these studies (the results of which are illustrated in Table 20), the vaccination efficacy rates were 66% in Lome, Togo; 53% in Bangkok, Thailand; 71% in Bangui, Central African Republic; and 74% in Seoul, Korea. Another important conclusion drawn by these studies is that the protective effects decrease in the course of time, decreasing from 79% in children under the age of 1 year to 46% in children aged 4 to 5 years in Seoul.

Meta-analysis of studies on BCG vaccination efficacy

Considering the marked differences among results of studies of the efficacy of BCG vaccination, an attempt has been made to obtain an

objective interpretation of the combined results of the different studies using meta-analysis statistical techniques. Ten controlled clinical studies were analysed, obtaining statistically significant heterogeneity in vaccination efficacy (P < 0.001), and it was therefore considered inappropriate to calculate a global protection effect. Vaccination efficacy was high (86%) against tuberculous meningitis and miliary TB. In casecontrol studies, heterogeneity was also high, although less in pulmonary TB (P < 0.02), presenting greater homogeneity against meningitis and miliary TB with a global protection effect of 75% against these forms of the disease.

Colditz and colleagues have performed two meta-analyses. In the first, a protective effect of 51% was obtained in controlled clinical studies and 50% in case-control studies. The second study, which analysed vaccination efficacy in children, reported an average protective rate of 74% in four controlled studies and 52% in nine case-control studies. However, these studies have received much criticism because they did not adjust for possible heterogeneous factors influencing the use of the BCG vaccination in the different studies, such as differences in populations, risks of infection and/or TB disease, vaccines, and methods of case-control selection.

Factors influencing the differences in BCG vaccination efficacy

The BCG vaccine, which is employed in different parts of the world, differs considerably in biological and even genetic characteristics, as well as in other factors such as conservation method and transportation. These differences have been used to explain the discrepancy in results from the various efficacy studies. The most important factors that may cause such differences are detailed in Table 22. As can be seen, there are many factors involved, among which one of the most significant is infection by environmental mycobacteria. These mycobacteria may afford a slight degree of protection in non-vaccinated individuals, which would therefore lower vaccination efficacy. This is one of the most important factors that has been used to explain the fact that studies conducted in tropical regions, where in theory there are more environmental mycobacteria, have always reported a lower degree of protection than studies performed in non-tropical areas.
Table 22. The most significant factors that may cause the marked differences found in the efficacy of the BCG vaccine in different studies.

- 1. Attributable to the vaccination:
 - Vaccination technique
 - Dose administered
 - Vaccination age
- 2. Attributable to the vaccine:
 - Transportation
 - Conservation
 - Viability of the vaccine
 - Different classes of vaccine used
 - Different culture methods employed in its preparation
- 3. Variables that may influence the host-vaccine interaction:
 - HIV infection
 - Immunosuppression
 - Severe malnutrition
 - Others
- 4. Previous infection from environmental mycobacteria
- 5. Methodological differences in the planning and development of studies
- 6. Possibility of reinfection by highly virulent strains of *M. tuberculosis* in individuals protected by the BCG vaccine
- 7. Differences amongst individuals in different populations

Conclusions on BCG vaccination efficacy

In conclusion, it is perhaps possible to summarise the following about the BCG vaccine:

- It does not prevent *M. tuberculosis* infection.
- It does not protect individuals with previous infection.
- It affords inconstant, temporary, and time-limited protection.
- It hinders the predictive value of the tuberculin test, and therefore interferes in the appropriate implementation of LTBI treatment, a more effective intervention strategy.
- It does appear to protect against disseminated TB and severe forms of the disease that can occur after primary infection.
- It affords inconstant protection against pulmonary TB.
- It does not protect against TB reinfection or adult TB.

- Even if implemented under the best conditions, and assuming maximum efficacy, it has scarce impact on prevalence rates of disease and does not contribute to a reduction in the annual risk of infection. It does, however, have protective effects in children, and 95% of these cases are smear-negative with a minimal capacity to infect others.
- It has a potential risk of dissemination if the patient acquires HIV, although complications are rare.

Contraindications to revaccination in schoolchildren

At present, there is no scientific evidence to justify revaccination in schoolchildren or in children over the age of 5 years. Furthermore, if it is acknowledged that BCG vaccination only protects against severe TB in children, it must then be remembered that this protection will disappear if the child develops adult forms of TB. If it is still believed that the BCG vaccination has a protective effect in children above the age of 5 years, and for this reason it is decided to recommend revaccination in schoolchildren, it should also be accepted that it would also be necessary to provide further protection by revaccinating at a later age. Hence, following this erroneous premise, not only would revaccination have to be recommended in schoolchildren, but also every 5 to 10 years thereafter. If it is acknowledged that this would not be the case, then revaccination should not be administered to schoolchildren.

Side effects of the BCG vaccine

As described earlier, the BCG vaccine is prepared from live bacilli, which is why its use is associated with certain side effects. Although these side effects are generally rare, they vary greatly, from disseminated infection from BCG to death. Table 23 shows a classification of possible complications that may result from the vaccine. For this reason, if BCG vaccination is indicated as an intervention strategy by an NTP, the programme must consider the need to monitor the development of possible side effects. Evidently, the likelihood of side effects, in particular the more adverse effects, increases in immunodeficient children and those who suffer from severe malnutrition, which is a very common situation in poorer countries.

It is well known that intradermal BCG vaccination causes a primary reaction at the injection site. However, some individuals suffer an excessive reaction, with ulceration, subcutaneous abscess, or suppurative adenitis. Despite the fact that BCG vaccination is practised widely and there is evidence of the poor evolution of vaccination lesions and generalised dissemination, it is not known what the risk of adverse effects is from the vaccination. This is mainly because local complications are not usually reported or diagnosed.

The IUATLD performed two studies to investigate the incidence of complications from BCG vaccination: a retrospective study (1975-1976) and a prospective study (1979-1981). Six European countries participated in the second study, which involved approximately 5.5 million vaccinated children, half of whom were over the age of 1 year when vaccinated. The observed risk of local complications and suppurated lymphadenitis was 387 cases per million in the younger age group, of which 93 per million registered positive histological or bacteriological results. In the older age group, the risk was 25 per million, and confirmation was attained in 18 cases per million. The risk of disseminated infection and hypersensitive reactions varied considerably amongst the countries. In the former German Democratic Republic, the risk ranged from 5.59 per million in children under the age of 1 year to 13.6 per million in children above this age. In Romania, the rates were 3.49 per million in those younger than 1 year and 0.99 per million in those above 1 year old. This major study suggested that both the number of notifications and risks calculated were underestimated, since there were cases of osteitis that were not recorded.

It has been observed that in certain BCG strains, the virulence and dose are more likely to cause bone and joint lesions. However, complications from regional ganglions are more related to the vaccine dose and the (younger) age of the child when vaccinated. **Table 23**. Classification of possible complications caused by BCG vaccination.

- 1. Abnormal primary reaction due to BCG vaccination:
 - 1.1. Ulcers, Koch's phenomenon, abscess
 - 1.2. Regional purulent adenitis
- 2. Disseminated BCG infection; generalised or local lesions; non-fatal cases
 - 2.1. Osteitis
 - 2.2. Retropharyngeal abscesses
 - 2.3. Specific tuberculous-type cutaneous lesions: lupus, others
 - 2.4. Metastatic subcutaneous and intramuscular abscesses
 - 2.5. Bone and joint complications (including synovial lesions)
 - 2.6. Renal and urogenital complications
 - 2.7. Pulmonary and hilar complications
 - 2.8. Mesenteric adenitis
 - 2.9. Multiple adenitis and/or hepatosplenomegaly, or other locations
- 3. Disseminated BCG infection; generalised lesions; fatal cases
- 4. Post-vaccination syndromes or pathologies associated with BCG vaccination
 - 4.1. Chronic local cutaneous complications (keloids, histiocytomas)
 - 4.2. Acute cutaneous eruptions (erythema nodosum and other eruptions)
 - 4.3. Ocular complications
 - 4.4. Other syndromes; non-fatal cases
 - 4.5. Other syndromes; fatal cases

BCG vaccination as an intervention strategy in the control of tuberculosis: indications according to the epidemiological situation

Of the three possible community interventions for TB control—chemotherapy, chemoprophylaxis, and BCG vaccination—the strategy on which all efforts should focus is chemotherapy, followed by LTBI treatment in a distant second place, and, lastly, BCG vaccination. This order of priority is based on factors related to efficacy, action, speed of action, duration of effect, capacity to eliminate infection sources, and possible community benefit. These factors, applied to each of the three intervention strategies, are discussed in detail in the chapter on TB control.

Having discussed the reasons for placing BCG in third place as an intervention strategy, and having analysed its efficacy and many limitations, several questions remain: is BCG vaccination, which is so widely recommended, of any real use? How can its use be justified as one of the vaccinations recommended by the WHO in its expanded programme on immunisation? To answer these questions, it should be remembered that

there are very different situations worldwide regarding TB prevalence and control. As in the case of treatment of LTBI, the indication for BCG vaccination differs greatly from one region to another, depending on TB endemic rates, achievements in disease control in recent decades, and available health resources.

Indications in countries with high prevalence rates

There is still a large part of the world population that lives in regions with high TB endemic rates, with a very high prevalence of disease and infection, and a probable annual rate of infection of over 1%. In these areas, the priorities are to reduce mortality from TB (2 million persons still die in the world from this curable disease) and to attain high cure and case detection rates, preferably for persons with positive smear microscopy. Here, there should be no doubt that there should be mass vaccination at birth for the entire population, not for the purpose of influencing the epidemiological impact of the disease, but rather to reduce the high infant mortality in these countries. In these areas, where 80% of worldwide TB cases live, BCG vaccination should become part of the intervention strategy, alongside chemotherapy (with directly observed treatment) and appropriate measures, to increase passive case detection.

Indications in countries with moderate prevalence rates

There is another significant segment of the world population that lives in zones of so-called moderate TB endemic rates, with an estimated annual rate of infection of 0.2% to 1%. Here, although it is likely that the problem of mortality has already been overcome, and the priorities should be on healing cases and increasing case detection, children are still at a high risk of TB infection, particularly those from the more socially and economically deprived sectors who have worse access to the health system. This is why in this group of countries, which consist of the great majority of middle-income countries, mass vaccination at birth should also be recommended, even though its impact on mortality will likely be lower and no epidemiological impact is to be expected.

Indications in countries with low prevalence rates

In the segment of the world's population that lives in low TB endemic zones, with very low disease rates and prevalence of infection, the annual

risk of infection is less than 0.1%. In these countries, which should already have attained success in the detection and healing of positive smear microscopy cases, much effort should focus on the early diagnosis of the disease, including negative smear microscopy cases; on achieving a 100% cure rate in TB patients; and on strategies to attack the TB endogenous reserve. Here, LTBI treatment should be implemented as an intervention strategy. As explained earlier, since BCG interferes with tuberculin test results and LTBI treatment management, mass vaccination at birth is not indicated. In these countries, which comprise most of the world's industrialised countries, BCG vaccination should not be used to support the health system, which is indeed often the practice; rather, BCG vaccination should be an individual decision.

BCG vaccination could possibly be indicated in children with negative skin test results who have had close and prolonged contact with chronic TB patients, those who have not complied with treatment, and those with multidrug-resistant (MDR)-TB and in whom no other prevention or control strategy can be implemented. It could also be indicated in members of risk groups with an annual rate of infection above 1% and in residents of countries where it is mandatory.

BCG vaccination in health care personnel

Some scientific societies also recommend BCG vaccination in health care personnel who are at high risk of MDR-TB transmission and in whom other control strategies cannot be implemented or have failed. Thus, the vaccination is currently recommended in health care personnel who are in contact with persons with MDR-TB, since the possible efficacy of LTBI treatment is unknown in this context. However, it should be noted that the possible efficacy of BCG vaccination in this group of professionals also has not been demonstrated and, in theory, is likely to be highly questionable.

Epidemiological criteria for suspending BCG vaccination

As discussed earlier, BCG vaccination has specific indications in certain epidemiological circumstances. When such conditions improve, the almost zero benefit of mass BCG vaccination does not justify the cost of this measure, the interference it causes with other intervention strategies (e.g., treatment of LTBI), or its side effects. Therefore, international organisations have reached the conclusion that mass BCG vaccination should be suspended in a country where any of the following epidemiological situations are found:

1. An average annual rate of cases of pulmonary TB with positive smear microscopy equal to or less than 5 cases per 100,000 inhabitants during the previous 5 years.

2. In children under the age of 5 years: an average annual rate of tuberculous meningitis of less than 1 case per 10 million inhabitants during the previous 5 years.

3. An average annual rate of infection of TB equal to or less than 0.1%.

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Chapter 14. Childhood tuberculosis

Chapter summary

Childhood tuberculosis (TB), in comparison with adult TB, is clearly different in epidemiological development, clinical presentation, and diagnostic methods, particularly in children under the age of 5 years. The only similarity pertains to therapy, and even then only three drugs can be used in the first phase when treating children. For this reason, children should be managed at senior levels in National Tuberculosis Control Programmes and preferably be cared for in areas where a paediatrician is on hand.

Childhood TB almost always registers with smear-negative microscopy results, and it is therefore assumed that the condition is not infectious. Control of TB in this group of patients thus has little epidemiological impact in the community. However, childhood TB is always the result of recent transmission, and children represent a sentinel group for the consequences of poor TB control. Hence, epidemiological studies involving children are of utmost importance.

Childhood TB almost always proceeds from primary TB, meaning that in young children the disease frequently manifests with haematogenous and lymphatic dissemination. The low bacillary population often produces negative results in microbiological studies, which is why other indirect diagnostic methods are important. Diagnosis should be based on an overall evaluation of a series of epidemiological, clinical, radiographic, tuberculin, and microbiological criteria. Using these criteria, a scoring system can be drawn up for the purpose of diagnostic decision making and, frequently, for determining which therapeutic tests have to be used.

Perhaps the first key issue in this Chapter should be to define up to what age an individual can be considered a child, in order to distinguish the different forms of tuberculosis (TB). In the great majority of countries, paediatrics covers care up to the age of 14 years, which may be too high an age limit in the case of TB management. Children above the age of 5 to 7 years already reproduce adult forms of TB, with a higher occurrence of pulmonary involvement or cavitary TB, and the possibility of registering a smear-positive result. When differentiating between the management of childhood and adult TB, a marked difference is found in children under the

age of 5 to 7 years, a difference that increases further with younger age. This chapter will therefore focus on the management of children under the age of 5 to 7 years.

The priority of all National Tuberculosis Control Programmes (NTPs) is the management of smear-positive cases, since they spread TB and perpetuate the epidemic. Because most cases of childhood TB are associated with smear-negative microscopy results, childhood TB is hardly ever a priority for control measures in an NTP and is thus often excluded from management in these programmes. However, although the level of infectiousness is not of epidemiological significance, childhood TB would probably reap the greatest benefit from appropriate individualised clinical management. Indeed, the children of today are the future of all countries.

The management of a child with TB differs in several major ways from that for adult TB. TB in children, in comparison with in adults, has a different clinical presentation, and the diagnostic methods also vary—this is especially so in children under the age of 5 years. The only similar characteristic is therapeutic management, except that fewer drugs should be used in the first phase, owing to a lower bacillary burden in children. For this reason, children should be managed at senior levels in NTPs, in contrast with the care provided to adults with smear-positive microscopy. Preferably, children should be cared for in areas where there is a paediatrician.

This chapter will discuss the conditions that characterise childhood TB, dividing these conditions into four major areas: epidemiological factors, clinical presentations, utility of diagnostic methods, and treatment.

Epidemiology

It is assumed that childhood TB, which almost always results in smearnegative microscopy, is not infectious, and if so, is much less infectious than adult TB. This low level of infectiousness means that childhood TB control is of little epidemiological relevance in the community and that its management is basically a result of clinical interest. However, childhood TB always reflects recent transmission, suggesting therefore that there is an infectious adult for every childhood case. This fact does acquire epidemiological importance and is significant for protecting public health. For this reason, TB in children is an indication of poor TB control in the community. In fact, children with TB represent a sentinel group for the advances of an NTP against the disease. Furthermore, epidemiological studies involving children are of tremendous importance with regards to the analysis of infection parameters, the disease, and mortality, as well as the observation of the presence of recent transmission. For example, if a drug resistance survey was performed in children under the age of 5 years who had TB (a difficult study because of the low bacillary burden), not only would information on drug resistance be obtained, but also information on the number of resistant strains being transmitted in the community and the possible virulence of these strains. For this reason, this age group is ideal for performing tuberculin studies, which focus on assessing the prevalence of tuberculous infection in the community, and for estimating the extent to which TB is transmitted in the community.

The best example of children as the sentinel population for demonstrating the success or failure of anti-TB efforts can be found in global statistics for TB. Of the 8 million new TB cases that occur each year worldwide, 1,300,000 are children under the age of 15 years. Likewise, of the 2 to 3 million deaths due to TB each year, 450,000 are children in this age group. Furthermore, the severe impact of HIV in low- and middle-income countries affects children.

Clinical presentations

In the great majority of childhood TB cases, the disease proceeds from primary TB, which means that haematogenous and lymphatic dissemination is common. The marked trophism from lymphatic involvement in children under the age of 5 years is of particular importance, since this can lead to a severe form of adenopathy. Furthermore, in these cases, 65% to 75% of tuberculous adenopathies have an intrathoracic location, particularly with hilar and/or mediastinal adenopathy. The rest are extrathoracic. One particular manifestation is adenopathy affecting the cervical ganglionic chain, which is commonly referred to as scrofula. When scrofula affects children younger than 5 years of age, 75% to 80% of cases are caused by environmental mycobacteria and not *M. tuberculosis*. Studies reporting this finding, however, have all been conducted in developed countries; it is not known whether this is the case in countries with high TB incidence. The *M. avium* complex and *M.* scrofulaceum are the species that are most commonly involved in this clinical presentation, and they are known to be highly resistant to antituberculous drugs. Surgical removal is therefore indicated in these cases. For this reason, it is important to culture all biopsy samples from presentations that are compatible with scrofula, because if the culture

confirms *M. tuberculosis*, medical treatment will suffice and it will not be necessary to resort to surgery.

Very young children, especially infants younger than 6 months, have an undeveloped cellular immune system and are therefore particularly susceptible to haematogenous dissemination and possible presentation of miliary TB. It is necessary to be on the alert for the possible development of meningeal TB, which is the most severe and fatal form that affects children.

Despite the above, the most common clinical presentation of TB in children is pulmonary TB, although, as mentioned, miliary and extrapulmonary TB is more common than in adults and therefore a keen diagnostic suspicion must always be maintained.

Diagnostic difficulties

While the diagnosis of adult TB is fundamentally based on microbiological studies, in childhood TB only a small percentage of these techniques are useful, which is why other indirect diagnostic methods are used more often in children. The lack of microbiological support makes the diagnosis of childhood TB far more difficult, and very often the diagnosis has to be based on other much less specific methods. This means that the diagnosis of TB can only be confirmed on very rare occasions, and it is therefore essential to possess an in-depth knowledge of what these indirect methods offer. Because a diagnosis cannot be confirmed, under- or over-diagnosis is a possibility, depending on the importance given to evaluation with alternative methods.

In short, the diagnosis of childhood TB should be based on an overall evaluation of a series of epidemiological, clinical, radiographic, tuberculin, and microbiological criteria. Using one of these criteria, a decision can be made to commence treatment and a therapeutic test may be performed to confirm cure.

Epidemiological factors

The frequency and importance of a focal point of transmission of the infection within the family to a child has been widely discussed in the literature. The World Health Organization considers contact studies to be one of the most practical methods of detection, especially in children. This means that when pulmonary TB is diagnosed in an adult, it is essential to examine all the children with whom the adult has had close contact.

Likewise, whenever TB is diagnosed in a child, it is necessary to determine if there is an undiagnosed infected adult in the family. Finding the source of TB transmission becomes more important in countries with a low TB prevalence, since in these countries there are few infectious focal points in the community, and a child with TB can readily lead to the suspected adult index case. In countries with a high TB incidence, where there are numerous infectious cases in the community, although there may not be a clear contact, it is also possible to determine who the contact source may be, with less room for error.

Clinical criteria

Clinical, physical, and analytical symptoms are unspecific and may provide very little aid in diagnosis. Furthermore, considering the significant degree of radiological involvement observed in many children with pulmonary TB, physical signs and symptoms are surprisingly rare. Clinical presentation depends on the location of the disease. The most common presentation in children is pulmonary TB, which initially manifests with dry cough, dyspnoea, sweating, and weight loss. Since clinical presentation is extremely unspecific in children, much more so than in adults, it is necessary to be on the alert for the possibility that a child may have TB when he or she shows signs and symptoms, such as cough, febricula, weight loss or absence of weight gain, irritability, or erythema nodosum. Miliary and extrapulmonary TB (i.e., in sites such as the lymph nodes, intestines, meninges, and bones) is more common in children and must also be considered. Findings on exploration are also very rare, except in newborns, who present with symptoms of chronic spastic bronchitis that do not respond to standard treatment. Manifestations of primary infection should be taken into account. Phlyctenular conjunctivitis and erythema nodosum may remain undiagnosed if they are not suspected. Blood tests provide very few findings. The erythrocyte sedimentation rate, which is highly variable at the onset of disease, may be useful in assessing disease evolution and treatment response in the initial months.

Tuberculin testing

As explained in Chapter 6, despite its limitations, the tuberculin test is actually one of the mainstays in TB diagnosis in children, and therefore any suspected cases should be confirmed by a positive tuberculin test. Owing to the importance of the tuberculin test in this population group, the test must be correctly administered and interpreted. Indeed, correct interpretation is essential, and the circumstances surrounding a child having to undergo the test should be carefully considered in order to detect a false-positive or false-negative result.

With regards to false-negative results, there are two very important conditions that must always be taken into account in children:

- 1. Recent infection. It takes 4 to 8 weeks for delayed hypersensitivity to be established after *M. tuberculosis* enters the host. During this period, the tuberculin test may yield negative results. This is the "window period".
- 2. Severe forms of TB. It is common to find a negative result in miliary localisation and meningitis.

Other situations involving immune deficiency, whether congenital, acquired (HIV), or temporary, as in viral diseases; or use of corticoid or immunosuppressant treatment; may result in a negative tuberculin test. When a false-negative result is suspected, the tuberculin test must be repeated as many times as required; the test does not produce sensitivity.

False positives should be taken into account when assessing the tuberculin test, particularly with regard to reactivity to the tuberculin on the part of environmental mycobacteria and, above all, from BCG vaccination.

In children, the tuberculin test increases the positive predictive value considerably for the diagnosis of tuberculous disease. All positive tuberculin test results in children are the result of recent infection, and the greatest risk of suffering from the disease is found during the first weeks and months after the infection occurs. For this reason, the younger the child, the faster the infection is detected by tuberculin test, and there is therefore a higher probability that the disease is proceeding from a primary infection. A positive tuberculin test in a child is always an indication for intervention, either by administering treatment of latent tuberculous infection (LTBI) if active disease has been ruled out, or by administering treatment if the child is considered to have the disease. If there is any doubt as to whether the child has the infection or the disease, the best recommendation is treatment, particularly if there are compatible symptoms.

Radiology

In the diagnosis of adult TB, radiology should always be backed up with microbiology, owing to its lack of specificity. In children, radiology is of much greater significance and actually becomes an essential diagnostic tool. However, interpretation of radiographs is a highly complex matter.

In the pathogenic pattern, it has been seen that the infection commences by depositing bacilli in the alveoli. Lung parenchymal inflammation is not usually visible on the chest radiograph, but an unspecific localised infiltrate may be observed. All the lobar segments in the lungs are at the same risk of being the initial focus of infection, and although there is usually just one focal point of infection, in 25% of cases there are multiple foci. In the initial phase, the infection disseminates to the regional lymph nodes. The characteristic lesion of initial tuberculous infection is a relatively large adenopathy, in relation to the relatively small size of the initial parenchymal focus. Because of lymph drainage, a focus in the left pulmonary parenchyma often causes bilateral hilar adenopathy, and a right-sided focus is accompanied by adenopathy on the same side.

Mild parenchymal opacities and adenitis often resolve spontaneously, occasionally with calcification, giving rise to the so-called bipolar complex if the calcification occurs in the inoculation chancre and to mediastinal adenopathy. In other instances, particularly in children younger than 3 years, because of the characteristics of the lymph system, the lesion progresses and causes much enlargement of the mediastinal adenopathy, which compresses the bronchi (extrinsic compression), in turn causing bronchial obstruction (Figure 16). When the adenopathy grows, it may infiltrate and break the bronchial wall, causing a granuloma in the bronchial lumen (intrinsic compression), which may also lead to obstructive problems. These granulomas may rupture in the bronchus, causing bronchogenous dissemination.

All these changes will have an effect on the chest radiograph, and may take on highly varied forms: atelectasis, hyperclarity, or mediastinal or parenchymatous limits. They may appear between chest radiographs, or change in the course of evolution. It is impossible to describe the appearance of a primary infection pattern since any abnormal image may have this aetiology. Correct interpretation by the physician is thus essential.

Sometimes there may be necrosis of pulmonary parenchyma, causing the formation of a thin-walled tuberculous cavity. Localised

pleural effusion often accompanies the primary pulmonary focus, but significant pleural effusion is rare in children under the age of 2 years. Reactivated TB, as observed in adults, with typical thick-walled apical cavities, is very rare in children, although it can be found in teenagers.

It is advisable to take two radiographs: one anteroposterior and the other lateral on inspiration. Occasionally, it may be necessary to complement the study with computed tomography or magnetic resonance imaging. These techniques are contraindicated in the routine clinical management of these cases in low- and medium-income countries because of their high cost. Additionally, due to the high prevalence of TB in these countries, where an assumed diagnosis of active disease can be made with greater confidence, a therapeutic trial can be implemented without having to use further imaging techniques. However, in industrialised countries where similar symptoms may represent alternative diagnoses, computed tomography and magnetic resonance imaging may aid in the diagnosis. Indeed, computed tomography has frequently been shown to reveal mediastinal adenopathies in patients with suspected TB and a normal chest radiograph. It should be noted that many of these patients also have positive results by polymerase chain reaction (PCR) for *M. tuberculosis*.

Microbiology

The isolation of *M. tuberculosis* is that which confirms the diagnosis of pulmonary TB in the adult, but this measure frequently cannot be used in children because childhood TB is associated with a very low bacillary burden that does not yield a positive smear microscopy result. Still, microbiological studies should be performed in all cases of suspected disease.

The problems surrounding microbiological diagnosis of childhood TB are based on two major areas: the impossibility of obtaining appropriate study samples, and the low quantity of bacilli contained in such samples. Since children do not expectorate, especially young children, and they simply swallow secretions, gastric juice can be used instead, obtained through aspiration with a nasogastric tube. This procedure should be performed on a fasting child and on 3 consecutive days. The sample must be processed as soon as possible. Results of cultures obtained via this method vary greatly, from 14% to 41%. The disadvantage of the gastric lavage sample is that if the maximum yield is to be obtained, by collecting all secretions aspirated during the night, the procedure must be performed as soon as the child wakes up, because if

there is a delay the secretions will be quickly absorbed in the stomach. For this reason, the child has to be admitted to hospital, which is a course that is rarely justified if the patient is not very ill, especially in low- and medium-income countries.

According to the most important studies, direct smear microscopy of the sputum or of gastric lavage is only positive in 5% to 10% of children with pulmonary TB, and the sensitivity decreases with the (younger) age of the patient. Sensitivity of the culture from sputum and gastric lavage does not exceed 50%, which is very low considering that it is necessary to wait several weeks to confirm the diagnosis. It may sometimes be necessary to return to sample taking by means of bronchoscopy, particularly if any obstructive process has to be ruled out. In this case, the bronchial exudate and other suitable samples must be collected. The problem of diagnosis increases even more in extrapulmonary forms of TB, particularly meningeal TB.

Therapeutic tests

The decision to diagnose TB and recommend treatment is often based on indirect and relatively unspecific data. For this reason, a therapeutic trial is often initiated in a suspected case, i.e., by commencing an antituberculous treatment regimen and observing whether the child is cured. These therapeutic trials are more justified in low- and medium-income countries than in wealthier nations. In the latter, it is common to find an adult TB case in a contact study, since the epidemiological clues are of greater value (there are fewer infectious cases in the community) and there are more resources available to reach a diagnosis (e.g., computed tomography, PCR). Furthermore, because TB is less common in wealthier countries, it is necessary to resort to these diagnostic methods more frequently to rule out other possible conditions that may have similar signs and symptoms. In poorer countries with a high TB prevalence, it is more common not to find epidemiological clues. Further, there are fewer resources in these countries, and when the clinical presentation is suggestive of TB, it is indeed almost always the case because of the high prevalence of the disease in the community. This is why a therapeutic trial is much more justified and recommended in suspected childhood TB in low- and medium-income countries.

Other diagnostic methods

The possibility of employing other diagnostic methods has two purposes: to obtain better study samples and to increase the utility of microbiology. The possibility of resorting to gastric lavage or specimens obtained through bronchoscopy has already been commented on, in order to improve the quality of samples to be studied. Samples obtained via biopsy or aspiration in suspected extrapulmonary TB cases can also be considered.

PCR can be used directly on the clinical microbiological samples obtained in children. However, the sensitivity of a diagnostic PCR test ranges from 25% to 83%, but specificity is almost 100%. The reason is that a positive result with PCR in children cannot differentiate between recent infection and disease; this is one of the biggest disadvantages of this technique. Furthermore, a PCR test is very costly and complicated to perform and therefore cannot be indicated in low- and middle-income countries. In these countries, when confronted with a suspected case of TB, a therapeutic trial is recommended instead. A PCR test could be indicated in industrialised countries, mainly because these countries have more resources and because alternative diagnoses are more common.

The other method that was suggested earlier (in Chapter 8) as being able to contribute to the diagnosis of childhood TB is serology. Although it has very low sensitivity (less than 21% to 40%), the test yields few false positives in children. In addition, a positive serological test could provide strong support in diagnosing the disease. In any event, results obtained via this method must always be interpreted in conjunction with results of other tests.

The approach to tuberculosis diagnosis in children: scoring system

As explained earlier, both a thorough evaluation of all the diagnostic tools discussed and a management decision must be made. Because the decision must be individualised, the management of childhood TB should take place at a higher level than the management of adults with smear-positive results. To standardise the diagnosis of TB in children, which would facilitate management at the peripheral level in the health system, scoring systems have been devised to assess all the topics discussed in this chapter. These scoring systems aim to achieve a more or less close approach towards TB diagnosis in children. They are artificial diagnostic tools that

may be both highly useful and, at the same time, the subject of debate. This is why when childhood TB is suspected, it is best to have the child evaluated by a paediatrician who can decide which approach to take.

Treatment and prevention

Treatment is the only similar characteristic between adults and children with TB, except that since children suffer from forms of TB with low bacillary populations, it is much more unlikely for them to have the bacillary load necessary for selecting naturally resistant mutants. This means that only three drugs—isoniazid, rifampicin, and pyrazinamide—can be administered in the initial phase in children and maintained in the continuation phase. The use of ethambutol is to be avoided, since its main side effect is altered visual acuity, which cannot be assessed in children. The other difference in the treatment of children is the need to adjust the dose according to weight. Doses for children are shown in Table 7. Fortunately, side effects are less common in children than in adults, but if any occur they should be managed in the same way as for adults.

The best way of preventing childhood TB is through good control of adult TB. As explained, the level of infectiousness is very low in children and the epidemiological impact is therefore also very low. However, treatment compliance must be ensured until the patient is cured. The search for TB cases can be conducted at a passive level among children who present for consultation in the health system with signs and symptoms that are suggestive of TB. However, active searching for cases should also be conducted among contacts between the ages of 5 and 15 years who have positive smear microscopy (depending on the resources of, and level of TB control in, the country). Tuberculin screening or mass radiological studies in the healthy population are not justified, since they are very costly and inefficient.

In low- and medium-income countries, LTBI treatment should be administered in all children aged 5 to 15 years (see Chapter 13) who have been in close contact with patients with smear-positive pulmonary TB. In developed countries, before initiating LTBI treatment, the discriminatory element of the tuberculin test should be introduced, as recommended in Chapter 13. Finally, in all low- and medium-income countries, all infants should be vaccinated at birth in order to lower infant mortality due to severe forms of TB. This vaccination is not justified in countries with low endemic rates, as was explained in Chapter 13.

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Chapter 15. Tuberculosis in the elderly patient

Chapter summary

Tuberculosis (TB) is much more frequent in the elderly than in any other age group. This is because the highest prevalence of *M. tuberculosis* infection is found in the elderly, such prevalence being cumulative in the course of a lifetime, and because a degree of natural immune deficiency develops with age, with a reduction in the number and function of the T-helper lymphocytes and an increase in the presence of T-suppressor cells, thereby augmenting the possibility of endogenous reactivation of TB disease.

There is controversy over whether elderly subjects present with different clinical and/or radiological manifestations, although the condition seems to be linked to the degree of possible immune deficiency. What is known, however, is that the utility of tuberculin testing decreases in this population group, while the performance of microbiological techniques is similar to that recorded in younger patients. It does seem to be accepted that the diagnostic delay is greater in elderly subjects—this phenomenon possibly conditioned by factors such as memory problems, mental confusion, isolation, and other concomitant diseases with similar symptoms—and there is an increased risk of contagion.

Treatment is the same and cure can be achieved in all cases, although in view of the additional problems typically found in elderly patients (other pathologies and treatments), close observation is required, with attention to possible side effects. Mortality due to TB is clearly greater in elderly patients and is also related to the possible immune deficiency, associated diseases, concomitant treatments, increased rates of treatment abandonment, and delays in establishing the diagnosis in the elderly.

In view of the above, an increased diagnostic suspicion of TB is warranted in persons over the age of 60 to 65 years.

Epidemiology

The age distribution of tuberculosis (TB) cases in a given community is an excellent epidemiological study parameter that reflects the effectiveness of the control measures implemented. It should be remembered that tuberculous disease is more common in population groups containing an increased number of individuals infected with M. tuberculosis. Thus, in countries that have effectively fought against TB in past decades, early diagnosis and the healing of most TB cases has been achieved, thereby shortening the epidemiological chain of the disease by eliminating the infectious sources in the community. As a result, population cohorts born in the last four to five decades have had low community infection risks and very few infections have resulted. This is why in these countries (which include most industrialised nations), TB is essentially found in the older age groups, where there is a persistent presence of individuals with M. tuberculosis infection-witnesses to the poor epidemiological and social conditions they had endured during their childhood when effective treatment measures were not yet available. In these elderly individuals, TB develops as a result of endogenous reactivation of the bacteria acquired in the past, which have remained in a latent or dormant state in the host for decades.

However, countries where no such effective TB control measures were employed in past decades continue to have numerous sources of contagion in the community, and the population cohorts born in these subsequent years represent high infection risks. As a result, infection and, consequently, disease have persisted. This is why in these countries, which include most low- and middle-income nations, TB is fundamentally found in younger individuals. In contrast to the situation found in more developed parts of the world, TB in poorer countries is more a consequence of progression of infection or of exogenous reinfection.

Nevertheless, the above considerations refer to absolute numbers of patients. All studies conducted in developing countries and that have reported a predominance of disease in young patients almost never express the figures in rates corrected per 100,000 inhabitants in each age group. This form of expression in absolute numbers is the most valid approach for comparison purposes. Not presenting data as corrected per 100,000 inhabitants can lead to the false belief that elderly individuals in poorer countries are relatively free of the disease. Normally, in countries with low- or middle- level incomes, there are clear discrepancies between the

age distribution curve expressed in absolute numbers of cases (Figure 51) and the curve obtained when these values are corrected per 100,000 inhabitants in each age group (Figure 52). The example of the age distribution of new TB cases diagnosed in Honduras in 2000 (Figures 51 and 52) illustrates the differences between the two ways of presenting the data.



Figure 51. New cases of TB in Honduras in 2000. Distribution by age, in absolute numbers. The numbers next to the diamonds (♦) indicate the number of cases. Source: National Tuberculosis Control Programme of Honduras.

The elderly population is the sector of the community that suffers the most from TB, regardless of the development of the country or the efficacy of the antituberculous strategies employed in the past. However, life expectancy is lower in poorer countries (an average of 20 years less than in wealthier countries), as a result of which there are fewer elderly individuals. Still, these elderly subjects have had to suffer even worse epidemiological and social conditions than their counterparts in the developed parts of the world, and, as a result, have a higher prevalence of *M. tuberculosis* infection and greater possibility of developing TB.



Figure 52. New cases of TB in Honduras in 2000. Distribution by age, in rates per 100,000 inhabitants in each age group. The numbers next to the diamonds (♦) indicate the rate per 100,000. Source: National Tuberculosis Control Programme of Honduras.

That the oldest segment of the population suffers tuberculous disease the most can be attributed to two reasons. First, the elderly have the highest prevalence of *M. tuberculosis* infection, since the prevalence is cumulative in the course of a lifetime. Thus, the older the individual, the greater the probability of infection. Second, immune defects in cellular immunity tend to be found with advancing age.

Pathogenesis

Estimations based on tuberculin anergy results from studies of elderly patients with TB infection or disease, and research involving animal models and healthy or TB-afflicted elderly individuals (in comparison with younger patients), have demonstrated the existence of the abovementioned discrete immune deficiency in older individuals. This natural immune deficiency is most likely established after the age of 60 years, increasing in probability and in intensity with age. This defect may be related to a reduction in the number and function of T-helper lymphocytes, and to an increase in the presence of T-suppressor cells—together with a possible increase in humoral immunity.

The way in which the disease develops will depend on the epidemiological situation found in the different communities. Thus, in areas with high rates of TB disease and infection in young subjects, a high infection rate is to be assumed in the older age groups. Due to their immune deficiency, these older people are at a greater risk of developing the disease as a result of endogenous reactivation of the bacteria acquired in the past, and which have remained in a latent or dormant state in their bodies for decades. However, in some areas with lower rates of TB disease and infection in young subjects (e.g., industrialised nations), the prevalence of *M. tuberculosis* infection in individuals over the age of 60 years, while still comparatively higher, may not exceed 10% to 25% of the total. This percentage of infected individuals is at an increased risk of developing TB disease due to endogenous reactivation. Moreover, globally this age group shows increased sensitivity towards developing the disease through exogenous infection, as has been demonstrated in the nosocomial epidemics reported in developed parts of the world. Consequently, in these situations, if the elderly live in closed environments, a case of TB (with its corresponding diagnostic delay) will cause many new infections by affecting a relatively immunodeficient segment of the population, and will considerably increase the risk of developing TB. In some homes for the elderly in developed nations, a TB disease rate of close to 7000/100,000 has been recorded, compared with the community rate of only 16/100,000.

Clinical manifestations and diagnosis

The immune deficiency characterising the elderly population might condition the growth and multiplication of *M. tuberculosis* and the different clinical and radiological presentations reported by some authors. Many studies have reported an increased frequency of disseminated forms of the disease (either demonstrated or suspected), with a clinical presentation in which syndromes of systemic involvement tend to predominate. However, this point remains open to controversy, as many authors have also reported no differences between young and older patients regarding the organs affected and the initial clinical syndrome involved.

Controversy likewise persists regarding the radiological presentation, with some studies reporting an increased frequency of lower lung lobe involvement and of non-typical or disseminated TB lesions. In contrast, other authors have reported no significant differences in comparison with TB found in younger subjects. This discrepancy in findings may have a pathogenic explanation based on the degree of immune deficiency found in the patients studied—a fact that has not been addressed in studies of clinico-radiological differences with respect to elderly TB patients. In this context, the greater the immune deficiency of the individuals, the greater the probability of disseminated presentations of the disease, systemic syndromes, and non-typical radiographic lesions. Furthermore, immune deficiency in the elderly is not only conditioned by old age but also by the frequent and numerous associated pathologies that are found in the elderly, and by the poorer living conditions and possible malnutrition in many instances.

Another characteristic of elderly TB patients is the increased diagnostic delay, which in turn implies an increased risk of contagion, particularly if the individual lives in closed institutions. Such delays in establishing a diagnosis can be attributable to a variety of reasons, such as memory problems, mental confusion, isolation due to partial loss of the senses (hearing, sight), and even the ability to communicate. However, the fact that elderly subjects often suffer from other concomitant diseases with symptoms that are similar to those of TB still constitutes the main cause of diagnostic delay.

Some diagnostic techniques are less sensitive in the elderly. For example, tuberculin testing clearly loses its utility in both diagnosing disease cases and identifying infection—a deficit that is related to the onset of age-related immune defects. For this reason, in wealthier countries it is never sufficient to perform a single tuberculin test in the elderly (when studying infection or TB disease); in effect, a second or even a third skin test is needed (spaced 1 week apart) if previous results are negative. The aim here is to identify the possible booster effect that these tuberculin doses may exert upon immune memory loss—a procedure that has been shown to be effective in all studies involving elderly persons. This procedure is not indicated in countries with low- or middle-income levels, since in these regions the great majority of elderly persons are infected with *M. tuberculosis* and tuberculin testing is associated with many limitations (Chapter 6).

Regarding the performance of microbiological tests in the elderly, the results seem to be similar to those obtained in younger patients, provided the same types of lesions are involved. In studies showing an increased frequency of non-typical forms, with a greater presence of infiltrations versus cavitary lesions, the performance of smear microscopy and culture may decrease. Performance is thus related to the bacillary population of the lesions.

Lastly, some studies have described a different presentation of tuberculous granulomas in some forms of TB in the elderly, including the repercussions this may have for the histopathological study of biopsy specimens. Thus, a variant described in elderly patients is referred to as "areactive" TB, often with a miliary presentation. Here, the classic caseous granuloma is not surrounded by the usual abundant inflammatory cells, but instead by numerous bacilli. This form of presentation, attributed to an inadequate immune response, is most frequently seen with fever and weight loss, but without the typical pathological signs.

Treatment and prognosis

TB in the elderly is healed with the same treatment regimens indicated for younger patients, although some studies have described decreased efficacy of such treatments in the elderly. Other studies have described a longer time to negative culture conversion, again possibly due to the diminished immune support found in the elderly, although it is not known if a comparatively lesser effect of treatment could also be involved. Elderly patients pose increased treatment adherence problems because of the possibility of memory lapses (difficulty remembering the prescribed dosage), mental confusion, a decreased ability to comprehend the scope of the disease and the precise drug doses required, and even eyesight problems that make it difficult to recognise which tablet to take. Some type of treatment supervision is thus required.

The most important consideration in the treatment of TB in the elderly is the increased toxicity of antituberculous medication in these patients, and the interactions of some of these drugs. The risk of liver toxicity, largely associated with isoniazid, is much greater in elderly patients (more than double the toxicity in younger individuals), as a result of which periodic liver function tests may be advisable. Similarly, nephrotoxicity is a consideration when streptomycin is used—a situation requiring monitoring of renal function and dose reduction. In addition, rifampicin has an enzyme inducer effect, and consequently the dosage of drugs commonly prescribed in the elderly (e.g., digoxin, antiepileptics, corticoids, tolbutamide) should be monitored closely.

Based on the above considerations, and despite the fact that healing can be achieved in all cases, TB mortality is clearly greater among elderly patients. In this population sector, mortality has increased in recent years, even in the developed parts of the world. This increased mortality is influenced by many factors, including some of those already mentioned above, such as immune defects, associated pathology, increased treatment abandonment, and delays in establishing a diagnosis.

Control

In view of the above considerations, an increased diagnostic suspicion of TB is indicated in people over the age of 60 to 65 years. This is the age group with the highest TB disease rates, in whom the clinico-radiological presentation, associated diseases, and different performances or yields of the diagnostic techniques imply an increased delay in diagnosis. This delay is not only of great importance in terms of public health (related to increased infectious periods), but also for the patient, which explains why TB is found with some frequency in necropsy-based studies. Such studies have reported a considerably greater frequency of TB among older population groups. On the other hand, the most important finding possibly is that almost half of these TB cases identified at necropsy did not have the opportunity to receive treatment because the disease had not been diagnosed when the patient was alive.

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Chapter 16. Tuberculosis and HIV

Chapter summary

The epidemiological impact of human immunodeficiency virus (HIV) infection on tuberculosis (TB) depends on four main factors: 1) the prevalence of HIV infection in the community; 2) the prevalence of TB infection, the risk of infection, and the infection trend among individuals between the ages of 15 and 49 years; 3) the transmission pattern of HIV infection; and 4) the risk of developing TB disease among HIV and *M. tuberculosis* co-infected individuals. Based on these variables, it can be seen why the impact of the acquired immunodeficiency syndrome (AIDS) on the problem of TB varies dramatically from the poorest nations (severe impact) to the richest parts of the world (little influence).

Immunosuppression induced by HIV can increase the incidence of TB via three mechanisms: 1) endogenous reactivation; 2) progression of recent infection; and 3) exogenous reinfection.

In turn, the diagnosis of these patients can be problematic, depending on the degree of immunosuppression at the time of diagnosis. In HIVinfected patients with reasonably good immune defences (CD4+ lymphocyte counts >300 cells/mm³), diagnostic test performance will be similar to that in patients not infected with HIV. In contrast, in subjects with advanced immunosuppression, increased clinical and radiological diagnostic difficulties may be encountered, and both tuberculin test results and histopathological findings may be more complicated to interpret. Nevertheless, the microbiological tests afford similar performance, although maximum care is necessary to ensure that multiple, good samples are obtained.

Treatment is the same as with normal TB patients, although in view of the increased rate of complications and particularly of drug interactions in these patients, management by expert physicians is required. Special attention should be paid to the pharmacological interactions between antituberculous drugs (particularly rifampicin) and antiretroviral agents (especially protease inhibitors). Chemoprophylaxis is always indicated in such individuals and should preferably involve 9 months of isoniazid.

Throughout history, the human species has periodically been attacked by different microorganisms that have threatened its very existence. Some of these microorganisms, such as the agent causing tuberculosis (TB) or

malaria, cause millions of deaths each year. Occasionally, the emergence or re-emergence of a microorganism can cause an unexpected and catastrophic pandemic of unpredictable consequences. During the twentieth century, two such unexpected health care catastrophes occurred. The first was the influenza epidemic beginning in 1918 that caused approximately 25 million deaths throughout the world in the course of 3 years. The second is thought to have begun in the summer of 1981, when a man in the United States presented with an opportunistic infection due to a case of severe immunodeficiency that could not be explained at the time. It is astounding that the virus that subsequently became known as the human immunodeficiency virus (HIV) could spread so extensively in less than two decades, leading to so many infected and diseased persons and deaths. The speed at which HIV has spread is all the more surprising because the virus is not transmitted through the air or via the digestive tract, but through human interrelations, which in theory should have caused its spread to be slow in the community. At present, there is no place in the world where this pathogen is not found, and its future devastating effects are still difficult to estimate. These considerations suggest that the disease was probably already present for some time in the human species, perhaps in some poorer areas of the planet, such as sub-Saharan Africa.

It is difficult to imagine a microorganism better suited than HIV to function as an ally of *M. tuberculosis*. The virus selectively destroys or alters the function of precisely those immune cells that defend the host against Koch's bacillus. Thus, the oldest of human infectious diseases, represented by TB, and the most recent pandemic to affect humans, represented by HIV, have combined their pathogenic effects to become the leading cause of death in large parts of the world. It is estimated that sizeable areas of the poorest countries will be literally deprived of their young populations in the coming decades as a result of the deadly association of these two pathogens.

The present chapter provides a detailed account of how these two epidemics are merging in the world, with a review of the pathogenesis, diagnosis, and treatment of TB associated with acquired immunodeficiency syndrome (AIDS). Some of these points have already been addressed in other chapters, as a result of which only the salient facts will be emphasised here.

Confluence of two epidemics

The AIDS epidemic is interfering with the natural balance that existed between *M. tuberculosis* and the host, which, even before the introduction of antituberculous treatment, favoured the human host. It is now evident that resources for controlling TB are proving to be insufficient for averting a new epidemic of this disease on a global scale.

The epidemiological impact of HIV infection on TB depends on four main factors:

- 1. The prevalence of HIV infection and its trend in the community.
- 2. The prevalence of TB infection, the risk of infection, and its trend among individuals between the ages of 15 and 49 years.
- 3. The transmission pattern of HIV infection.
- 4. The risk of developing TB disease among HIV and *M. tuberculosis* co-infected individuals.

Prevalence of HIV infection and its trend in the community

Towards the end of 2002, the World Health Organization (WHO) estimated that over 42 million people worldwide were living with HIV infection/AIDS, and that this disease had already caused 26 million deaths. The number of AIDS cases continues to grow each year, although in 2002 there was an estimated 5.6 million new cases of HIV infection, a figure very similar to that recorded for 2000. The best indication of the devastating spread of this disease is possibly afforded by the fact that in both 2000 and 2002, 2.6 million people were estimated to have died of AIDS. Similar to TB, the global distribution of AIDS is very heterogeneous, with 95% of all cases being located in the poorest parts of the globe. Thus, both pathogens have gradually spread to the most vulnerable populations of the world. At the end of 2002, it was calculated that 70% of all cases of HIV infection/AIDS were confined to sub-Saharan Africa, 20% to Southeast Asia and the Western Pacific, and 5% to Latin America and the Caribbean. According to the predictions of the Population Division of the United Nations, between 2010 and 2015 the life expectancy of the populations in the nine African countries with the highest prevalence of HIV infection will be reduced by an average of 16 years. This adds another obstacle to the development of these countries, where poverty can be expected to increase owing to the weakening of the economically active stratum of the population.

The scenario is completely different in the industrialised world, where only 5% of all HIV infection/AIDS cases are found, together with 5% of the cases of dual HIV-TB infection (Figure 53). These countries possess the resources and infrastructure necessary to institute good National Tuberculosis Control Programmes. Owing to a unique set of circumstances, the association of these two pathogens in the developed world also does not have the same effects as in poor countries, a topic that will be discussed later in this chapter. Moreover, since 1996, highly active antiretroviral therapy (HAART) has been available for the treatment of HIV infection. These drugs, however, are very expensive, and their use is largely confined to industrialised countries. HAART affords substantial and sustained increments in peripheral blood CD4+ lymphocyte counts, together with reduction of the plasma HIV viral load to undetectable levels.

Although it is well accepted that the HIV epidemic is completely out of control in the world, there is much that we still do not know, such as:

- 1. The number of HIV-infected individuals in the world, and the proportion that will develop AIDS.
- 2. The number of individuals with high-risk behaviours for HIV infection in the different countries.
- 3. The true efficacy of the preventive programmes designed to modify risk behaviours under different epidemiological conditions.

Prevalence of tuberculous infection, infection risk, and its trends in subjects aged 15 to 49 years

TB has been the principal plague of mankind since the first half of the twentieth century. As explained in Chapter 4, it is estimated that one third of the world population (close to 2 billion people) are infected with M. *tuberculosis*. In most developing countries, over half the adult population is infected, which represents an enormous reservoir from which new cases of TB disease may develop if these individuals suffer a deterioration in their immune defences.





The appearance of AIDS occurred during a bad epidemiological juncture in many countries, when there was still a high proportion of M. tuberculosis-infected individuals. Regrettably, in countries with a high prevalence, TB predominantly affects younger individuals between the ages of 15 and 49 years (Figure 51), which is also the age range at which persons are most vulnerable to HIV infection. Furthermore, the incidence of TB-the so-called annual rate of infection-is still extremely high and shows no signs of decreasing significantly in many poorer parts of the world. To illustrate these differences between the situation in the developed and developing world, take The Netherlands, for example, where the risk of TB infection decreased by 10% to 14% yearly in the last 40 years, as a result of which the infection prevalence is presently less than 0.5% in those aged 20 years and 15% in those aged 50 years. This suggests that it may become possible to "eradicate" TB in a few more decades. In contrast, in some African countries, successive tuberculin surveys have shown the risk of TB infection to continue to be high (about 2-3%) in the last 10 years. Similar considerations apply to Asia, the largest reservoir of M. tuberculosis-infected individuals.

Thus, while TB remains completely out of control in most poor countries, in the developed world its prevalence has been declining for over two centuries, coinciding with improved socioeconomic conditions in the community. This drop has been particularly apparent in the past 40 years, with the possibility of curing all TB cases provided effective therapeutic strategies are implemented. However, while developed nations have instituted successful TB control programmes for the early detection and cure of patients during this time, developing countries have achieved little in the struggle against the disease-in fact, a large number of infective cases continue to appear that elude detection and healing. Consequently, the different population cohorts born in the past 40 years have been exposed to very different TB infection risks—a situation that in turn has contributed to the fact that while 80% of infected persons in the developed world are older than 50 years, in developing countries 75% of infected individuals are below 50 years of age. This difference in the distribution of infected subjects is influencing the added problem posed by HIV infection, since 90% of those infected with the virus are younger than 50 years.

In short, the gains associated with several decades of ensuring good TB control throughout the world have not been used advantageously. The mistakes of the past in relation to antituberculous measures are now

exacting a high price in most countries, particularly with the appearance of the HIV epidemic.

Dynamics of HIV transmission

Three HIV transmission patterns have been described. Type I fundamentally affects homosexuals and intravenous drug users, and is mainly found in the United States and Europe. Type II involves heterosexual transmission. Type III is less well defined and corresponds to those parts of the world where the prevalence of AIDS is still low. All kinds of combinations of these patterns may be observed, and account for the important variations in HIV epidemiology seen in different parts of the world.

Type I transmission, particularly that affecting homosexuals with high-risk behaviours, is very unlikely to worsen the TB problem significantly, since it affects a population that often has a low and decreasing TB infection prevalence. From the TB perspective, transmission among intravenous drug users is more dangerous, since this is an already weakened population with a higher prevalence of M. tuberculosis infection. However, in view of its limited magnitude, transmission among this group of individuals is likewise not believed to have a significant influence on the epidemiological trend of the disease in developing countries. Moreover, drug users can be regarded as a relatively self-limiting transmission group, since those who have been previously infected will ultimately die or control the disease with antiretroviral drugs. Still, it is difficult to predict an increase in the disease due to this group because new drug users tend to avoid the parenteral route out of fear of contracting AIDS. In turn, the campaigns developed for the prevention of AIDS among homosexuals with high-risk behaviour have been shown to be much more effective than similar campaigns targeted at heterosexuals.

In contrast, type II (heterosexual) transmission is having a considerable impact on the problem of TB, especially in countries whose young adult populations are already largely infected with *M. tuberculosis*. Type II transmission is presently the dominant pattern in Africa, Asia, the Caribbean, and some regions of Latin America, where 50% to 80% of prostitutes are already infected with the virus and where perinatal transmission is leading to an alarming increase in AIDS-related infant mortality. On the other hand, it is estimated that the time to double the number of AIDS cases is shorter in the case of type II transmission.
However, the preventive measures (which are estimated to be able to reduce type I-mediated infection by 50%) would only afford a reduction of about 30% in the best of cases among those infected via type II transmission. This is in part due to the difficulty of identifying bisexual individuals, a situation that tends to be predominant in restricted societies, with a male-female infection ratio closer to 1—which also increases the risk of maternal-offspring transmission.

Unfortunately, heterosexual transmission is also becoming increasingly frequent in other countries with a high prevalence of TB infection among the sexually active adult population, to the point that the WHO indicates that heterosexual transmission of HIV is now the predominant form of transmission in the world. Theoretically, the greater the number of sexual relations with different persons, the greater the risk of HIV infection.

Risk of developing tuberculosis among patients with HIV and M. tuberculosis co-infection

By knowing the percentage of HIV-infected individuals and M. *tuberculosis*-infected subjects in the 15- to 49-year-old age group in a population, we can estimate the proportion of individuals simultaneously infected with both microorganisms in the community.

HIV infection increases by 20- to 40-fold the possibility that an *M. tuberculosis*—infected individual will develop TB disease. It is estimated that 4% to 8% of co-infected individuals will develop TB disease yearly, as a result of which— depending on how long they live—it can be expected that about half of these subjects will fall ill with TB. The average increased survival of HIV-infected patients thus constitutes a new vital factor concerning the impact that the AIDS epidemic may have on the problem posed by TB.

In some countries, an increasing number of recently diagnosed M. *tuberculosis*—infected individuals are also infected with HIV, in proportions ranging from 30% to 60% in some parts of Africa, the United States, and Latin America. On the other hand, the proportion of AIDS patients presenting with some form of TB already exceeds 50% in some regions of Africa, reaching 20% in large cities in Latin America. Even in more developed countries, despite the very low tuberculous infection rates in the age groups believed to be free of risk, the number of HIV-infected individuals presenting with TB in groups such as intravenous drug users, immigrants, and ethnic minorities remains significant.

In this way, in some regions and particularly in certain groups of people, TB has become the main infection complicating AIDS. Not surprisingly, epidemiological studies are indicating an alarming increase in TB incidence in large parts of the world where the epidemic is far from under control. In some parts of Africa, for example, the incidence of TB has more than doubled recently.

Not surprisingly, the map illustrating the world distribution of cases of HIV and *M. tuberculosis* co-infection (Figure 53) indicates that 95% of dual-infected patients are from the poorest parts of the world.

Impact of HIV on the prevalence of tuberculosis

Based on these considerations, it can be seen that the impact of HIV on the problem of TB differs greatly throughout the world. In industrialised countries, which represent only 5% of the HIV problem and of the dual HIV-TB infection cases (Figure 53), HIV transmission is largely through homosexual relations and intravenous drug use. As previously mentioned, intravenous drug users constitute a relatively small closed group whose numbers tend to decrease. The measures designed for HIV control can be as effective among homosexuals as in heterosexuals (the main transmission source in the developing world). Moreover, in developed countries, 80% of M. tuberculosis-infected individuals are over the age of 50 years, while 85% to 90% of HIV cases are under this age. Consequently, the two population groups with TB and HIV infection are unlikely to coincide, as a result of which the impact of HIV on TB has been (and can be expected to remain) limited in industrialised parts of the world. In contrast, developing countries are characterised by large numbers of individuals infected with both pathogens. Further, both M. tuberculosis and HIV are found to affect the same age groups, specifically the sectors of the population between the ages of 20 and 45 years. This and the unlimited pattern of (heterosexual) HIV transmission in these areas attest to the terrible impact that HIV has on the problem of TB. For example, in sub-Saharan Africa and some parts of the Caribbean where the prevalence of both diseases is the highest in the world, the recent substantial increase in TB cases has caused the already precarious health care services to fail, contributing to a severe lack of hospital beds, drugs, and personnel. There is no doubt that this situation will continue to worsen in the coming decades.

Pathogenesis

HIV is known to be a lymphotropic virus that infects and eventually destroys the CD4+ or helper T lymphocytes via the intervention of a glycoprotein in its envelope (GP 120) which is complementary to the CD4 receptor. The function of the infected lymphocyte is altered as a result, and the cell is no longer able to respond to soluble antigens or antigens bound to other cells, with a decrease in the release of interferon γ , interleukin 2, and other macrophage-activating lymphokines. Moreover, the infected cells begin to express GP 120 on their surface, and bind via this viral component to other uninfected lymphocytes to form pathological syncytial cellular formations. HIV also infects macrophages and other phagocytic cells without destroying them, but instead compromising their functions; as a result, chemotaxis is reduced, along with the bactericidal potential against intracellular microorganisms.

These macrophages operate as true Trojan horses, transporting the virus to more inaccessible organs such as the central nervous system. It has been calculated that each HIV-infected individual who is not undergoing antiretroviral treatment reduces his or her CD4+ helper lymphocyte population by about 70 cells/mm³ each year—which in turn leads to a progressive decrease in cell-mediated immunity that facilitates the reactivation of intracellular infections such as TB. This is how it has become established that HIV infection is presently the greatest risk factor for the progression of a patient with *M. tuberculosis* infection (recent or old) to actual TB disease.

Immune suppression caused by HIV can increase the incidence of TB via three mechanisms:

- 1. Endogenous reactivation from dormant foci derived from remote infections. This would be the most frequent mechanism that, in the absence of severe cellular immune defects, would lead to typical cavitary and bacillary TB presentations.
- 2. Progression of recent infection, with the development of more acute and atypical forms of TB, difficult diagnosis, and frequent haematogenous dissemination reminiscent of the more serious forms of primary TB in children.
- 3. Exogenous reinfections in subjects previously infected with *M. tuberculosis*, who, on losing much of their immune memory, would progress to manifest TB.

Molecular biological studies conducted in the last decade have pointed to the importance of infections, particularly of exogenous reinfections, in seriously immunocompromised AIDS patients with TB.

On the other hand, TB may exert a deleterious effect on the natural course of HIV infection. In effect, stimulation of CD4+ lymphocytes carrying dormant viruses may activate these viruses. In this sense, it has been seen that while TB responds equally well to chemotherapy in both HIV-infected and uninfected subjects, patients with AIDS who develop TB, with similar levels of immunodeficiency, have shorter survival.

Diagnosis

The diagnosis of TB in patients infected with HIV may present certain difficulties, depending on the degree of immunosuppression at the time of diagnosis (see Chapter 7). In patients with reasonably good immune defences (CD4+ lymphocyte counts >300 cells/mm³), diagnostic test performance will be similar to that in non–HIV-infected individuals. However, the situation becomes more complicated in those with advanced immune suppression.

Regarding the clinical manifestations (Chapter 7), if HIV-positive individuals have not yet developed significant immune deficiency, TB symptoms tend to be similar to those observed in the rest of cases. However, in the immunosuppressed AIDS patient, the initial manifestations tend to be non-specific, with a predominance of general symptoms (e.g., nocturnal fever, asthenia, weight loss, peripheral adenopathies), a high likelihood of tuberculin test negativity, and significant involvement of extrapulmonary TB locations. All AIDS patients should therefore undergo thorough screening to identify TB disease or infection.

The utility of radiology in HIV-infected subjects again depends on the degree of immune suppression involved (Chapter 7). If immunosuppression is not severe and the patient was previously infected with the bacterium, endogenous reactivation of these bacilli is typically observed, with production of lesions typical of post-primary TB. If immune suppression is severe, any exposure to a source of contagion, and even endogenous reactivation, will encounter practically no opposition on the part of the host defences—resulting mainly in the production of lesions typically associated with primary TB, with frequent lymphatic involvement and haematogenous dissemination. In this latter group of patients, normal chest radiograph findings are common, and extrapulmonary involvement is frequent.

As with all cases of TB, the most important concern is to reach a firm diagnosis based on microbiological techniques. As a general rule, the performance of such methods will be similar to that performed in patients without HIV infection, although it is highly advised to obtain as many good-quality samples as possible. Thus, in AIDS patients, in whom disseminated TB is much more common, all possible samples should be considered for confirming the diagnosis, including specimens from sputum, urine, cerebrospinal fluid, and biopsies of different organs. In

cases of severe immune deficiency and fever of unknown origin, three haemocultures for *M. tuberculosis* may be useful.

It should also be emphasised that in severely immunosuppressed AIDS patients, the information afforded by the histological study of biopsy specimens decreases, owing to the inability of these patients to generate granulomas in the face of *M. tuberculosis* aggression.

Treatment and chemoprophylaxis

HIV-infected patients should receive the same treatment regimen as uninfected patients. The only additional requirement is closer supervision, because of the increased risk of complications in the form of adverse reactions, intolerances, and drug interactions. Consequently, the management of such patients should be in the hands of physician specialists. Drug interactions between rifampicin and antiretrovirals, particularly protease inhibitors, should especially be noted. Presently, the use of protease inhibitors is not practical in poorer countries owing to their high cost; however, prices are expected to fall in the near future, as a result of which their use will become more widespread. The possibility of such drug interactions must therefore be considered. The most important examples are presented in Table 24.

Another problem is the greater probability of relapses, which increase as the host defences weaken and are not able to prevent the multiplication of the latent bacteria that persist when TB is healed. In this setting, some have suggested that the best course of action would be to prolong treatment, or to maintain the patient on isoniazid for life. However, these measures are not justified. It would be reasonable to administer the same treatment as in immunocompetent individuals and to maintain close supervision and follow-up, as well as an increased suspicion for relapse. Some prefer to prolong the treatment to 9 months, particularly in patients who take longer to show culture negative conversion.

In turn, it has been demonstrated that intervention with chemoprophylaxis in subjects with dual HIV and TB infection is highly effective; as a result, there should be no doubts concerning the utility of such intervention in these subjects. In these cases, the efficacy of isoniazid treatment for 9 months has been confirmed. As was explained in Chapter 13, although evidence suggests that this group can also benefit from the regimen comprising 2 months of rifampicin and pyrazinamide, recent research has suggested that increased liver toxicity may be an adverse effect. For the time being, this regimen is not indicated. Some medical societies also recommend rifampicin for 4 months, although there is no firm scientific evidence to support this approach.

Table 24. Principal drug interactions between antituberculousmedications and antiretroviral agents. Source: reference 16, Tuset,2000.

Antirotrovinol	Mathadana	Difomnioin	Difabutin	Clarithromyoin
	Can [†] Cn ^A 7T	NDAR	NDAP	NDAR
ddI	57% AUC ddI	Space 2 h	NDAR	
uui	↓57% AUC dui	Space 2 II	NDAK	
ddC	No data			NDAR
uue	No data			NDAR
d4T	123% AUC d4T			
	NDAR			
3TC	Unlikely interaction			
Abacavir	May require slight	NDAR	NDAR	
	↑MT			
Nevirapine	↓60% AUC MT	Not	NDAR	NDAR
-	Possible withdrawal	recommended		
	syndrome.			
	↑dose MT of 8 th -			
	10 th day			
Delavirdine	Could ↑Cp MT	Contraindicat	Contraindic	Monitor CLM toxicity
		ed	ated	~
Efavirenz	↓60% AUC MT	Assess dose	Rb 450-600	Contraindicated
	Possible withdrawal	increase EFV	mg/day or	
	syndrome.	800 mg/day	2-3	
	May require dose		unies/wk	
	MI of 8 -10 day			
Indinavir	In vitro: $+30\%$	Contraindicat	IDV 1000	NDAR
	AUC MT	ed	mg/8 h and	
	Probably NDAR		Rb 150	
			mg/24 h or	
			300 mg 2-3	
			times/wk	
Ritonavir	<i>In vitro</i> : x2 AUC	-35% AUC	Rb 150 mg	Adjust CLM dose if alt. renal
	MT L L LOCOL ALIC	RTV	2-3	function*
	In vivo: ↓36% AUC	NDAR (RFP	times/wk	
	MI Adore MT	2.3 times/wk		
Sequinevir HCC	uose MI In vitro: NDAP	2-3 times/wk)	Contraindia	NDAP
Saquillavii 110C	In vitto. NDAK	ed‡	ated	NDAK
		cu	aicu	
Saquinavir SGC	In vitro: NDAR	Contraindicat	150 mg/dav	NDAR
1		ed†	or 2-3	
			times/wk‡	
Ritonavir +	↓40% AUC MT	RFP 600	Rb 150 mg	
saquinavir	with RTV/SQV	mg/day or 2-3	2-3	
	400/400	times/wk	times/wk	
		Probably		
		NDAR		

Antiretroviral	Methadone	Rifampicin	Rifabutin	Clarithromycin
Nelfinavir	↓40% Cp MT	Contraindicat	NFV 1250	NDAR
	However, in most	ed	mg/12 h	
	cases NDAR		Rb 150	
			mg/24 h or	
			300 mg 2-3	
			times/wk‡	
Amprenavir	↓Cp MT	Contraindicat	Rb 150	NDAR
	Consider ↑ dose	ed	mg/24 h or	
	MT		300 mg 2-3	
-			times/wk‡	

*ClCr 30-60 ml/min: reduce 50%; <30 ml/min: reduce 75% (max. 1 g/day).

 \pm n 2 patients, the use of RTV/SQV 400/400 mg/12 h or 100/1000 mg/12 h allowed for the rapeutic levels of SQV in association with rifampicin.

[‡]In intermittent regimens, the use of rifampicin is preferred.

AUC = area under the curve; CICr = creatine clearance; CLM = clarithromycin; Cp = plasma concentration; EFV = efavirenz; HGC = hard gelatin capsules; IDV = indinavir; MT = methadone; NDAR = no dose adjustment required; NFV = nelfinavir; Rb = rifabutin; RFP = rifampicin; RTV = ritonavir; SGC = soft gelatin capsules; SQV = saquinavir.

Lastly, in patients with severe immunosuppression as a result of AIDS, BCG vaccination is contraindicated, since the vaccine considerably increases the risk of complications, some of which are serious. However, after assessing the risks and benefits, mass vaccination of newborns is advised—without having to conduct the virus detection test—in countries where the prevalence of HIV infection is high.

As a final comment, it should be emphasised that TB is the most preventable and treatable of all the infections that complicate HIV, even though it is also the most contagious. On the other hand, since TB is one of the first infections to affect HIV patients, it can be regarded as a form of sentinel infection of HIV infection.

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Chapter 18. Diseases caused by environmental mycobacteria

Chapter summary

Since it is not mandatory to declare diseases caused by environmental mycobacteria, data regarding their incidence and prevalence must be considered approximate. All of these mycobacteria can be found distributed widely throughout the environment, particularly in the water and ground, which are the principle reservoirs. The prevalence of the disease and the responsible species are found in a wide range of geographic locations, and there has been a considerable increase in the incidence over the last 15 years, most of which have been HIV related.

At present, the concept of "colonisation" is rejected, although there are many clinical manifestations that would be difficult to explain without using this term. However, the considerable limitations of sensitivity tests to antitubercular drugs are unanimously accepted (the majority of these species are resistant to these drugs *in vitro*), as is the fact that these are only helpful in specific cases and should include macrolides and quinolines.

Treatment will therefore depend on the sensitivity of the mycobacterium towards different drugs. In the case of disease caused by the *M. avium* complex, currently the most common type described, important progress has been made, especially with the incorporation of clarithromycin and rifabutin in treatment and prophylaxis. Patients with these forms of the disease must be treated in specialised centres by qualified personnel.

One important aspect is how these diseases are managed in low- and middle-income countries, where they are less common and where there is a lack of diagnostic and therapeutic resources. This chapter includes a simple explanation of how these diseases can be correctly handled even in the poorest of countries.

Fifty years ago, a series of clinical manifestations, many of which were similar to tuberculosis (TB) and that were caused by mycobacteria other than *M. tuberculosis* and *M. leprae*, began to appear. They were initially classified based on their growth characteristics *in vitro* and were considered "atypical" mycobacteria for many years, although perhaps a

more suitable name for these would be environmental mycobacteria. This group of pathogens has been also known as "non-tuberculous mycobacteria", "mycobacteria other than *M. tuberculosis*", "opportunist mycobacteria", "unclassified mycobacteria", or "anonymous mycobacteria". For many years, there was only an occasional, almost anecdotal, description of the diseases caused by these mycobacteria, collectively known as "mycobacteriosis", the majority of which involved immunodeficiency. Nevertheless, over the last 15 years, this has become quite a common pathology, especially with the start of the HIV epidemic, which has led to an increase in the amount of research on these microorganisms, which in turn has led to the standardisation of diagnostic and therapeutic criteria.

Epidemiology

It is not mandatory to declare diseases caused by environmental mycobacteria; therefore, data regarding their incidence and prevalence must be considered approximate. In many cases, these data should be considered in the context of the local laboratories reporting the data, which may involve inconsistent reporting and limited resources for mycobacterial identification. In any event, it has always been accepted that there is a wide geographic variability in the prevalence of the disease and in the species involved. Therefore, the frequency with which each species is isolated varies from one part of the world to another, and even in the same area, over any one period of time.

The majority of papers published on this group of pathogens cite that the risk factors most commonly associated with these diseases are smoking and an underlying lung pathology, such as chronic obstructive lung disease, silicosis, residual TB, or bronchitis.

There has been a significant increase in the incidence of mycobacteriosis in the majority of developed countries over the last few years, even in children, and this has been related to the following factors:

- An increase in the prevalence of chronic obstructive lung disease.

- Improvements in diagnostic techniques.

- The nature of the microorganisms.

- An increase in the clinical awareness of the disease.

- The description in immunocompromised patients (neoplasias, transplant receivers, and steroid users).

- The HIV epidemic. At present, it is unanimously accepted that HIV has led to a marked increase in the incidence of disease caused

by environmental mycobacteria, both in number and in species involved.

The disease caused by the *M. avium* complex is the most common form of mycobacteriosis in AIDS patients, and the risk is closely related to the degree of immunosuppression. It is curious to note, however, that there are hardly any cases of the disseminated type caused by the *M. avium* complex in Africa and in the majority of low-income countries where TB is highly prevalent. This phenomenon is difficult to explain, although it has been speculated that the possible immunity to these environmental mycobacteria could be due to the widespread prevalence of the infection and disease caused by *M. tuberculosis*, as well as to wide-scale BCG vaccination. This last hypothesis is supported by data suggesting that infection by *M. tuberculosis* may protect against infection disseminated by the *M. avium* complex.

Table 25 shows the various species of *Mycobacterium*. These are classified according to their speed of growth, principal reservoir, and capacity to cause disease in humans and animals.

Epidemiological chain of transmission

Environmental mycobacteria are widely distributed throughout the environment, fundamentally in water and the ground. In the majority of cases, their reservoir is water. In the case of the *M. avium* complex, it is water taps; in fact, there have even been cases of nosocomial epidemics of the disseminated disease (in AIDS patients) transmitted through hospital taps. *M. kansasii* has been repeatedly isolated in water systems and taps, and *M. xenopi*, which needs temperatures over 28°C to grow, is isolated almost exclusively in hot water or hot water systems, which may lead to intra-hospital cases. *M. marinum* also has a reservoir and is transmitted via salt water, fresh fish, reservoir water, and swimming pools, while fast-growing species such as *M. fortuitum*, *M. chelonae*, and *M. abscessus* can be isolated from the ground and from water, although the most common cause of disease involves nosocomial transmission.

Table 25. Classification of the species of the *Mycobacterium* genus, according to reservoir, speed of growth, and pathogenic capacity. Source: reference 15, Ruiz Manzano et al, 1998.

Slow-growing mycobacteria	Fast-growing mycobacteria		
Species whose reservoir is infected	Species with an environmental reservoir		
mammals			
Pathogens for man:	Associated with human disease:		
M. africanum	M. abscessus		
M. bovis	M. chelonae		
M. leprae	M. fortuitum		
M. tuberculosis	M. mucogenicum		
	M. novocastrense		
Pathogens for other animals:	M. peregrinum		
M. lepraemurium			
M. microti	Associated with animal disease:		
M. paratuberculosis	M. porcinum		
Species with an environmental	Never or rarely associated with human		
reservoir	disease:		
Associated with human disease:	M. agri		
M. asiaticum	M. aichiense		
M. avium	M. alvei		
M. bryeri	M. aurum		
M. celatum	M. austroafricanum		
M. conspicuum	M. brumae		
M. genavense	M. chitae		
M. haemophilum	M. chlorophenolicum		
M. interjectum	M. chubuense		
M. intermedium	M. confluentis		
M. intracellulare	M. diemhoferi		
M. kansasii	M. duvalii		
M. malmoense	M. fallax		
M. marinum	M. flavescens		
M. scrofulaceum	M. gadium		
M. shimodei	M. gilvum		
M. simiae	M. hassiacum		
M. szulgai	M. holderi		
M. triplex	M. komossense		
M. ulcerans	M. madagascariense		
M. xenopi	M. mageritense		
	M. moriokaense		
Associated with animal disease:	M. neoaurum		
M. farcinogenes	M. obuense		
	M. parafortuitum		
Never or rarely associated with	M. phlei		

Slow-growing mycobacteria	Fast-growing mycobacteria
human disease:	
M. cooki	M. proriferae
M. gastri	M. pulveris
M. gordonae	M. rhodesiae
M. hiberniae	M. senegalense
M. lentiflavum	M. smegmatis
M. nonchromogenicum	M. sphagni
M. terrae	M. thermoresistible
M. triviale	M. tokaiense

Although some data regarding the pathogenesis of the infection and disease caused by this group of mycobacteria still need to be clarified, several studies suggest that person-to-person transmission is rare, and that the majority of cases are caused by microorganisms distributed in the environment. The most accepted transmission mechanism is the aerosolisation of microorganisms in respiratory tract infections, which are then introduced into the digestive system in the case of lymphadenitis in children and disseminated forms in AIDS patients (colonisation of the digestive tract). Direct inoculation of microorganisms from water and other materials has been observed in patients with soft tissue infections.

It is still not known if there is a latent period after infection, but the clinical forms of presentation most commonly described have always been pulmonary involvement, lymphadenitis, skin and soft tissue abscesses, and osteomyelitis. The mycobacteria most commonly involved in these clinical manifestations are *M. avium*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. fortuitum*, *M. chelonae*, and *M. scrofulaceum*.

The pathogenesis of the infection caused by the *M. avium* complex is still not clearly understood. While in *M. tuberculosis* the disease can be caused through endogenous reactivation or through primary progression after an exogenous infection, depending largely on the degree of immunodeficiency, in the case of *M. avium* the disseminated disease is thought to generally occur through progression of the primary infection. Some results indicate that the *M. avium* complex is acquired after ubiquitous environmental exposure, which is very difficult to prevent.

Clinical manifestations: diagnostic criteria

Traditionally, these environmental mycobacteria have been classified according to their growth characteristics and the pigments they produce. A

more recent classification is based on the organs affected and the diseases caused (Table 26).

Table 26. Classification of environmental mycobacteria isolated in humans, according to organ affected and pathology. Source: reference 13, Medina, 1999.

Disease	Common species	Geography	Morphology	Unusual
Pulmonary	M. avium	Worldwide	Slow growth,	M. simiae
	complex		non-pigmented	M. szulgai
				M. fortuitum
	M. kansasii	USA, Europe	Pigmented	M. celatum
				M. asiaticum
	M. abscessus	Worldwide, but	Fast growth, non-	M. shimodei
		mostly in USA	pigmented	M. haemophilum
				M. smegmatis
	M. xenopi	Europe, Canada	Slow growth,	
			pigmented	
	M. malmoense	North Europe,	Slow growth,	
		England	non-pigmented	
Lymphadenitis	M. avium	Worldwide	Generally non-	M. fortuitum
	complex		pigmented	M. chelonae
				M. abscessus
	M. scrofulaceum	Worldwide	Pigmented	M. kansasii
				M. haemophilum
	M. malmoense	North Europe,	Slow growth	
		England		
Cutaneous	M. marinum	Worldwide	Photochromogen,	M. avium
			low temperatures	complex
				M. kansasii
	M. fortuitum	Worldwide, USA	Fast growth, non-	M . nonchromo
			pigmented	genium
				M. smegmatis
				M. haemophilum
	M. cheionae			
	M. abscessus	A	C1	
	M. ulcerans	Australia, tropics,	Slow growth,	
Discominated	M mium complex	Alfica, Asia Worldwide	HIV oultures	M abaaaaa
Disseminated	M. avium complex	worldwide	HIV cultures,	M. abscessus M. nononi
			pignienieu,	M. xenopi M. malmoonse
	M kansasii	USA	Non nigmented	M. maimoense M. ganavanse
	M. chelonge	USA	Non pigmented	M. genuvense M. simiae
	M haemonhilum	USA Australia	Requires hemira	M conspicuum
	м. пасторнийт	USA, Australia	low	M marinum
			temperatures	M fortuitum
			and CO ₂	

,

Lung involvement

Chronic pulmonary involvement with a variable, non-specific presentation is the most frequently identified clinical manifestation. The most commonly implicated source is *M. avium*, followed by *M. kansasii*, although in patients not suffering from AIDS *M. kansasii* or other species may be involved, depending on geographic variability. There is often an underlying pulmonary pathology (chronic obstructive lung disease, pneumoconiosis, active or residual TB, cystic fibrosis, smoking, or bronchitis), which makes symptoms difficult to interpret.

Radiological findings are also non-specific (Figures 33 to 36), although thinner wall cavities are more often found than in TB, as well as a more tenuous surrounding pulmonary infiltrate. The pleura are usually preserved at the bases, and effusion is rare. For this reason, it is often difficult to differentiate between active TB and disease caused by other mycobacteria in patients not infected with HIV. Occasionally, when a clinical-radiographical manifestation suggests TB and the smear microscopy is positive, TB may be diagnosed in the patient and antituberculous treatment started; however, this will be followed by great surprise when the patient's responds poorly to therapy and when the report on the culture shows growth of an environmental bacteria, meaning that treatment will have to be modified.

Over the last few years, it has been noted that high-resolution computed tomographic scan of the thorax may help to diagnose disease caused by M. avium, sometimes revealing the presence of bronchitis and nodules, a syndrome that is more commonly found in older women who do not smoke.

With regards to intradermoreaction tests, there is a wide variety of cross-reactions among the antigens of different species, although at present there are promising studies being carried out to obtain reactants with a higher specificity for the *M. avium* complex. This information will depend on the geographic variability of the different mycobacteria. They are known as the sensitins of the different species.

That these microorganisms can be found everywhere in nature suggests the possibility of contamination or transitory infections, particularly in patients with underlying pulmonary pathologies and in those whose sputum samples contain very few microorganisms. This type of case has been called colonisation and is characterised by minimum pulmonary infiltrators, stability, with no cavities and with sporadic isolation of the mycobacteria in the sputum. More recent studies have tried to show that colonisation is, in its strictest sense, quite rare. Consequently, the American Thoracic Society has considered it in its latest recommendations on the criteria for the diagnosis of disease caused by these mycobacteria. However, rejection of the concept of colonisation is sometimes difficult to accept, especially in the immunocompetent patient. It is also difficult to accept the disease caused by environmental mycobacteria (meaning prolonged treatment) in healthy individuals or in individuals with previous bronchial or pulmonary complaints, who, although showing no symptoms or signs, have repeatedly positive sputum cultures for some of these species, especially when these species are non-pathogenic, such as *M. gordonae*. The topic of colonisation will undoubtedly be brought up for further discussion in the future, particularly with regards to immunocompetent patients.

To diagnose lung disease caused by environmental mycobacteria, it is advisable to follow the criteria defined by the American Thoracic Society, which is based on the following clinical, radiological, and bacteriological data:

1. Clinical data:

- i) Compatible symptoms and signs (cough, temperature, weight loss, haemoptysis, dyspnoea) with a deterioration of the clinical state.
- ii) Exclusion of other diseases or treatments of other pathologies that may produce clinical deterioration.
- 2. Radiological data:

or

- i) In simple chest radiology:
 - Infiltrators with or without nodules
 - Cavitation
 - Single or multiple nodules
- ii) In high-resolution computed tomography of the chest:
 - Several small nodules

– Multi-focal bronchiectasis with or without small pulmonary nodules

- 3. Bacteriological data. Provided that one or more of the following apply:
 - i) Provided that at least three samples of sputum or bronchoalveolar lavage can be obtained in 1 year:
 - Three positive cultures and a negative smear microscopy,
 - Two positive cultures and one positive smear microscopy.ii) If unable to obtain sputum, one bronchoalveolar lavage:

- With a positive culture (2+, 3+, 4+), or

Positive culture with a positive smear microscopy (2+, 3+, 4+).

iii) Biopsy:

– Any growth in the sample cultures obtained from bronchopulmonary biopsy specimens.

- Granulomas and/or observation of acid-alcohol-resistant bacilli in a pulmonary biopsy specimen with one or more positive cultures of sputum or bronchoalveolar lavage.

Any growth obtained from sterile extrapulmonary samples.

The same criteria apply for immunosuppressed patients, although in this case a positive culture showing growth of 1+ or more is considered diagnostic.

Peripheral lymphadenitis

Another clinical manifestation frequently caused by these mycobacteria is peripheral lymphadenitis, which is most frequently found in children between the ages of 1 and 5 years. It especially affects the adenopathies of the head and neck, although it can affect any other area. As discussed in Chapter 17 (extrapulmonary TB), the most important differential diagnosis is with lymphadenitis caused by *M. tuberculosis*. The *M. avium* complex is isolated in 70% to 80% of lymphadenitis cases caused by environmental mycobacteria. In Australia and the United States, the second most common species is *M. scrofulaceum*, while in Northern Europe, it is *M. malmoense*. In children under 5 years, M. tuberculosis is isolated in only 10% of cases of peripheral lymphadenitis caused by mycobacteria, with M. avium and M. scrofulaceum isolated in the remaining 90%. Conversely, M. tuberculosis is isolated in 90% of adults with this clinical manifestation. Knowledge of these epidemiological differences between adults and children is very important, since the majority of environmental mycobacteria that cause lymphadenitis in children are very resistant to antituberculous drugs. In addition, since it is a localised disease, surgical excision is required. In contrast, when *M. tuberculosis* is isolated, this type of surgery is not recommended in both adults and children, and medical treatment is preferred. These data emphasise the importance of culturing the samples obtained by biopsy or thin needle aspiration (the only way to obtain a definite diagnosis), rather than just sending the specimens to the anatomic-pathological laboratory.

Infections of the skin, soft tissue, and bones

The species that most frequently cause infections of the skin, soft tissue, and bones are *M. fortuitum*, *M. abscessus*, *M. marinum*, and *M. ulcerans*. These normally occur after trauma injuries, although nosocomial infections in intravenous or intraperitoneal catheters, mammoplasty, or heart bypass surgery have also been described. *M. marinum* causes swimming pool granuloma, which is characterised by a single papule-shaped lesion on an extremity (knee, shoulder, the back of hand, or sole of the foot).

Disseminated disease

There are two possible presentations of disseminated disease. The first affects patients who are not suffering from AIDS but who are immunosuppressed (e.g., those with neoplasias, who have received a transplant, or who are undergoing prolonged steroid treatment), and the most frequently isolated organisms are the *M. avium* complex and *M.* kansasii. M. avium complex causes fever of unknown origin, while M. kansasii generally causes subcutaneous nodules and abscesses that drain spontaneously. Mortality is directly related to the type and severity of the underlying disease. The second presentation affects severely immunosuppressed AIDS patients (CD4 count <50 cells/mm³), and the most commonly isolated organism is again the *M. avium* complex, which also causes a disseminated infection accompanied by a high temperature, night sweats, weight loss, abdominal pain, and diarrhoea. M. kansasii can also be a common cause of disease. The diagnosis can frequently be made with a haemoculture of a blood sample (sensitivity of approximately 90%), making it necessary to culture the samples.

Culture methods, identification, and sensitivity tests

Culture methods

At least three sputum samples are necessary, and these must be incubated in one or more solid media and one liquid medium. In the case of blood samples, a simple medium using Bactec 13 A broth or lysis centrifugation with 7H10 or 7H11 can be used. There are two solid media available: an egg-potato base medium (Löwenstein-Jensen agar) and a free agar base medium (Middlebrook 7H10 or 7H11 agar). Quantification of growth (normally from 0 to 4+) is important in order to assess clinical significance and response to treatment. The Middlebrook 7H10 or 7H11 medium is the solid medium of choice due to the easy recovery and quantification of the *M. avium* complex. The Löwenstein-Jensen medium, although excellent for recovering *M. tuberculosis*, is generally inferior to the Middlebrook agar in the case of the *M. avium* complex. In low- and middle-income countries, only solid media are indicated, preferably the Löwenstein-Jensen agar.

The greatest difference in culture techniques for environmental mycobacteria is the need to incubate skin or soft tissue samples at two different temperatures: 35° C and $28-32^{\circ}$ C. This is because a considerable amount of common pathogens found in these tissues, including *M. haemophilum*, *M. ulcerans*, *M. marinum*, and *M. chelonae*, only grow at low temperatures.

Identification

Traditional identification of environmental mycobacteria was based on a series of characteristics and a range of biochemical reactions. The niacin test was the most useful for differentiating between these mycobacteria (niacin-negative) and *M. tuberculosis* (niacin-positive). Runyon described the first classification of nontuberculous mycobacteria based on their growth characteristics and pigments produced. Traditional biochemical reactions are extremely slow, so most laboratories in industrialised countries use other methods to identify the different species, such as high-performance liquid chromatography (HPLC) (fingerprint patterns), the Bactec-NAP test (selective inhibitor of *M. tuberculosis* growth), and DNA tests.

The above-mentioned identification methods are not recommended for use in low- and middle-income countries because they are very expensive and because the diseases caused by these mycobacteria occur very infrequently. In these countries, the classification of mycobacteria according to their speed of growth in Löwenstein-Jensen medium (slowand fast-growing) and their capacity to produce pigments (scotochromogen, photochromogen, and non-chromogen) can still be used. Based on this simple and rudimentary classification, it is possible to obtain an accurate description of the species and, more importantly, a guide to the type of treatment needed.

Susceptibility tests

As far as antimicrobial sensitivity tests are concerned, there are some recommendations on when, how, and which species these should be carried out. In the case of the *M. avium* complex, this is a matter of some controversy, as *M. avium* complex strains are almost always resistant to low doses of isoniazid, rifampicin, streptomycin, and ethambutol, which are normally used in *M. tuberculosis* sensitivity tests. For this reason, the sensitivity tests for normal antituberculous agents are not recommended for the *M. avium* complex. Neither should they be carried out during initial treatments with clarithromycin or rifabutin. Their use is only indicated in samples from patients who have received previous treatment or prophylaxis containing a macrolide.

M. kansasii is initially sensitive to rifampicin, but acquired resistance has been described. Consequently, *in vitro* sensitivity tests should be carried out when a patient relapses or when treatment fails. A more controversial issue surrounds the question of whether these sensitivity tests should be carried out at the beginning of treatment, due to the different behaviour of the drugs *in vitro* (frequent resistance) and *in vivo* (effective if combined with others). For this reason, at least in the case of immunocompetent patients, these sensitivity tests may serve to confound rather than help. They may be useful at the beginning of treatment for AIDS patients, provided the results are interpreted correctly. In addition, the sensitivity of cultures resistant to rifampicin must be determined with the new macrolides, quinolines, and aminoglycosides.

For other slow-growing mycobacteria, the sensitivity tests should include macrolides (clarithromycin, azithromycin), quinolines (ciprofloxacin, ofloxacin, levofloxacin), rifampicin, ethambutol, and isoniazid—some of these drugs could be useful in specific cases if the results are correctly interpreted.

Sensitivity tests are also very useful in diseases caused by fastgrowing mycobacteria, and can form the basis for treatment. These sensitivity tests should be carried out with antibiotics such as clarithromycin, azithromycin, cefoxitin, and doxycycline, and not antitubercular drugs.

Treatment of diseases caused by environmental mycobacteria

The choice of treatment will depend on three fundamental factors: 1) the type of clinical presentation; 2) the species of mycobacteria responsible; and 3) the patient's immune state (Table 27). *In vitro* resistance to the majority of front-line antituberculous drugs is one of the most surprising characteristics of these mycobacteria, which, until recently, justified the need for aggressive treatments using up to five or six drugs for long periods of time, as well as the need for surgical excision in cases of localised lung disease. Despite current progress in this area, current treatment of diseases caused by environmental mycobacteria is quite complex, and should only be carried out in specialised centres by expert staff.

Table 27. Treatment of diseases caused by the principal environmental mycobacteria

Type of environmental mycobacteria	Clinical form	First-choice treatment	Alternative treatment	AIDS
<i>M. avium</i> complex	Disseminated	Clarithromycin; azithromycin + rifabutin; rifampicin + ethambutol	Quinolines Clofazimine Amikacin Streptomycin	Rifabutin or clarithromycin
	Pulmonary	Clarithromycin; azithromycin + rifabutin; rifampicin + ethambutol	Isoniazid Streptomycin Ethionamide Quinolines Clofazimine Amikacin	
M. kansasii	Pulmonary	Isoniazid + rifabutin; rifampicin + ethambutol	Clarithromycin Streptomycin Sulphamethoxazole	Rifabutin or clarithromycin
	Disseminated	Isoniazid + rifabutin; rifampicin + ethambutol	Clarithromycin Streptomycin	
	Lymphadenitis	Surgical	Sulphamethoxazole	
M. fortuitum	Cutaneous	Surgical	Imipenem	
M. chelonae M. abscessus	infections Surgical wounds	Amikacin + cefoxitin	Tobramycin	
M. marinum	Cutaneous	Clarithromycin + aminocycline; doxycycline + trimethoprim- sulphamethoxazole; rifampicin + ethambutol; surgical		

Treatment of disease caused by the M. avium complex

Not all mycobacteria have the same range of sensitivity; the *M. avium* complex stands out as having the highest degree of resistance. The first studies on the treatment of the *M. avium* complex in HIV-infected patients showed that treatments involving four drugs (ethambutol, rifampicin, ciprofloxacin, and clofazimine) were effective in reducing bacteraemia and improving symptoms. However, the most important progress in therapeutics was made in the early 1990s when new macrolides were introduced. These drugs were quickly used for the treatment of this infection, owing to their excellent *in vitro* action and because they reached high intracellular concentrations, which is important given that most

mycobacteria reside inside the phagolysosome of the macrophages. Rifabutin, a semi-synthetic derivate of rifampicin, has also become a basic component in the treatment of the disease caused by the *M. avium* complex and has proved to be more active *in vitro* than rifampicin.

These data suggest that macrolides are currently a fundamental part of the treatment used for disease caused by the *M. avium* complex as well as by the majority of other slow-growing environmental mycobacteria. Treatment is based on the combination of a minimum of three drugs: 1) clarithromycin (500 mg, twice daily) or azithromycin (250 mg/day or 500 mg three times/week); 2) rifabutin (300 mg/day) or rifampicin (600 mg/day); and 3) ethambutol (25 mg/kg day for 2 months, followed by 15 mg/kg per day). Patients older than 70 years and those with a low body mass tolerate clarithromycin well at doses of 250 mg/12 hours, or azithromycin at doses of 250 mg/three times per week. Other drugs that could potentially be used are quinolines, clofazimine, amikacin, and streptomycin. These drugs, however, are frequently associated with adverse effects that require changes in the treatment. Furthermore, there are drug interactions between rifampicin and protease inhibitors in HIVinfected patients. Rifampicin and, to a lesser extent, rifabutin increases the hepatic metabolism of these drugs, thus resulting in subtherapeutic levels, treatment failure, and acquired resistance to these protease inhibitors.

Patients with a CD4 count of less than 50 cells/mm³ are considered to have an increased risk of disseminated infection caused by the *M. avium* complex. These patients may therefore benefit from primary prophylaxis, which can be carried out using three drugs: 1) rifabutin, which induces the metabolism of antiproteases and requires that infection by *M. tuberculosis* (masked monotherapy) be ruled out; 2) clarithromycin, which is well tolerated and more effective; and 3) azithromycin, which is more effective than rifabutin. Macrolides are associated with one inconvenience—the appearance of resistant strains in 11% to 58% of all cases in which prophylaxis fails, a phenomenon that has not been observed with rifabutin. The primary role of macrolides in the treatment of disseminated infection, as well as cross-resistance between the different drugs in this group, greatly limit prophylaxis. No specific guidelines have been defined, and treatment should be determined on a case-by-case basis.

In immunocompetent patients it is possible to observe various clinical forms of presentation (e.g., pulmonary, lymphatic), and when the lungs are affected there may be different patterns with different clinical evolutions. The most common type has been the fibrocavitary form of the disease, which affects both upper lobes and can be found predominantly in older men (frequently alcoholics) with underlying lung disease. These cases evolve progressively over 1 to 2 years. Another type of presentation is the bilateral or interstitial nodular form, which is found mainly in older women with no previous lung pathology. This form predominantly affects the medium lobe and lingula, and has a characteristically slow clinical and radiological evolution of 5 to 10 years. It is not known at what stage these patients should be treated. The heterogeneity of the clinical forms of lung disease caused by the *M. avium* complex; the stability of the disease; patient age; and the sequelae, tolerance, and long duration of the antibiotic treatment all make it necessary to carry out a thorough evaluation before deciding on when to begin treatment. Treatment is based on the same three-drug combination described before: 1) clarithromycin or azithromycin; 2) rifabutin or rifampicin; and 3) ethambutol. Patients with an extensive form of the disease are treated at intervals with streptomycin during the first 2 to 3 months, and the dose will depend on the age and weight of the patient. No maximum treatment time has been established, but it is generally accepted to be 12 months after the cultures have proved negative. There should be some clinical improvement after 3 to 6 months of treatment. If this is not the case, resistance or intolerance should be suspected. If the treatment is not successful (due to intolerance or resistance), treatment using a combination of four drugs can be tried: isoniazid (300 mg/day), rifampicin (600 mg/day), ethambutol (25 mg/kg/day for the first 2 months and then 15 mg/kg/day), and streptomycin during the first 3 to 6 months of treatment.

Surgery may be attempted in cases of localised pulmonary involvement by the *M. avium* complex in patients who respond poorly to treatment or who have become treatment resistant. However, the bilateral nature of the disease, the existence of pre-disposing diseases, advanced age, and high morbidity and mortality do not render this treatment very recommendable. Surgical removal in children with cervicofacial or mediastinal lymphadenitis caused by these mycobacteria can be recommended, with or without the need for additional treatment. Patients with a high surgical risk can undergo treatment similar to that used for the localised pulmonary form of the disease.

Treatment of disease caused by M. kansasii

M. kansasii is the second most common cause of lung disease caused by environmental mycobacteria, as well as its most common clinical manifestation. Although it is a less virulent pathogen than *M. tuberculosis*,

its clinical and radiological manifestation is very similar to TB (Figure 34). An increase in the number of *M. kansasii* strains resistant to rifampicin has been reported, due in part to the HIV epidemic as well as to inadequate treatment guidelines that use only one or two effective drugs.

Before the introduction of rifampicin, disease caused by M. kansasii was treated with isoniazid and ethambutol for 18 months, with the frequent recommendation of surgical treatment to better prevent relapses. Surgery was never proven to provide any benefits. At present, the American Thoracic Society and the British Thoracic Society are in disagreement over treatment guidelines. While the American Thoracic Society recommends the combination of isoniazid (300 mg), rifampicin (600 mg), and ethambutol (25 mg/day in the first 2 months, followed by 15 mg/day) daily for 18 months, including at least 12 months of negative cultures; the British Thoracic Society recommends a treatment of 9 months with rifampicin and ethambutol for immunocompetent patients, prolonging treatment to 15 to 24 months in patients with low host defences. The British Thoracic Society does not recommend the use of isoniazid because of (1) limitations in sensitivity tests and (2) no significant differences in the number of relapses among patients undergoing the British recommended treatment and those undergoing treatment that includes isoniazid. Despite these official recommendations, various studies have demonstrated the effectiveness of short-term treatments.

Clarithromycin shows excellent *in vitro* activity against *M. kansasii* and should be considered in cases of intolerance to any of the previously mentioned drugs. Pyrazinamide should never be used because of proven resistance to this drug.

The majority of patients with an *M. kansasii* infection that is resistant to rifampicin and isoniazid, including HIV patients, respond to a four-drug treatment comprising high doses of isoniazid (900 mg), ethambutol (25 mg/kg), sulphamethoxazole (1 g, three times daily), and an aminoglycoside other than streptomycin or amikacin, until 12 months of negative cultures have been obtained. It should also be evaluated whether or not clarithromycin should be included.

The treatment of extrapulmonary disease in adults is the same, although in the case of lymphadenitis in children surgical excision is the treatment of choice. Managing this disease in patients co-infected with HIV is complicated by the fact that they are also being treated with protease inhibitors, which suggests that it is advisable to replace rifampicin with rifabutin or clarithromycin.

Treatment of disease caused by other diseases produced by other slowgrowing environmental mycobacteria

The therapeutic focus of diseases caused by the remaining slow-growing environmental mycobacteria varies greatly, depending on the species that produces the clinical manifestation and antimicrobial sensitivity. There are many uncertainties about when and how treatment should be started, and how long it should be continued. What is certain is that expert personnel must carry out treatment in specialised centres. Most treatment for disease caused by these other environmental mycobacteria is based on that recommended for the *M. avium* complex, with certain modifications for each organism.

Treatment of disease caused by *M. marinum* may range from a simple search for small lesions, to the use of antituberculous and other antibiotic agents, or even surgical excision. Suitable treatment would include clarithromycin, aminocycline, or doxycycline; trimethoprim-sulphamethoxazole or rifampicin; and ethambutol for 3 months.

M. malmoense is characterised by a slow growth and nonpigmented colonies, with the majority of cultures sensitive to ethambutol, rifampicin, and streptomycin. A treatment regimen of four drugs is recommended.

M. simiae is the only niacin-positive environmental mycobacteria. The majority of the cultures of this type are resistant to the front-line antituberculous drugs; hence, treatment with clarithromycin, ethambutol, rifabutin, and streptomycin is recommended, and can be modified in relation to the results of sensitivity tests.

M. szulgai is generally sensitive to rifampicin and to high concentrations of isoniazid, streptomycin, and ethambutol, which is the recommended treatment.

The recommended treatment for *M. xenopi* should include ethionamide, streptomycin, and ethambutol or rifampicin.

Treatment of disease caused by fast-growing environmental mycobacteria

Most cases associated with infection by fast-growing environmental mycobacteria are sporadic and community acquired, although nosocomial epidemics and infections associated with mammoplasty and cardiac surgery wounds have been described. The majority of these infections (>90%) are caused by three species: *M. fortuitum, M. abscessus,* and *M. chelonae.* These species are resistant to all antituberculous drugs, although they are sensitive to a series of standard antibiotics. There are no clinical tests for comparing the different therapeutic treatments, but due to the variability in sensitivity among the different species and subgroups, it is necessary to carry out *in vitro* sensitivity tests (including the most of the standard antibiotics) so that appropriate treatment can be chosen. This will vary according to the form of presentation.

Cutaneous infection is usually due to trauma or surgical infection, and many cases may cure spontaneously or after surgical debridement. In cases of severe infections caused by *M. fortuitum* or *M. abscessus*, intravenous treatment with amikacin is recommended (10-15 mg/kg, given in two doses) and cefoxitin for a minimum of 2 weeks. Imipenem is a reasonable alternative to cefoxitin if the cultures of *M. smegmatis* and *M. chelonae* are resistant to this drug. For *M. chelonae*, tobramycin is more effective *in vitro* than amikacin, which in turn should be used in combination with cefoxitin or imipenem. In patients with serious infections, 4 months of treatment is recommended, whereas in the case of bone infections, treatment duration increases to 6 months. Surgery is recommended when there is widespread infection, formation of abscesses, or treatment is difficult.

In the case of pulmonary infections caused by fast-growing mycobacteria, M. *abscessus* is the responsible agent in 85% of isolations, although in patients with gastroesophageal involvement M. *abscessus* and M. *fortuitum* are isolated with the same frequency. Sensitivity tests are also essential when treating infections caused by M. *abscessus* and M. *fortuitum*, with 6 to 12 months of treatment being adequate. The natural history of the disease depends on the underlying disease. In the case of M. *abscessus*, most patients without an underlying disease have a painless, slowly progressive course, whereas in patients with gastroesophageal complaints, disease may progress rapidly and be devastating, causing death in 20% of cases.

Management of diseases caused by environmental mycobacteria in low- and middle-income countries

How this group of diseases is managed in developed countries is completely different with respect to low- and middle-income countries. In richer countries, although these diseases are more common (competing with *M. tuberculosis*), all the diagnostic (liquid culture media and a range of sensitivity tests) and therapeutic resources are available. Furthermore, procedures very similar to those described in this chapter can be used. In countries with fewer resources, however, these diseases are much less common, so dedication of limited resources to sophisticated diagnostic methodology cannot be justified and optimisation of current treatments is therefore necessary.

In low- and middle-income countries, cultures should only be done in solid Löwenstein-Jensen medium. Some simple biochemical tests, if available, can also be performed. Sensitivity tests will not be necessary if resources are not available. The important decision is whether the patient should be treated or not, and with which combination of drugs. The decision to treat (colonisation vs. disease) will depend on the physician caring for the patient. However, it is impossible to adapt the methods described in this chapter for use in poorer countries. Still, it could be argued that for adequate handling of this group of diseases, it is possible to make a rudimentary classification that will serve as a relatively accurate guide for treatment by using culture with the Löwenstein-Jensen medium and its exposure to light and darkness. In this way, the mycobacteria can be classified in four main groups:

- 1. The *M. tuberculosis* complex. Easily identifiable using simple biochemical tests (niacin). Patients will receive a standard treatment such as those outlined in Chapter 9.
- 2. Fast-growing mycobacteria. All of these grow in the Löwenstein-Jensen medium in less than 7 days, which distinguishes them from the other mycobacteria. All patients can be treated in the same way, with a minimum margin of error, using amikacin (10-15 mg/kg, given in two doses) and intravenous cefoxitin, for a minimum of 2 weeks. In patients with serious infection, a minimum of 4 months of treatment is recommended; in the case of bone infection, the length of treatment increases to 6 months. When treatment is prolonged, the addition of

vibramycin, clarithromycin (or azithromycin), and a second-generation oral cephalosporin should be considered.

- 3. Slow-growing mycobacteria (more than 1 week) that change colour when exposed to light (photochromogens). Most cases will be caused by *M. kansasii*; thus, a treatment including isoniazid + rifampicin + ethambutol for 12 months will cure most patients.
- 4. Slow-growing mycobacteria (more than 1 week) that do not change colour when exposed to light (non-photochromogens). This group will include all non-chromogens (do not change colour) and the scotochromogens (change colour in the dark). The most important species in this group is the *M. avium* complex, although the rest respond very well to the same treatment. A treatment of ethambutol + rifampicin (rifabutin is very complicated to use and costly to obtain) + clarithromycin for 18 months for this entire group offers a high probability of success.

Recommended reading

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