Chapter 6
Mycobacterial (Skin) Infections

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6.1 Introduction

Mycobacterium is a genus of the Actinobacteria, belonging to family Mycobacteriaceae. The genus includes pathogens known to cause serious diseases, including tuberculosis (*Mycobacterium tuberculosis*), leprosy (*Mycobacterium leprae*), and Buruli ulcer (*Mycobacterium ulcerans*).

Mycobacteria can be divided into those which are strict pathogens for humans and animals and those which are potentially pathogenic. The first group includes *M. tuberculosis* and *M. leprae*, and the second group comprises the nontuberculous mycobacteria of which *M. marinum* is the most common cause of skin disease (aquarium granuloma) and of which *M. ulcerans* may be considered a specific subgroup [1, 2].

The Greek prefix "myco" means fungus, since mycobacteria have been observed to grow in a mold-like fashion on the surface of liquids when cultured [3]. They are

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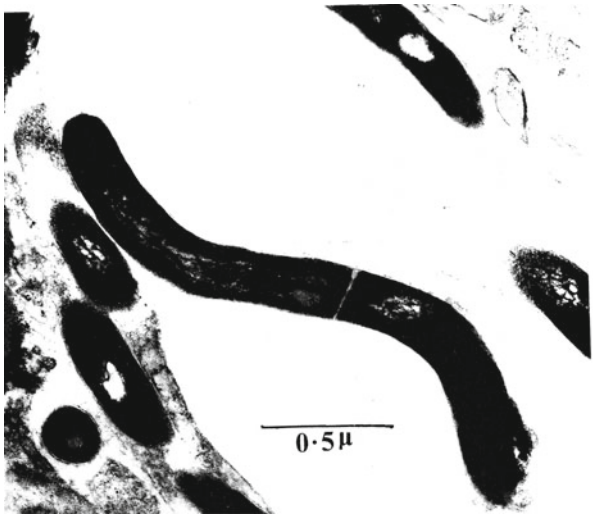

Fig. 6.1 Electron microscopic picture of mycobacteria in macrophage. One dividing bacterium. (Courtesy Dr. John Stanley)

thin, slightly curved to straight nonmotile bacilli, except for *Mycobacterium marinum*, which has been shown to be motile within macrophages. They are between 0.2 and 0.6 μm wide and 1.0 and 10 μm long (Fig. 6.1).

Mycobacteria are aerobic and are characteristically acid-alcohol-fast. Mycobacteria do not contain endospores or capsules and are usually considered Gram neutral. They stain very weakly Gram-positive or not at all (cells referred to as "ghosts") [4]. All *Mycobacterium* species share a characteristic cell wall, thicker than in most other bacteria, which is hydrophobic, waxy, and rich in mycolic acids/mycolates. The cell wall consists of a hydrophobic mycolate layer and a peptidoglycan layer held together by a polysaccharide, arabinogalactan. The cell wall makes a substantial contribution to the hardiness of this genus. The biosynthetic pathways of cell wall components are potential targets for drugs [5].

6.2 Clinical Features and Immunology of Mycobacterial Infections in General [6]

Skin infections caused by mycobacteria usually present as nodules which commonly show crusting, though ulcers and hypo- and hyperpigmentation may be seen. They may be single or multiple due to multiple inoculates or lymphatic spread; nodular lymphangitis is a well-known feature, even in the immunocompetent. In light-skinned people, granulomatous inflammation can be recognized by its so-called apple sauce appearance on diascopy. The clinical appearance does not reveal the causative microorganism; a causative microorganism may not be detected.

Mycobacteria responsible for most cutaneous disease are *M. marinum*, *M. ulcerans*, *M. fortuitum*, *M. chelonae*, *M. avium-intracellulare*, *M. leprae*, and *M. tuberculosis. M. leprae* infects the skin, nerve, and sometimes internal organs. *M. tuberculosis* usually causes internal disease, but skin manifestations may be present. More rarely skin infections are caused by *M. scrofulaceum*, *M. szulgai*, *M. kansasii*, or *M. haemophilum* [6].

Cutaneous mycobacterial disease occurs [6]:

- By inoculation (traumatic or iatrogenic)
- Contiguous with an underlying osteomyelitis or lymphadenitis
- As a manifestation of disseminated disease, which may be acquired through inhalation, ingestion, or trauma

As mycobacteria are intracellular microorganisms, the immunological response of the host is a cell-mediated immune (CMI) reaction, usually resulting in a granulomatous tissue reaction. Immune suppression in HIV-infected patients has caused an increase in the number of mycobacterial skin infections, besides TB, in particular of infections with the *Mycobacterium avium complex*. Antitumor necrosis factor-alpha inhibitors and other immunosuppressive drug treatments have also led to an increase of mycobacterial infections. Another group prone to infection are those with acquired or inherited immune deficiencies (severe combined immunodeficiency (SCID)), such as changes in the interferon-γ receptor 1 (IFN-gR1) and IL-12 receptor. As the interferon-γ and IL-12 pathways are crucial in the development of a CMI response to intracellular microorganisms, widespread involvement can be found [7, 8]. In the Mayo clinics over 30 years, the infections with nontuberculous mycobacteria (NTM) increased threefold [9].

Patients with intrinsic innate immunity against mycobacteria are protected [10]. Here, toll-like receptors play an important role [11]. The involvement of IL-10, Th17, and Tregs in the resulting inflammatory reaction is in discussion [12, 13].

6.3 How Are Mycobacterial Infections Diagnosed? (Table 6.1)

The most important issue is to have a high index of suspicion. One must suspect the possibility of a mycobacterial infection and recognize granulomatous processes. Sometimes there is only crusting but a prolonged history. Histopathology often directs the suspicion. Culture is still the golden standard but may be negative if not prompted by request: Many mycobacteria grow at lower than standard temperatures and often only on selected media. If not specifically requested, no growth will be the result. *M. leprae* cannot be cultured. PCR may be helpful but is regularly negative and may be positive while nonpathogenic. In some cases only clinical suspicion and the response to therapy “confirm” the diagnosis.

Table 6.1 Diagnostic tools in cutaneous mycobacterial infection

1. Clinical suspicion	Ulcerating macules, nodules, or plaques
	Longstanding
	Not particularly painful
	Non-painful lymph node involvement
	History of water contact, travel, trauma, immunosuppressed patient
	Unresponsive to treatment for other conditions, immuno
	Granulomatous aspect
	In leprosy nerve involvement
	In TB systemic disease
	In TB and leprosy contacts
2. Smear or fine needle aspiration for AFB	Ziehl-Neelsen, auramine
3. Biopsy	Histology, AFB (Ziehl-Neelsen, auramine)
4. PCR	Very variable sensitivity and specificity
	Phenotypic susceptibility testing sometimes possible
5. Culture	Appropriate incubation temperature (at 30–32 °C) and media
6. Tuberculin skin test	Latent infection, cross-reactivity
7. Interferon gamma release assays	Latent infection, cross-reactivity (TB, leprosy, several NTM)
8. Response to treatment	

6.3.1 Tuberculosis

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. orygis*, *M. canettii*, and *M. bovis BCG*), which are acid-fast in the Ziehl-Neelsen stain. They are aerobic (microaerophilic), unencapsulated, and 1–8 μm long by 0.3–0.6 μm wide and do not produce spores or toxins. The reproduction is strictly subject to oxygen pressure; therefore, unlimited *M. tuberculosis* multiplication is observed in the tuberculous cavities, while the multiplication under low oxygen pressure, e.g., in caseous lesions, is reduced [14]. TB is one of the world's deadliest diseases: One third of the world's population is infected with TB. In 2011, nearly nine million people worldwide contracted disease caused by *M. tuberculosis*. Most of these (82 %) live in 1 of 22 high burden countries. TB is a leading killer of people living with HIV [15].

Cutaneous TB was first documented in 1826, when Laennec reported his own "prosector's wart," a lesion that likely represented tuberculosis verrucosa cutis, a variant of TB that results from direct entry of the organism into the skin [16]. However, the causative organism of TB was unknown until Robert Koch discovered

Mycobacterium tuberculosis in 1882. Subsequently, the bacillus was detected in cutaneous lesions [17].

Scrofuloderma and lupus vulgaris are the oldest forms of cutaneous tuberculosis described in the medical literature and were known as the king's evil [18, 19] (see Box 6.1).

Box 6.1: King's Evil
King's evil: Scrofula, or struma, a tuberculous swelling of the lymph glands, once popularly supposed to be curable by the touch of royalty. The custom of touching was first adopted in England by Edward the Confessor and in France by Philip I. In England the practice was attended with great ceremony; and from the time of Henry VII, sufferers were presented with especially touched coins to be worn as amulets or charms. The custom reached its zenith during the Restoration: Charles II is said to have touched more than 90,000 victims between 1660 and 1682. The last royal healer in England was Queen Anne, who touched 200 victims in 1712. In France the ceremony persisted for another century and was even briefly revived by Charles X between 1824 and 1830 (*Encyclopedia Britannica*).
http://www.britannica.com/EBchecked/topic/318668/kings-evil

The range of clinical manifestations of cutaneous tuberculosis provides an example of the varying immune response of the host toward infection with mycobacteria, which is also dependent on previous exposure to other mycobacteria and the route of infection [20, 21].

M. tuberculosis, *M. bovis*, and *M. Bacille Calmette-Guérin* may cause "tuberculosis" involving the skin. Cutaneous tuberculosis can be acquired exogenously or endogenously and present as a multitude of differing clinical morphologies. Cutaneous tuberculosis has become a rare disease in the western hemisphere. The majority of cutaneous tuberculosis cases will be diagnosed in immigrants [6].

6.4 Diagnosis

The clinical diagnosis can be confirmed by smear, biopsy, culture, and/or PCR (Table 6.1). A polymerase chain reaction (PCR) assay has been validated for detecting *M. tuberculosis* and rifampicin resistance in microscopy-negative samples, especially in HIV-infected and drug-resistant tuberculosis (DR-TB) suspects; and a molecular line probe assay has been validated for detecting DR-TB in microscopy-positive samples and culture isolates in DR-TB suspects [22, 23].

6.5 Clinical Manifestations

Cutaneous tuberculosis can be classified according to four categories [6, 24]:

I. Primary infection (in tuberculin-negative persons)
II. Secondary infection (in tuberculin-positive persons)
    A. Exogenous inoculation
    B. Endogenous inoculation by contiguous spread
    C. Endogenous inoculation by hematogenous route
III. Bacille Calmette-Guérin (BCG), *M. bovis* infection
IV. Immunological reactions ("tuberculids")

6.5.1 Primary Infection: Tuberculous Chancre

The lesion starts 2–4 weeks after inoculation, with a smooth papule or nodule which enlarges in the course of several weeks to a plaque which subsequently ulcerates. The ulcer has undermined edges and is painless. Non-tender lymphadenopathy may ensue producing a clinical picture of a lymphocutaneous complex analogous to the Ghon complex seen in the pulmonary infection. This process generally heals spontaneously with atrophic scarring in 3–12 months.

Differential diagnosis: Other causes of ulceration, other mycobacteria (e.g., Buruli), and other chronic conditions like subcutaneous mycoses, cutaneous leishmaniasis, and malignancies. It includes also infections which show sporotrichoid spread, for example, sporotrichosis, cat scratch disease, and tularemia.

6.5.2 Secondary Infection

Secondary infection comprises the vast majority of all cases of cutaneous tuberculosis.

6.5.2.1 Warty Tuberculosis: Tuberculosis Verrucosa Cutis

Warty tuberculosis, known as tuberculosis verrucosa cutis, is the most common type of skin tuberculosis in the East, particularly India [25]. Due to a rapid cell-mediated response, the infection remains localized, and regional lymphadenopathy is not prominent.

The lesion develops from an asymptomatic reddish-brown papule into a verrucous plaque of varying shapes and sizes. The verrucous fissures may become superinfected (Fig. 6.2). The plaque may heal spontaneously in the course of months to years, with atrophic scarring and activity in different parts of the same lesion [26].

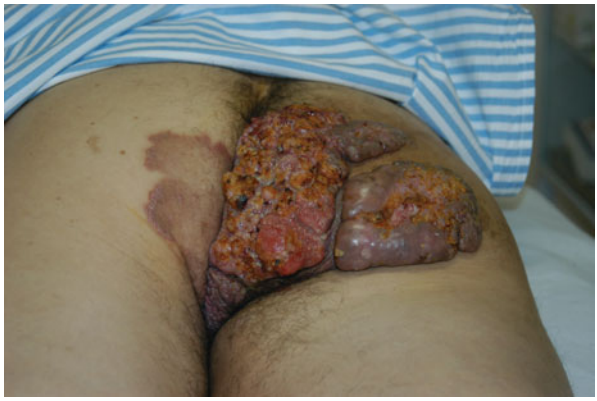

Fig. 6.2 Warty tuberculosis in a 46-year-old man (Courtesy ILSL, Bauru, Brazil)

An identical lesion may be seen among cattle workers. Here the species involved is most commonly *M. bovis*.

Differential diagnosis: Common warts during the initial stage, later hypertrophic lichen planus, and verrucous lesions caused by NTM or by deep mycoses like blastomycosis, sporotrichosis, chromomycosis, lobomycosis, some forms of leishmaniasis, verrucous rupial tertiary syphilis, rupioid psoriasis, and squamous cell carcinoma.

6.5.2.2 Scrofuloderma: Tuberculosis Cutis Colliquativa

Scrofuloderma is the result of contiguous spread from an underlying mycobacterial infection, commonly in lymph nodes or in some cases the bone. It is the most common cause of cervical lymphadenitis in children. Due to suppuration fluctuating nodules develop, which ulcerate. In the course of time, cordlike scars develop. The lesions heal over years with a characteristic pattern of fibrosis, atrophy, and scarring. Recurrence of drainage is common (Fig. 6.3). Other mycobacteria now known to cause scrofuloderma are *M. scrofulaceum*, *M. haemophilum*, and *M. avium-intracellulare* complex, but it is also still seen with *M. tuberculosis*.

Differential diagnosis: Deep mycoses such as sporotrichosis or coccidioidomycosis but also actinomycosis, hidradenitis suppurativa in axillary lesions, granuloma inguinale and lymphogranuloma venereum in inguinal lesions, and chronic bacterial osteomyelitis when localized over the bone.

6.5.2.3 Orificial Tuberculosis: Ulcerative Tuberculosis in the Mucosa

This rare form of cutaneous TB starts with single or multiple nodules which become fluctuant and ulcerate showing draining sinuses. The cause is autoinoculation from active tuberculous foci affecting the mucosa or skin near the oral, genital, or anal orifices. Pain is a cardinal feature [6].

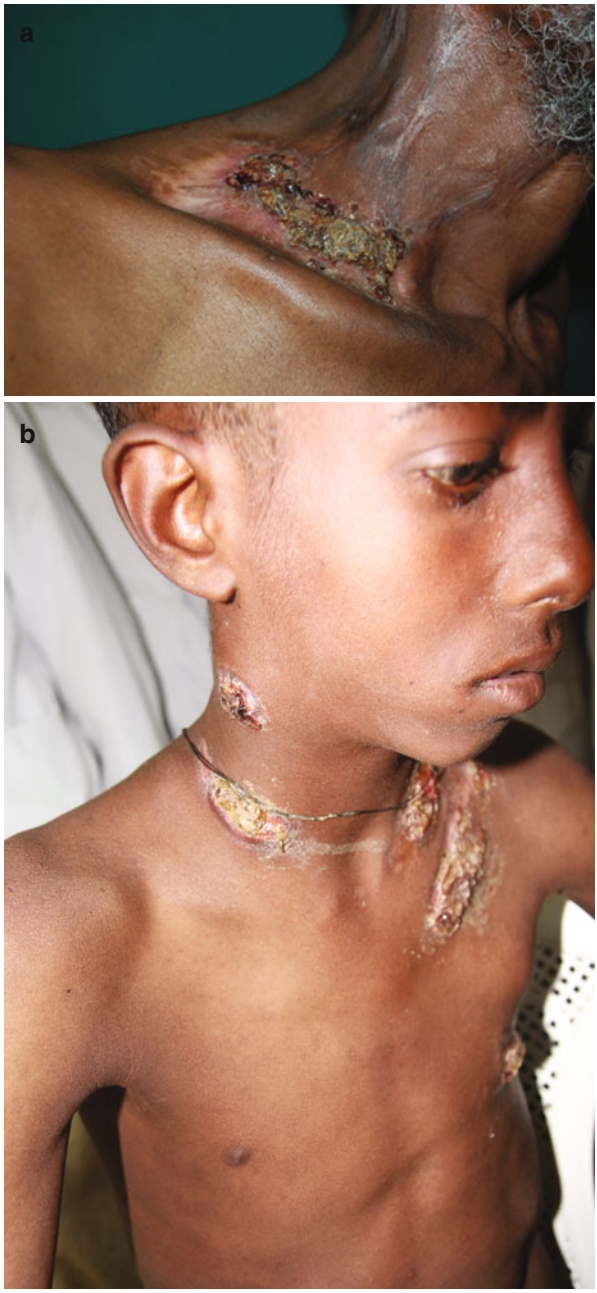

Fig. 6.3 (**a**) Scrofuloderma by *M. tuberculosis* (Courtesy of Dr. Dassoni, Ayder Hospital, Mekelle, Ethiopia). (**b**) Scrofuloderma in a 12-year-old boy. Regional Dermatology Training Center (RDTC), Moshi, Tanzania. The diagnosis was clinically made, but one of the NTM's is possible as cause as well. (Determination was not possible at that time in these institutions)

The primary foci of tuberculosis are the lungs, gastrointestinal tract, and/or genitourinary tract. The affected patient is usually in poor health with long-standing advanced tuberculosis involving multiple internal organs [27].

Differential diagnosis: Leishmaniasis, aphthous ulcers, dental and perianal abscesses, M. Crohn, paracoccidioidomycosis, malignancies, herpes simplex lesions, or ulcerating venereal disease. Painful anal ulcerations may also be seen in cutaneous amebiasis.

6.5.2.4 Lupus Vulgaris

Lupus vulgaris was a common disease in the early twentieth century. Today it is rare in the West and it also occurs less frequently in developing countries. It is due to reactivation of a mycobacterial infection in patients with a moderate to high degree of CMI, hence paucibacillary "tuberculosis."

Reactivation usually stems from cervical adenitis or pulmonary tuberculosis but sometimes from an old, apparently quiescent primary complex. Rarely, it follows primary inoculation or BCG vaccination. Lupus vulgaris lesions have been described around warty tuberculosis and scrofuloderma.

Classic lesions start as brown-red papules which extend to plaques with active, irregular borders and central healing with atrophic, depigmented scarring (Fig. 6.4). Spontaneous involution may occur and new lesions may arise within old scars. Complete healing rarely occurs without treatment. Squamous cell carcinoma may develop in these chronic lupus vulgaris lesions.

Differential diagnosis: Lupoid and recidivans variant of cutaneous leishmaniasis, subcutaneous mycoses, sarcoidosis, chronic discoid lupus erythematosus, and basal cell carcinoma.

6.5.2.5 Tuberculous Gumma: Metastatic Tuberculous Ulcer

The gumma is due to hematogenous dissemination from a primary focus, during periods of decreased immunity. A subcutaneous nodule or fluctuant swelling results in an undermined ulcer with sinus formation, also known as a "metastatic tuberculous abscess" or "metastatic tuberculous ulcer" which may resemble scrofuloderma. It is histologically characterized by massive necrosis [28]. Some of these patients may have an underlying malignancy (lymphoma) [29, 30].

Differential diagnosis: Cold abscess, scrofuloderma, tertiary syphilitic gumma, subcutaneous mycoses, and cutaneous leishmaniasis.

6.5.2.6 Acute Miliary Tuberculosis: Tuberculosis Cutis Miliaris Disseminata

Miliary tuberculosis is the result of hematogenous dissemination of *M. tuberculosis* to the skin and other organs in the absence of CMI reactivity against *M. tuberculosis* antigens. It shows a generalized eruption of small purplish macules and papules (1–5 mm), with vesicles on top which may break, forming crusts. Due to the absence

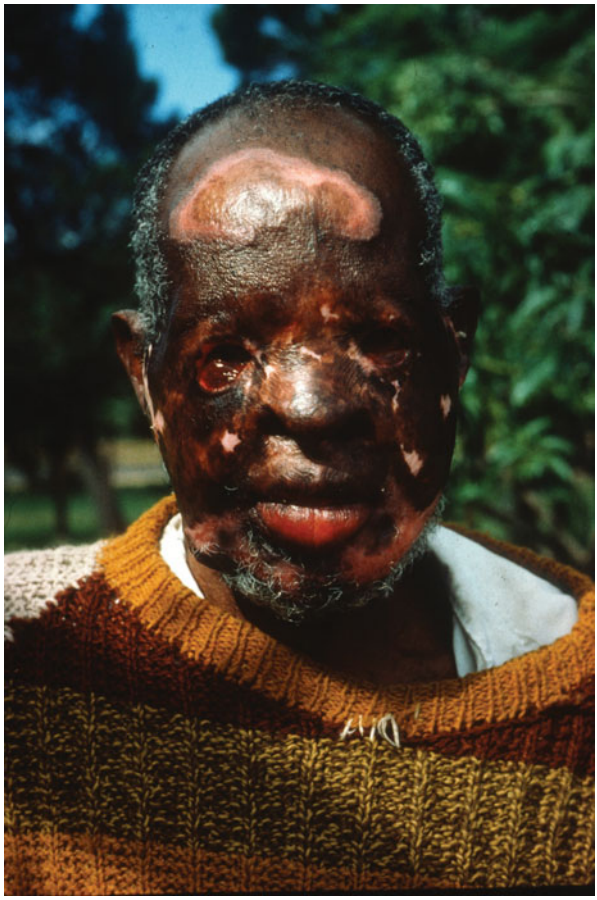

Fig. 6.4 Lupus vulgaris in a 50-year-old Zimbabwean trader

of CMI reactivity, the histopathological picture is that of a nonspecific inflammation with numerous acid-fast bacilli [6, 28]. A patient with miliary tuberculosis usually presents with nonspecific signs, such as low-grade fever, cough, and enlarged lymph nodes, and there may be an enlarged liver, enlarged spleen, inflammation of the pancreas, and multiple organ dysfunction with adrenal insufficiency [31].

Differential diagnosis: The skin eruption is nonspecific, but the patient is ill [6].

*6.5.3 BCG (*M. bovis*, BCG Inoculation)*

BCG vaccination, with an attenuated strain of *M. bovis*, is practiced in many areas of the world. The vaccination provokes a CMI reaction in susceptible persons. This is clinically observed as an infiltrated papule which develops in 10–14 days at the site of inoculation. It enlarges into an ulcerative lesion of approximately 1 cm at

10–12 weeks. It heals which scarring. After approximately 3 months, the tuberculin skin test reverses from negative to positive, except in people with an inherited protective innate immunity. Its protective prophylactic effect varies from less than 10–80 % probably depending on the presence or absence of boosting by environmental microorganisms. But at least it protects against tuberculous meningitis in infants [32].

6.5.4 Immunological Reactions to Tuberculosis Elsewhere: Tuberculids

Tuberculids are generally considered to be a CMI response to dissemination of *M. tuberculosis* or antigenic particles to the skin [33]. Papular necrotic tuberculids would represent the paucibacillary pole of blood-borne disseminated TB, as opposed to the multibacillary picture of acute miliary TB [24]. But because very often *M. tuberculosis* cannot be detected in the skin or elsewhere in the body, antigenic determinants of the host similar to those of *M. tuberculosis* may be the cause [34].

The following are nowadays accepted to be true tuberculids [6]:

1. *Papulonecrotic tuberculid*

   Papulonecrotic tuberculid occurs as crops of symmetric, small, inflammatory papules which have a predilection for acral and dorsal surfaces. Lesions may undergo central ulceration and heal spontaneously within weeks, leaving varioliform scars. Microscopically, a wedge-shaped area of necrosis is seen with underlying vasculitis and granulomatous infiltrate [35]. They may heal with antituberculous treatment but may also resolve spontaneously with a depressed scar with a hyperpigmented border. Some noticed phlyctenular conjunctivitis in children [36].

   The tuberculous etiology is suggested by a positive tuberculin skin test, the demonstration of *M. tuberculosis* DNA in the lesions, and prompt resolution of the condition on antituberculous treatment.

   Differential diagnosis: Prurigo papules, folliculitis, and papular lesions of syphilis. Necrotic lesions should be differentiated from pityriasis lichenoides acuta, necrotizing vasculitis, necrotic insect bite reactions, and self-inflicted injury.
2. *Lichen scrofulosorum*

   Lichen scrofulosorum, also known as "tuberculosis cutis lichenoides," is a rare tuberculid that presents as a lichenoid eruption of minute papules in children and adolescents. The lesions are usually asymptomatic, closely grouped, skin-colored to reddish-brown papules, are often perifollicular, and are mainly found on the trunk (abdomen, chest, back) and proximal parts of the limbs. The eruption is usually associated with a strongly positive tuberculin reaction [37]. PCR has also demonstrated the presence of *M. tuberculosis* DNA in lesions [38].

   Differential diagnosis: Lichen planus, lichenoid drug eruptions, secondary syphilis, and pityriasis lichenoides chronica. Due to the perifollicular distribution

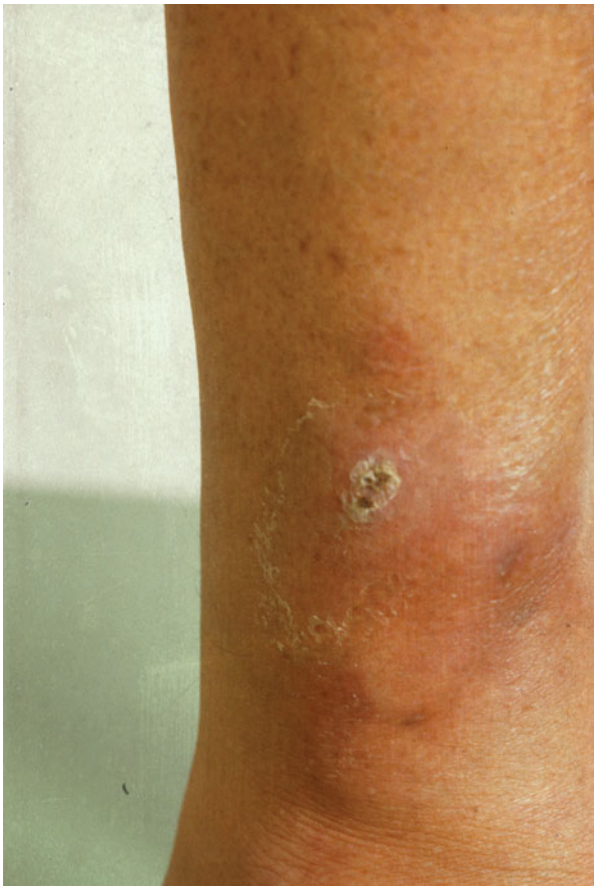

Fig. 6.5 Erythema induratum of Bazin in a 65-year-old Dutch nurse

also keratosis pilaris, lichen nitidus, lichen spinulosus, and pityrosporum folliculitis. Differentiation from the micronodular form of sarcoidosis may be clinically and histopathologically difficult [39].

3. *Nodular vasculitis* (erythema induratum of Bazin)

   Erythema induratum, described by Bazin in 1855, has been considered to be associated with tuberculosis [40, 41]. It can however be induced by numerous triggers including tuberculosis [42]. Erythema induratum presents during early adolescence and perimenopause as recurrent subcutaneous poorly defined erythematous plaques and tender violaceous nodules, sometimes ulcerating, on the calf of the legs of otherwise healthy, often heavy-set, women [43] (Fig. 6.5). At present it is the most prevalent tuberculid in the West. The histopathological picture is that of a nodular vasculitis. *M. tuberculosis* DNA has been demonstrated in lesional biopsies.

   Differential diagnosis: Erythema nodosum, cutaneous polyarteritis nodosa, pancreatic panniculitis, lupus profundus, subcutaneous sarcoid, and cutaneous T-cell lymphoma.

4. *Erythema nodosum*

   Erythema nodosum was frequently associated with tuberculosis in the past, while today it is most frequently caused by streptococcal infections, sarcoidosis, drug reactions, and inflammatory bowel disease. But tuberculosis still should be considered in patients from developing countries.

   Painful erythematous nodules present on the lower legs, especially on the shins. Sometimes the extensor sides of the arms are involved. The histopathological picture is a panniculitis with vessel involvement.

   Differential diagnosis: Panniculitis, polyarteritis nodosa, erythema induratum, nodular lymphangitis, and erythema nodosum leprosum.

6.6 Treatment of Tuberculosis

The first "treatments" of tuberculosis consisted of nutritious food, rest, and "pure" air. TB sanatoria were established from the middle of the nineteenth century onward, and their numbers grew in the early twentieth century. In 1903, Niels Ryberg Finsen was awarded the Nobel Prize for his invention of radiation therapy for skin tuberculosis (lupus vulgaris) [44].

The history of currently used antituberculosis drugs goes back to 1943, when Waksman with his research team from the Rutgers University isolated streptomycin from the actinomycete *Streptomyces griseus*. Streptomycin turned out to be the first drug that decreased mortality due to tuberculosis [45]. The next, not less important step was introduction of para-aminosalicylic acid (PAS) and isoniazid (INH) in the early 1950s – drugs that, similarly to streptomycin, significantly reduced mortality from tuberculosis.

However, drug resistance soon developed to single agents [46]. The synthesis of pyrazinamide [47] in the 1950s and ethambutol in 1962 [48] brought drugs that are used in antituberculosis therapy to this day [49]. At the same time, ethionamide, prothionamide, cycloserine, and thiacetazone were discovered.

Most important in the development of antituberculosis medications was the discovery of the soil bacterium: *Streptomyces mediterranei* in 1959, from which rifamycin was isolated [50]. During further studies, a semisynthetic derivative of the rifamycin antibiotic was synthesized – rifampicin (RMP). This was permanently included in the standard treatment of tuberculosis and many other mycobacterial infections.

Together with the discovery of rifampicin, the increasing problem of drug resistance was observed [51]. Multidrug (MD) treatment was the best way to avoid this. Unfortunately at present MD-resistant (MDR) TB is becoming a huge problem [52]. To date there is even extensively drug-resistant tuberculosis (XDR-TB), which is defined as resistance to at least RMP and INH (the definition of multidrug-resistant tuberculosis (MDR-TB)), in addition to resistance to any fluoroquinolone, and at least one of the three injectable antituberculosis (TB) drugs capreomycin, kanamycin, and amikacin [53]. Inadequate treatment of MDR-TB inevitably results

in high mortality and the development of XDR-TB [54]. About 3.7 % of new tuberculosis (TB) patients in the world have multidrug-resistant strains (MDR-TB). Levels are much higher in those previously treated – about 20 %. The frequency of MDR-TB varies substantially between countries. About 9 % of MDR-TB cases also have resistance to two other classes of drugs or extensively drug-resistant TB (XDR-TB). By March 2013, 84 countries had reported at least one XDR-TB case [55, 56]. TB treatment should be instilled according to local regional or national guidelines. Pulmonary TB is treated using a 6-month course of a combination of antibiotics. The usual course of treatment is two antibiotics (isoniazid and rifampicin) every day for 6 months and two additional antibiotics – pyrazinamide and ethambutol – every day for the first 2 months [57, 58].

Extrapulmonary TB, e.g., cutaneous TB, can be treated using the same combination of antibiotics. However, the treatment may take longer, 12 months.

The US recommendation is 2HREZ/7HR [59]. However, there is good evidence from randomized controlled trials that in tuberculous lymphadenitis [60] and in TB of the spine [61], a 6-month regimen is equivalent to the 9-month regimen.

6.6.1 Leprosy [62]

Leprosy, or Hansen's disease, is a chronic infection caused by *Mycobacterium leprae*. Leprosy takes its name from the Greek words "lepros," a scale, and "lepein," to peel, while the term "Hansen's disease" is named after the physician Gerhard Armauer Hansen who discovered the bacillus in 1873 [63]. It was the first bacterium assigned as a cause to a human disease. This being said, Koch's postulates are not established in leprosy as *M. leprae* cannot be cultured in vitro, and it has not been possible to infect someone willfully with leprosy, although there are some reports of leprosy following trauma or tattooing [64].

It is primarily a disease of peripheral nerves, the skin, and the mucosa, in particular the upper respiratory tract. Skin lesions are usually the first sign noticed. Left untreated, leprosy can be progressive, causing permanent damage to the skin, nerves, limbs, and eyes. Tissue damage may be caused by primary infiltration by *M. leprae* [65], but most of the damage is secondary to immunological phenomena: reactions [66]. Secondary infections can result in tissue loss causing fingers, toes, and nose to become shortened and deformed, as the bone and cartilage are absorbed.

Frequently leprosy is not in the differential diagnosis, since this subject is often not emphasized in medical curricula. Attention shifted from leprosy to TB and HIV infection in the late twentieth century, and the WHO leprosy program was toned down in the conviction that leprosy was all but eliminated. In 2005 WHO stated that leprosy was eliminated as a worldwide public health problem. Unfortunately this is not the case [67]. To this day worldwide incidence since 4 years remains stable at about 250,000 new patients annually, while the prevalence has also stopped decreasing. Often children are affected and new cases

present late, with permanent disabilities [68]. Due to increased travel, patients are diagnosed everywhere in the Western world, unfortunately often after long doctors' delay. But even in leprosy endemic areas, the diagnosis is often delayed, because leprosy was not in the differential diagnosis [69]. Dermatologists and neurologists are generally poorly trained in leprosy.

6.6.1.1 Diagnosis

Patients may complain of loss of sensation in skin lesions or of their hand or foot. They may have aches and pains in the face or limbs or describe a numb, sleepy, or dead feeling or sensations like "ants running under their skin" in the affected areas.

Skin lesions are usually hypopigmented or erythematous macules or papules and nodules and plaques which are skin colored or slightly red.

Most important for the diagnosis is awareness. Clinically, leprosy is diagnosed when the patient shows two out of three cardinal signs [70]. In endemic countries, one cardinal sign is considered enough [71].

The three cardinal signs of leprosy are:

1. Loss of sensation in a skin lesion
2. Enlarged peripheral nerve
3. Positive skin smears

Loss of sensation is tested with a wisp of cotton wool. The area in the lesion is tested by touch. With closed eyes the patient points to where he is touched. To make sure, the area outside the lesion is tested as well (Fig. 6.6).

Enlarged nerves can be cutaneous nerves, subcutaneous nerves in the vicinity of skin patches, or nerve trunks. At least the posterior auricular nerves, the ulnar, the radiocutaneus, the median, the lateral popliteal, and the tibial posterior nerves should be palpated. Nerve thickness, consistency, and tenderness should be appreciated (Fig. 6.7). Ultrasound is a good alternative [72].

Smears are taken to detect acid-fast bacilli from the earlobes and other cooler areas and from the rim of the lesion in paucibacillary (PB) and central in the lesion in multibacillary (MB) patients. The smear is taken while squeezing the skin, to numb and to diminish the bleeding while incising into the dermis. Only tissue fluid is required. The number of bacilli is counted and graded along a logarithmic scale (BI, bacillary index), and the percentage of solid bacteria, live (viable) bacilli, is estimated (MI, morphological index) [68]. It is important to decolorize shortly with 1 % hydrochloric acid in isopropyl alcohol (as in Fite stain), as opposed to the 3 % solution used for TB, because *M. leprae* is less acid-fast than *M. tuberculosis*. Using the common Ziehl-Neelsen stain (3 % hydrochloric acid) may make the smear negative. Another way to detect bacilli is by PCR or NASBA, which, like the smear, is often negative in PB patients. Smears and molecular techniques can however be very useful in the diagnosis of MB leprosy, in follow-up, and in detection of relapses.

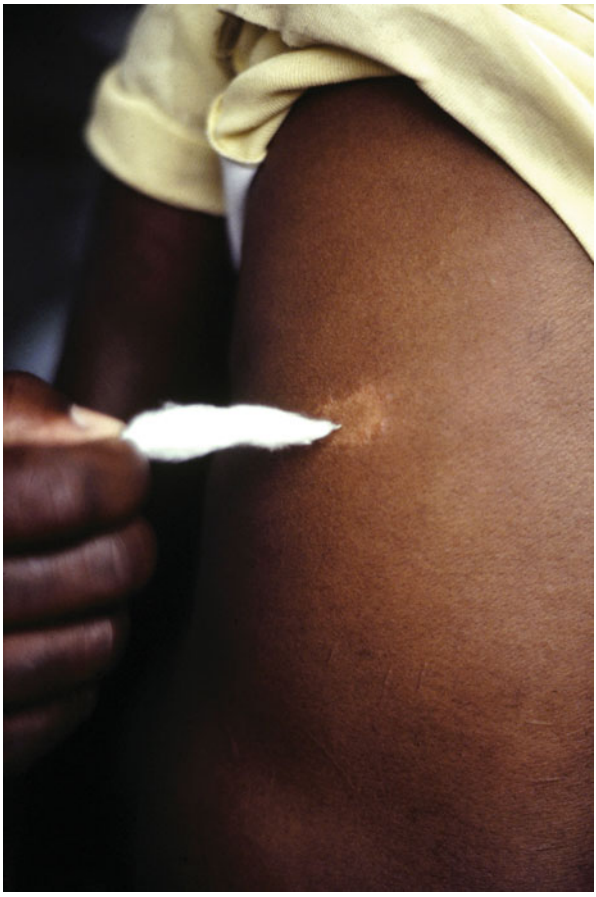

Fig. 6.6 Sensory testing in an 8-year-old Tanzanian boy at the Regional Dermatology Training Center (RDTC), Moshi, Tanzania

Other laboratory investigations are of some help in the diagnosis of leprosy, but none will be diagnostic in all cases. The antibody titer against phenolic glycolipid 1 (PGL-1), a cell wall species-specific glycolipid, is useful in MB leprosy. However, this can be positive in contacts and negative in PB leprosy. It helps to classify leprosy into PB and MB, and it can be used to follow the effect of treatment in MB patients and to detect relapses [73]. The value of the recently introduced "LID" is still not clear.

Lymphocyte transformation tests against different antigenic determinants have been a disappointment up to now. The lepromin test (Mitsuda), an old test, is positive in PB leprosy and negative in MB leprosy. But in healthy people, it can be positive and negative. Thus, it helps only with the classification. Because it is made from biological material, theoretically it may sensitize; therefore, many oppose its use [62].

Histopathology can be very helpful, as can immunopathology, but the latter is still experimental. A problem is that even within lesions, the histopathology of one spot may differ from the other [62].

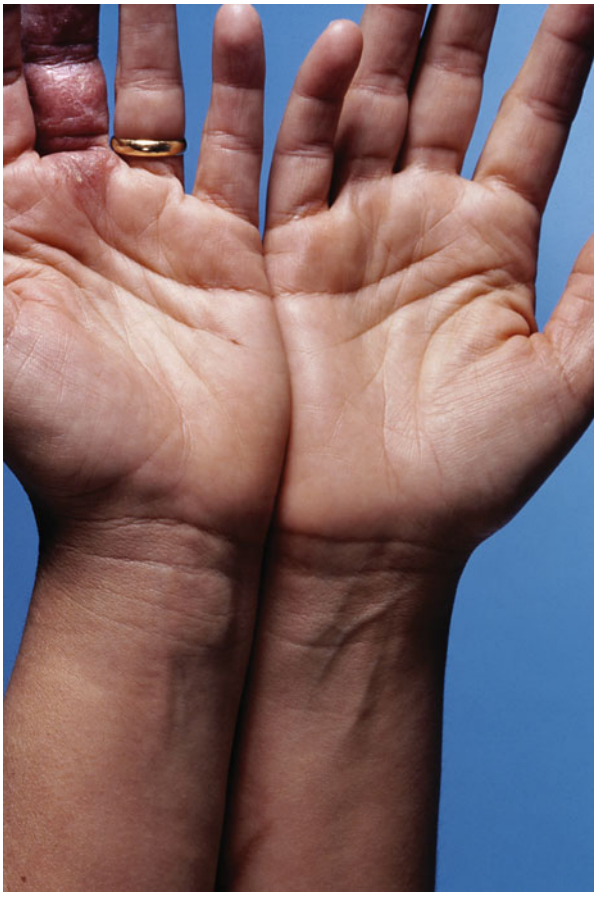

Fig. 6.7 Enlarged median nerve in a Brazilian patient who presented with swelling of the median nerve which was attributed to carpal tunnel syndrome. After unsuccessful surgical and moderately successful treatment with systemic steroids, she developed the skin lesion. Diagnosis: BT leprosy

6.6.1.2 Infection and Classification

Leprosy is highly infectious [74], but the attack rate is low. The major reason for this low attack rate is that most people are genetically unable to supply the mycobacteria in their cells with what they need to survive, because they lack the type of genes the bacterium needs [64, 75].

In order to predict complications and to stratify according to CMI, the Ridley-Jopling scale (Fig. 6.8) is important, with on one side of the spectrum polar tuberculoid (TT) (Fig. 6.9) leprosy with a single well-described lesion or an enlarged nerve and with no bacilli detectible and a high CMI against *M. leprae* antigenic determinants, and on the other side, polar lepromatous (LL) leprosy with nodules and/or plaques (Fig. 6.10), with symmetrically enlarged nerves or even only an infiltrated skin (lepra bonita), and with an absence of CMI against *M. leprae* antigenic determinants and many bacilli. Between the TT and LL leprosy is the borderline group, which comprises the majority of the patients: borderline tuberculoid (BT) (Fig. 6.11a, b) with

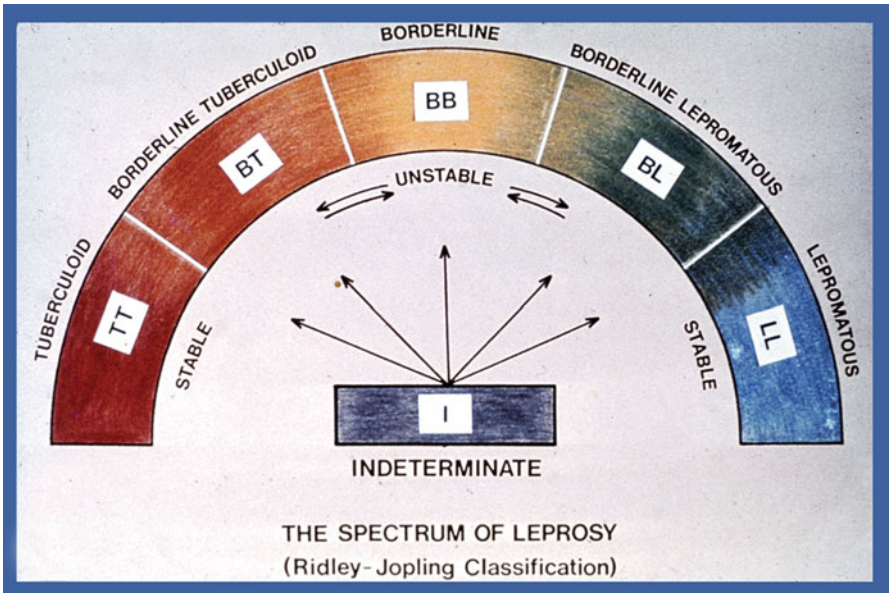

Fig. 6.8 Ridley-Jopling classification of leprosy (Courtesy of Dr. DL Leiker) [76]

predominantly tuberculoid features or borderline lepromatous (BL) (Fig. 6.12a, b) with predominantly lepromatous features. Between those two is a small group of mid-borderline (BB) (Fig. 6.13) patients with typical punched out or dome-shaped lesion [77].

Sometimes it is not possible to classify leprosy. The lesions in those cases are then clinically and histologically indeterminate.

The WHO classified leprosy into just two groups for practical purposes in the field (They count the number of lesions): five or less classified as paucibacillary leprosy (PB leprosy) and more than five as multibacillary leprosy (MB leprosy) [77]. Although this is a very practical approach, several reports have shown that by just counting, up to 30 % of the patients may be wrongly classified as PB and therefore undertreated [78].

6.6.1.3 Treatment

The first treatment known with some effectiveness was chaulmoogra oil, mentioned already in the *Sushruta Samhita* 600 BC. The effect was minimal in different preparations used; however, some success was obtained in PB leprosy [79]. The first effective antibiotic, intravenous sulfone, promin, appeared in 1943 [80]. Soon afterward, a new oral derivate called dapsone (diamino-diphenylsulfone, DDS) became the standard treatment. Upon the appearance of secondary DDS resistance in the 1970s together with the ready availability of rifampin (RMP), the use of combined

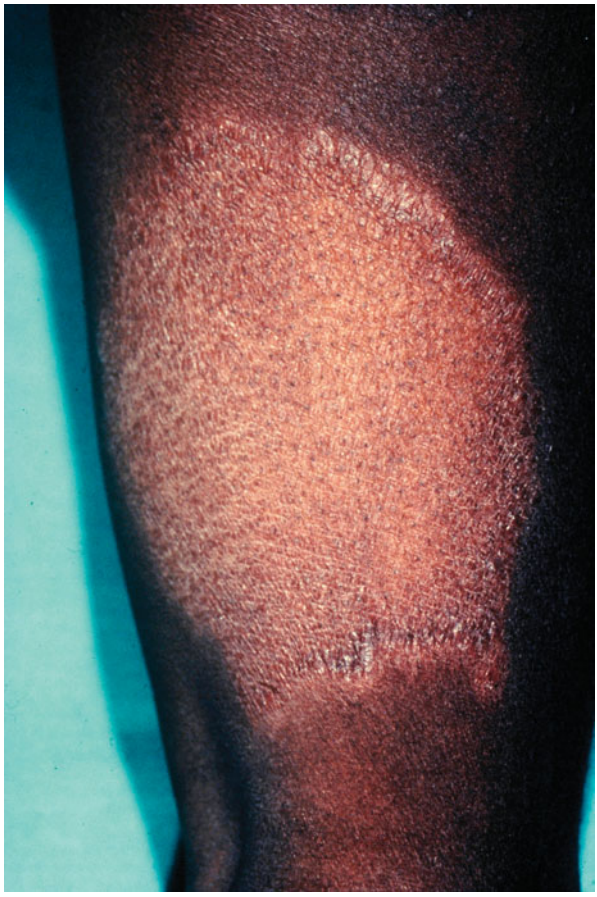

Fig. 6.9 TT leprosy (Courtesy of Dr. DL Leiker)

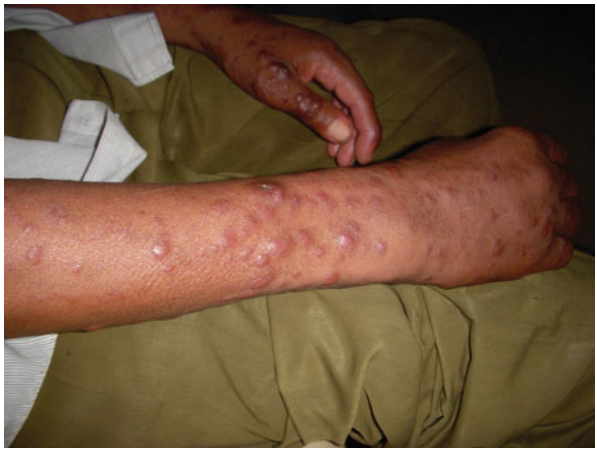

Fig. 6.10 A 20-year-old Tanzanian with LL leprosy (Courtesy of Regional Dermatology Training Center (RDTC), Moshi, Tanzania)

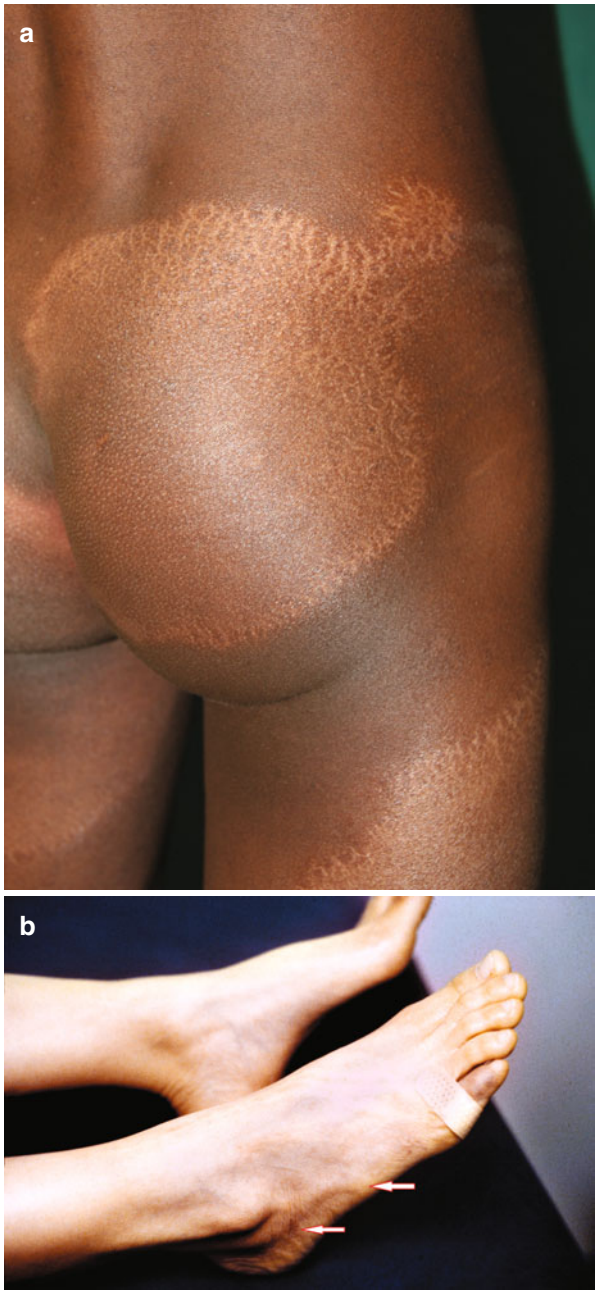

Fig. 6.11 (**a**) BT leprosy (Courtesy of Dr. Workalemahu, Ayder Hospital, Mekelle, Ethiopia). (**b**) BT leprosy showing enlarged sural nerve (*arrows*) in a 40-year-old Chinese woman

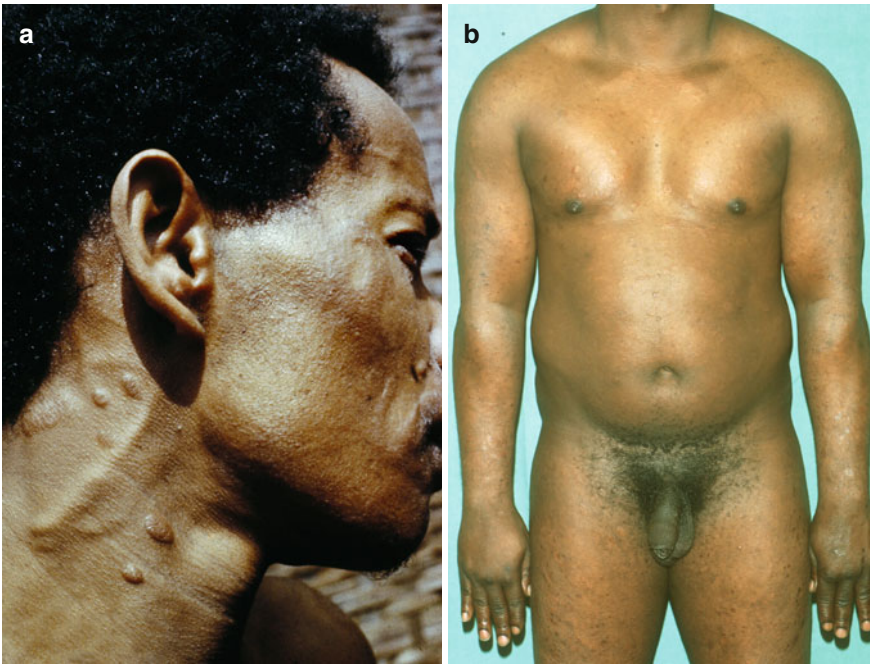

Fig. 6.12 (**a**) BL leprosy, downgrading from BB, enlarged nerve, nodules partly still edematous. (**b**) BL leprosy, small nodules with outside the nodules frequent low BI in smear or biopsy

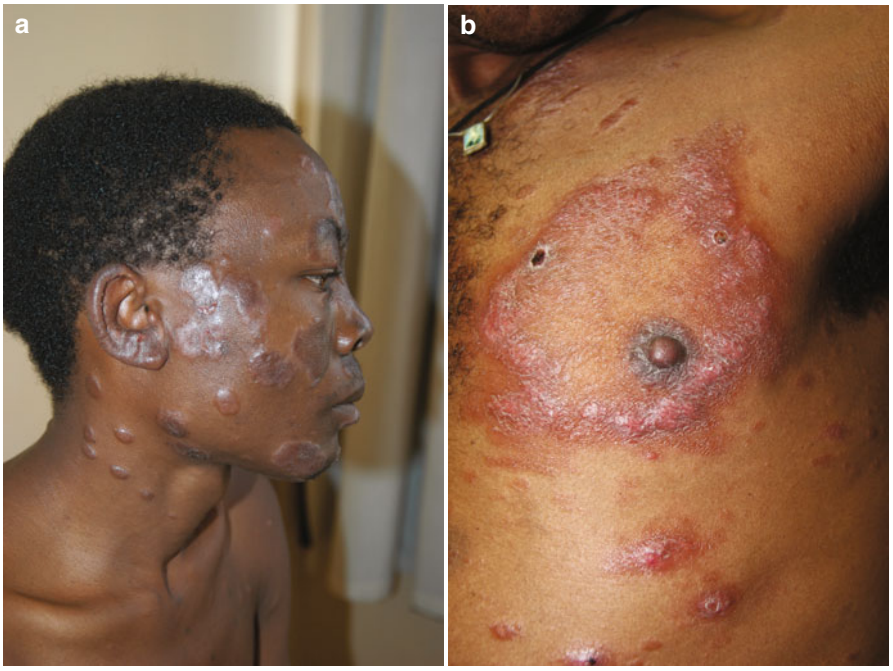

Fig. 6.13 (**a**) BB leprosy, downgrading to BL, nodules become firm showing "immune areas" in the center of the lesions. (**b**) BB leprosy downgraded from BT immune area's, involvement of the palm of the hand is typical. Lesions are still edematous

regimens was recommended [81]. Several treatment combinations, mainly based on previously proven effective tuberculosis therapy, were proposed to combine with DDS, such as rifampin, thioamide drugs, and isoniazid. The latter is however not active against *M. leprae*.

Combined therapy was implemented by several national programs. For instance, in Paraguay and Malta, isoprodian® (175 mg of prothionamide, 50 mg DDS, and 175 mg isoniazid) and RMP were extensively used with only a few reported relapses [82, 83]. It was also used in Ethiopia and Tanzania where many side effects were noticed, gastrointestinal disturbances and particularly liver toxicity. But it was not until 1982 that the WHO's Chemotherapy Study Group recommended the combined use of RMP and DDS with or without clofazimine [81]. WHO-MDT is the current standard treatment and continues to be widely administered.

6.6.1.4 Multidrug Therapy (MDT)

Paucibacillary leprosy: 600 mg rifampicin once monthly under supervision and daily 100 mg dapsone for 6 monthly doses within 9-month time. The dose is for a 60 kg patient.

Multibacillary leprosy: 600 mg rifampicin and 300 mg Lamprene (clofazimine) once monthly under supervision and 100 mg dapsone and 50 mg Lamprene daily. Twelve monthly doses should be given within 18 months for low-BI patients and 24 monthly doses in 36 months for patients with a BI of 4 or more. The doses are for 60 kg patients.

These treatment regimens have proved sturdy; hardly any relapses (4%) are seen. However, be careful with dapsone in Nordic Caucasians who easily develop hemolysis and with Nepalese and Chinese patients who have a greater risk of developing dapsone hypersensitivity syndrome. This is independent of G6PD. Fifty milligram of dapsone is effective in the majority of patients and causes much less anemia. It is probably genetically determined [84].

As alternative for daily treatment and as once-only treatment for single-lesion leprosy, a combination of RMP, ofloxacin, and minocycline was advocated. For BT and LL leprosy, it was given once per month, but it showed to be less effective than WHO-MDT [85].

6.6.1.5 Reactions

Reactions belong to the normal course of a leprosy infection. Treatment can prevent or precipitate them. There are three types of reactions: type I leprosy reaction (T1R), also called reversal reaction (RR); type II leprosy reaction (T2R), also called erythema nodosum leprosum (ENL); and Lucio's phenomenon, a reaction occurring specifically in patients from Mexico.

T1R is a CMI reaction, a type IV Gell and Coombs reaction against *M. leprae* antigenic determinants [86]. Clinically, there is increased inflammation of lesions, which become more visible and erythematous, are raised or enlarged (Fig. 6.14), and even may ulcerate. Nerves may be inflamed, enlarged, and tender, causing diminishing strength, sweating, and sensitivity. There may be acroedema.

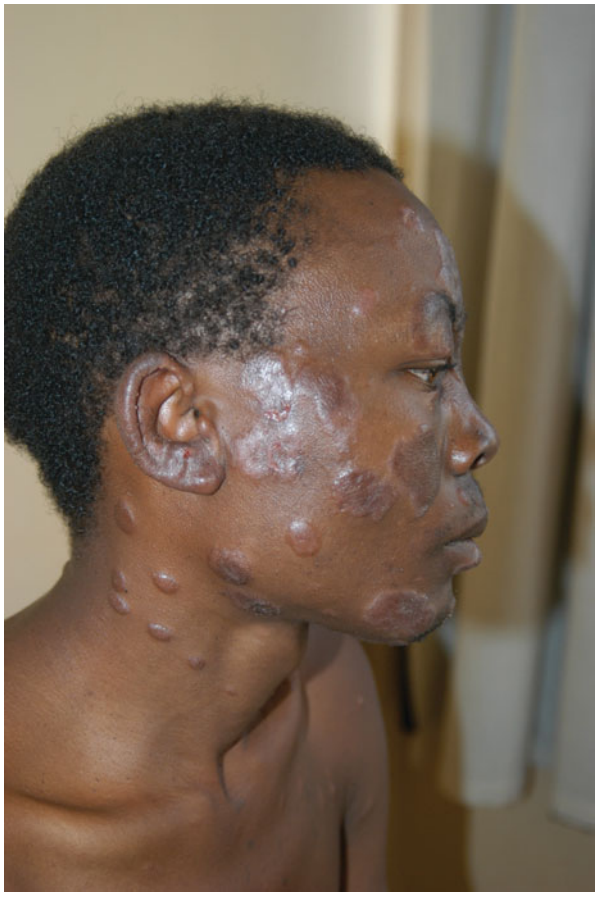

Fig. 6.14 Type 1 leprosy reaction (T1R) in a 25-year-old man (BB-BL leprosy) (Courtesy of RDTC, Moshi, Tanzania)

T2R is an antigen-antibody immune complex reaction in the tissues, particularly in the skin and nerve [87]. The skin shows the characteristic red painful, tender nodules (Fig. 6.15). It is a multi-organ disease; all types of tissues can be inflamed. There may be fever and leukocytosis.

The treatment of T1R primarily consists of corticosteroids, 30–40 mg prednisone starting dose, tapering down, guided by, for instance, graded sensory testing, in 6–12 months, in which the dose needs to be 20 mg at least to be effective. Adequate immune suppression (>0,25 mg/kg) should be given at least for 3 months for BT leprosy to 18 months or even longer for BL leprosy [88]. In some cases dapsone helps to prevent a reaction [89].

T2R treatment is difficult. The reaction is episodic, 95 % of ENL episodes last less than 1 month [90]. Mild reactions can be treated with NSAIDs; arthritis with antimalarials, but severe reactions, needs high dose steroids (60–120 mg) for a short period, diminishing to zero in a month or less. A new attack should be treated the same way [91]. Clofazimine may prevent a T2R or can be used as treatment [92]. Thalidomide as treatment is superior above all and can be used as prophylaxis. But even thalidomide may not be effective in every T2R. The combination of low-dose steroids with

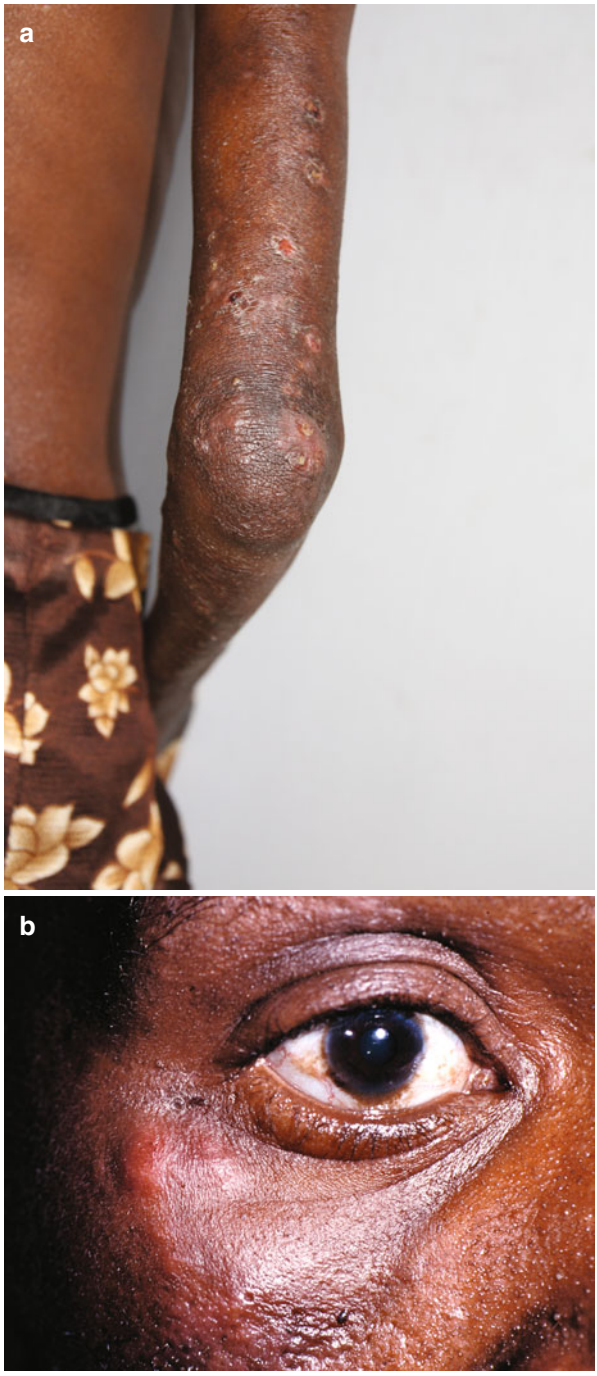

Fig. 6.15 (**a**) Type 2 leprosy reaction (T2R) in a 24-year-old man. (**b**) Type 2 leprosy reaction (T2R) in a 31-year-old woman (Courtesy of RDTC, Moshi, Tanzania)

low-dose thalidomide is counterproductive [93]. When thalidomide is not available, for the prevention of new ENL episodes methotrexate (MTX) could be used [94].

When nerves continue to deteriorate despite proper medical treatment, a nerve release operation needs to be considered. This also can be done for nerves without a reaction that remain tender after treatment.

The Lucio phenomenon presents as an infarction in the skin, when a huge amount of bacilli is blocking the venous return in the small venules. This is only seen in untreated diffuse lepromatous leprosy and may be triggered by sudden cold. The treatment is MDT; RMP is the crucial drug.

The results of nerve damage, loss of sensation, and muscle strength are the sequelae or the stigmata of leprosy. These should be countered with supplying special padded tools, utensils, and shoes. Sometimes in order to increase grip or to improve foot movement, a tendon transfer may be considered, but always with an experienced physiotherapist present.

6.6.1.6 Prevention

Despite many efforts to develop a universal active vaccine, involving DNA techniques, BCG vaccination remains the best prophylactic in many areas. Protection ranges from less than 20 % in some areas to up to 80 % in other, probably depending on the presence and characteristics of environmental microorganism [95]. Treating of contacts with a single 600 mg dose of rifampicin has proven to be effective for a few years, and BCG vaccination may extend this [96].

6.6.2 Buruli Ulcer [97, 98]

Buruli ulcer (BU), also known as the Bairnsdale, Searls, or Daintree ulcer, is an infectious disease caused by *Mycobacterium ulcerans* [97, 98].

The disease was named after the area of the first large epidemic in Uganda (1961), in an area named "Buruli," near Lake Kyoga [99]. *M. ulcerans* grows optimally at 30–32 °C and contains a large plasmid that encodes for enzymes to produce a polyketide-derived macrolide toxin called mycolactone which mediates tissue necrosis, immunosuppression, and apoptosis [97, 100–102].

BU is a public health problem, mainly because of the severe disabilities it causes when diagnosed late and the stigma it carries [103]. Since 1998, WHO has highlighted the growing problem of BU and developed improved treatment and control programs [104].

BU afflicts all age groups but most cases occur in children younger than 15 years of age. There is no gender preference [105]. Most lesions are on the lower extremities, a relatively cooler site which is also prone to traumata.

BU is focally endemic in rural wetlands of tropical countries of Africa, America, Asia, and Australia. BU is most common in West Africa, with highest incidences in

Benin, Ghana, and Côte d'Ivoire [106]. A BU focus in Kenya is confirmed [107]. The disease has been reported in over 30 countries. About 5,000–6,000 cases are reported yearly from only 15 of these countries [106]. A few cases also have been reported in nontropical areas of Australia, Japan, and China. Imported BU has been seen in industrialized countries where BU is not endemic [97].

In the Americas, BU seems most common in French Guyana, with about 200 cases reported since 1970 [108]. The incidence of BU is low in Asia and Oceania. Since 1971, about 400 cases have been reported in Papua New Guinea, whereas in other Asian countries very few cases have been confirmed. In Australia, the main focus is North Queensland, with 92 cases reported over the past 44 years [109].

BU is directly related to environmental factors and thus considered noncontagious [110]. The epidemiology of BU is strongly associated with wetlands, especially with slow-flowing or stagnant water. A plausible mode of transmission is a minor, often unnoticed skin trauma that permits inoculation of *M. ulcerans*. The mode of transmission may be related to the geographic region [111].

M. ulcerans DNA is detectable in some aquatic insects, prompting investigation into biting insects as vectors infecting humans. Portaels et al. reported the first direct isolation of *M. ulcerans* from a water strider, an aquatic insect that however does not bite humans [112]. In Australia, BU may be a zoonosis transmitted by mosquitoes, from indigenous marsupials such as the koala bear and opossum to humans [113]. There may be mammals involved in Africa too [114]. Recently amoebas were implicated as a possible reservoir [115].

6.6.2.1 Clinical Picture

Like in other mycobacterial diseases, exposure of the skin to *M. ulcerans* may lead to one of three outcomes: clearance of the infection, clinical disease soon after infection (primary BU), or subclinical or asymptomatic infection (latent BU) that may later reactivate and produce disease. It is most likely that many individuals exposed to *M. ulcerans* clear the infection and never develop disease [97, 98].

The incubation period of primary BU is estimated to be 2–3 months. Delayed onset of disease, i.e., ≥3 months after leaving an endemic area, may represent activation of latent infection [97, 98]. In contrast, the incubation period may be short (≤15 days), with lesions developing proximal to a bruise or sprain, without clinically detectable damage to the skin. This could be an activation of latent *M. ulcerans* infection caused by local trauma [97].

BU presents with a spectrum of symptoms, which may depend on time of consultation, host immune status, inoculum size, inoculation depth, geographic area, and strain virulence. There can be a striking discrepancy between the complaints and the symptoms as even impressive lesions may be painless. Together with stigma and fear of hospital admittance and surgery, this led as to delayed care-seeking behavior [103]. Delayed care results in more ulcerative forms. The disease develops through two active stages, non-ulcerated and ulcerated lesions (Fig. 6.16) to the third stage, the healed or scarred lesion. There may be mixed forms however, with

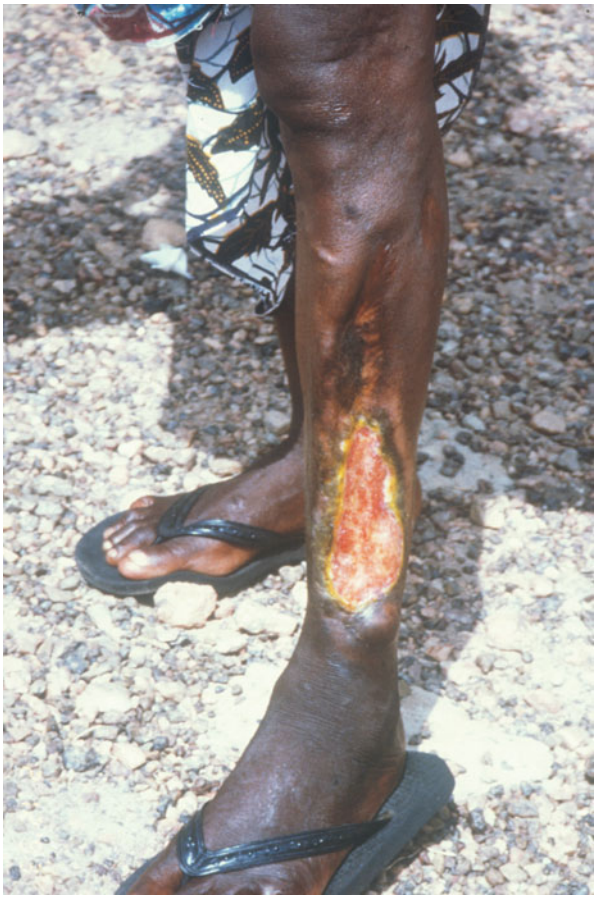

Fig. 6.16 Buruli ulcer (Courtesy of Father George, Ghana)

different stages presenting in the same site or at a different body site. Also disseminated forms occur, through spread by continuity or by lymphohematogenous spread. Bone involvement presents as osteomyelitis and occurs in up to 10 % of patients in Africa [116, 117]. As such, it is important to examine patients thoroughly, looking for new and old lesions. The patient may be unaware of scars from healed BU [97]. HIV seropositivity may be associated with aggressive BU [118].

Non-ulcerative forms often occur in early stages and may heal spontaneously. Non-ulcerative lesions may progress to ulcers after a few weeks to months, bringing patient to the doctor.

Clinical criteria supporting the diagnosis of BU include [97]:

- ≥1 painless ulcers lasting at least several weeks, undermined edges (Fig. 6.17)
- Nodule, plaque or wheal, or depressed scar
- Swelling over a painful joint, suggesting bone involvement
- No fever or regional lymphadenopathy (assumes no bacterial superinfection)

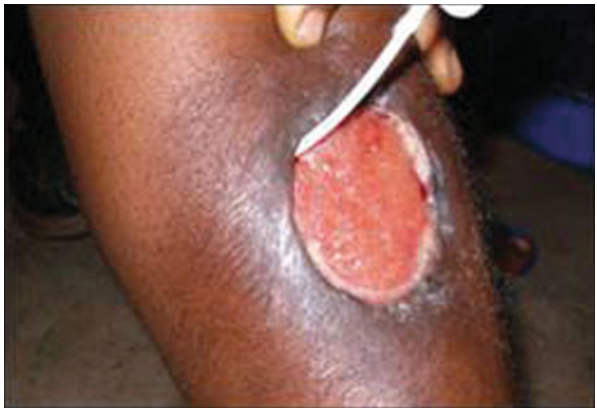

Fig. 6.17 Buruli ulcer with undermined edges (Courtesy of Mr. Vandi, Ivory coast)

- Patient <15 years of age
- Patient lives in, or traveled to, a BU endemic region, particularly West Africa

The disease may also be classified in three categories, according to lesion size, which may be helpful for choosing a treatment regimen:

Category I: single lesion, <5 cm in longest diameter
Category II: single lesion, 5–15 cm in longest diameter
Category III: single lesion, >15 cm in longest diameter, multifocal lesions, lesions at critical sites (eye, breast, genitalia), or bone involvement [87]

6.7 Diagnosis [106]

Many conditions resemble BU. Differential diagnoses include bacterial, deep fungal and parasitic infections, inflammatory lesions, and tumors. For ulcerative and edematous BU, the differential diagnosis includes tropical phagedenic ulcer, leishmaniasis even anthrax, and necrotizing fasciitis [119]. Most of these conditions, unlike BU, are painful, and a phagedenic ulcer emits an unpleasant odor. Painful ulcers may indicate secondary infection.

6.7.1 Collection of Clinical Specimens for Laboratory Testing

For routine assessment of suspected BU, for culture or PCR, ulcers should be swabbed or scraped at the undermined rims. Fine needle aspiration can be used [120]. Lesion biopsies, punch or excisional, are appropriate for suspected imported BU in an industrialized country. If surgery is conducted, specimens should be collected from excised tissues for bacteriological and histopathological analyses.

Curetted bone samples should be cultured to determine the cause. Sampling of at least two sites of each lesion is suggested, which may increase sensitivity over a single sample by up to 25 % [121, 122].

6.7.2 Laboratory Confirmation

Confirmation of BU is important because treatment may involve a moderately toxic antibiotic (streptomycin) and sometimes surgery.

Two out of four laboratory tests should be positive in order to confirm the diagnosis [97].

Lesion swabs or preferably scraping material or material obtained by biopsy may be used for:

1. Direct smear examination for AFB, i.e., Ziehl-Neelsen or auramine stain
2. In vitro culture on mycobacteriological media, at 30–32 °C
3. PCR amplification of insertion sequence 2404 (IS*2404*), which is considered to be specific for *M. ulcerans*
4. The fourth technique, punch biopsy, that allows for histopathologic examination

Laboratory tests vary in sensitivity. Sensitivity is 60–80 % for direct smear examination for AFB, 20–80 % for culture, and >90 % for PCR and histopathology. Direct smear and culture provide about 60 % sensitivity for nodules versus up to 80 % for edematous forms. PCR and histopathology provide >90 % sensitivity for all forms [123].

Histopathology may confirm BU or suggest another diagnosis. Culture can be useful for tracking treatment response [123]. At community level, direct smears are useful, but rapid diagnostic tests are needed. Simple methods for the detection of mycolactone or *M. ulcerans*-specific proteins in lesions or other fluids are under investigation [124].

6.8 Treatment

Historically, BU treatment has consisted mainly of wide excision. Antibiotics were generally considered ineffective, although, already by the 1970s, Meyers indicated that RMP could be used for early lesions [125].

In 2004, supported by data [126], WHO advocated a provisional antibiotic regimen, composed of oral RMP (10 mg/kg) + intramuscular streptomycin (S) (15 mg/kg), given daily for 8 weeks under supervision, with surgery as needed [127].

In 2010, the first randomized trial of RMP+S for “early, limited” BU, defined as lesions of <6 months duration composed of nodules or ulcers <10 cm in diameter, was reported [128]. RMP+S was given daily for 8 weeks or daily for 4 weeks, followed by all-oral RMP + clarithromycin (CLR) daily for 4 weeks, all without surgery. >90 % of the BU patients were cured after 1 year.

The current WHO recommendations for treatment are [129]:

- A combination of specific antibiotics for 8 weeks as first-line treatment for all forms of active disease
- Wound care
- Prevention of disability
- Surgery to remove necrotic tissue, cover large skin defects, and correct deformities

In general, recurrence rates in Category I and II disease after completing an RMP+S-based regimen are low (1–2 %) [97].

Despite the encouraging success of antibiotics for BU [130], extensive disease still requires surgery. However, the point in time at which surgery should be performed in relation to antibiotic treatment is not clear [97]. Twelve weeks of RFM+S for osteomyelitis did not prevent dissemination to other bones, despite one or more surgical procedures [130, 131].

Clearly, management of severe BU, such as length of antibiotic treatment and when to perform surgery, needs further investigation [132]. Physiotherapy, especially for Category III disease, should be instigated to prevent contractures [133].

Small case series describing 4–8 weeks of all-oral regimens for BU, including RMP+CLR in Benin and RMP + moxifloxacin in Australia, are encouraging [134, 135]. The all-oral regimens are less toxic and are relevant in pregnancy, in which streptomycin is contraindicated [136]. Recent investigations showed that RMP lowers CLR serum levels by 65 % and moxifloxacin serum levels by 30 %. The exact clinical relevance of these findings is still to be determined [137, 138].

Sometimes BU worsens during antibiotic treatment, and this may be due to an increased CMI response [139, 140]. Lesions developing after treatment is completed may represent immune responses to subclinical foci of *M. ulcerans*, treatment failures, or reinfections [141]. Sometimes steroids are needed [142].

6.9 Prevention

In tropical rural settings where BU is endemic, protection against contamination of the skin is virtually impossible. Wearing protective clothing, immediate cleansing of any skin injury, and the use of protected water sources in villages may reduce BU [143].

BCG vaccination may protect against BU, estimated 6–12 months after vaccination, and neonatal BCG vaccination may reduce the risk of BU osteomyelitis [144].

M. ulcerans, as an intracellular organism, triggers CMI [145, 146]. BURULIVAC, a collaborative project funded by the European Union under the Seventh Framework Programme, supports efforts to identify vaccine candidates based on DNA engineering and virulence factors, including mycolactone [147]. A mouse model for research has been developed [148].

6.9.1 Nontuberculous Mycobacteria

Nontuberculous mycobacteria (NTM) (synonyms atypical mycobacteria (ATM) and mycobacteria other than tubercle bacilli (MOTT)) are implicated in cutaneous infection [6, 147, 149].

NTM are usually transmitted from environmental sources by ingestion, inhalation, or inoculation [1]. These environmental sources may include aerosols, water (surface water, ponds, streams, municipal waters), soil, dust, food products, and contaminated medical equipment.

6.9.1.1 **Diagnosis** (Table 6.1)

Culture is the golden standard in the diagnosis of NTM. Culture may be negative if the laboratory is not informed of the clinical suspicion because specific conditions are required for culture. Mycobacterial infections usually have some specific features in a skin biopsy, so this may help direct suspicion. The histologic findings of an infection vary by the age of the lesion. Scanning a developed lesion shows a typical granulomatous dermatitis, which forms an extensive inflammatory nodular infiltrate within the dermis. Early lesions may show acute suppurative inflammatory processes with little granuloma formation and sometimes extensive neutrophils. The epidermis may show pseudoepitheliomatous hyperplasia with or without ulceration. Sometimes there are tuberculoid granulomas with varying degrees of abscess formation. The principal infiltrate however is mixed lymphohistiocytic with a few multinucleated giant cells and scattered neutrophils. Acid-fast bacilli may be scarce and are often not found. Molecular techniques are now available but sensitivity and specificity varies. A positive tuberculin test is not specific for tuberculosis but may direct toward NTM as well. To narrow down the possibilities in such cases, interferon gamma release assay (IGRA) may also be performed; however, this is known to cross-react with the NTM *M. kansasii*, *M. marinum*, and *M. szulgai* (and also with *M. leprae*). It is not a standard test in skin disease.

Detection of new species and subspecies of NTM is a constant issue as at the same time it has to be realized how limited the molecular tools are in determining and classifying mycobacteria [150]. This leads to new classifications, for example, of clinical disease which was formerly always attributed to *M. tuberculosis*: Lichen scrofulosorum may also be caused by *M. avium*, papulonecrotic tuberculid reaction by *M. kansasii*, lupus vulgaris by *M. xenopi*, and scrofuloderma by *M. haemophilum*. Pulmonary infections which are attributed to *M. tuberculosis* worldwide are to date also partly attributable to NTM [150].

A natural division occurs between slowly and rapidly growing species. Mycobacteria that form colonies clearly visible to the naked eye within 7 days on subculture are termed rapid growers, while those requiring longer periods are termed slow growers.

The common cutaneous disease-producing rapid-growing species are *M. abscessus*, *M. chelonae*, and *M. fortuitum*. The most common slow growers that cause skin disease are *M. marinum*, *M. avium* complex, *M. haemophilum*, and *M. kansasii* [150].

Mycobacterium marinum Infections [4]

Swimming pool or fish tank granuloma is caused by *M. marinum*, a mycobacterium which causes disease in fresh, brackish, and saltwater fish and occasionally in humans. It is found in aquariums, pools, natural water supplies, and salt water and is among the most common NTM known to cause opportunistic infection in humans. It has an incubation time of 2 weeks to several months.

Clinical Picture

The infection is preceded by trauma, often there is history of cleaning a fish tank or swimming in open water. The initial lesion starts as an inflammatory papule after an incubation period of 2–6 weeks. The papule then gradually enlarges into violaceous nodules or plaques which may ulcerate or show a warty surface. These lesions are painless. They may heal spontaneously in the course of months to years.

M. marinum infections are one of the causes of nodular (also called sporotrichoid after the lymphatic spread of sporotrichosis) lymphangitis where nodules and/or ulcerating lesions are seen along the lymphatic vessels. Deep infections such as tenosynovitis, osteomyelitis, arthritis, and bursitis may occur. They are unusual but more common in immune-deficient patients.

Diagnosis

Diagnosis is based on the clinical picture; the preferential localization in combination with a history of aquatic activity coinciding with skin trauma should give a high index of suspicion (Fig. 6.18). The diagnosis should be confirmed by diagnostic tests. Histopathology can be nonspecific in the early stage of the disease. After 6 months a granulomatous reaction develops. Acid-fast bacilli may be seen; the absence does not rule out a *M. marinum* infection. Cultures can be performed from aspirates or biopsies. Growth is optimal at 30–32 °C and cultures should be maintained for 6 weeks. PCR techniques from biopsy material may provide a diagnosis within days.

Treatment

In superficial cutaneous infections, monotherapy with minocycline, clarithromycin, doxycycline, and trimethoprim-sulfamethoxazole can be effective, but drug resistance varies, and therefore combination therapy of usually two or three drugs is recommended. Ciprofloxacin has shown considerable effectiveness. In cases of severe infection, including those with a sporotrichoid distribution pattern, a combination of RMP and ethambutol (ETM) is the recommended regimen.

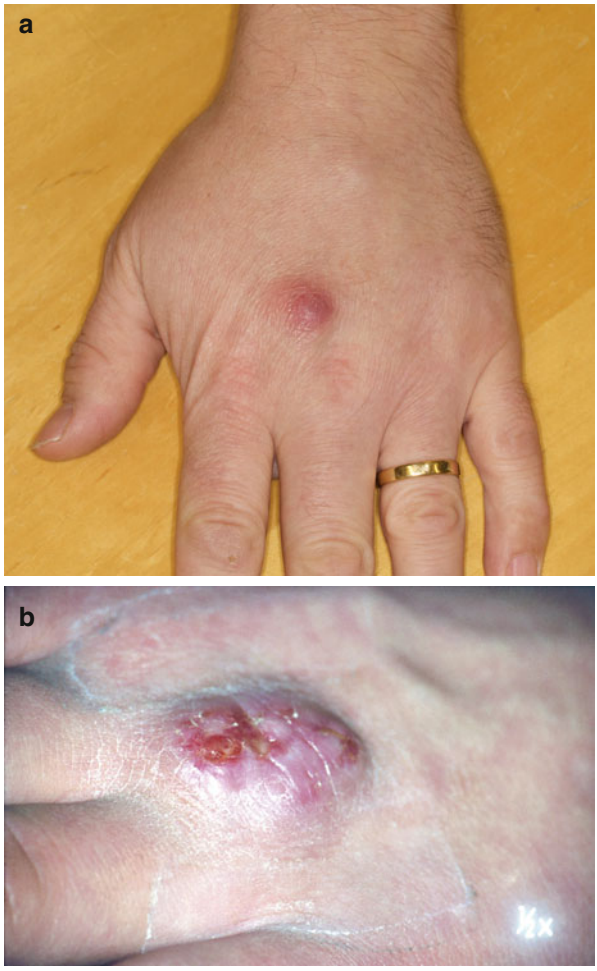

Fig. 6.18 Early *M. marinum* infection

Response to treatment is slow. Treatment is continued until 4 weeks after clinical cure and usually takes 4–9 months. Surgical treatment is not usually recommended. Cryotherapy, X-ray therapy, electrodessication, photodynamic therapy, and local hyperthermic therapy have been reported as effective alternatives. *M. marinum* infection should always be included in the differential diagnosis of patients with poor-healing plaques, nodules, or ulcers on the upper extremities and a history of exposure to aquariums [151].

Mycobacterium kansasii Infections [4]

M. kansasii causes disease in humans throughout the world and is often associated with AIDS. It has been isolated from cattle and swine. However, water is most likely its true habitat. It affects patients of all ages.

Clinical Picture

The most common manifestation is chronic pulmonary disease. Inoculation of the skin is in general through a small wound. Cutaneous lesions include erythematous to violaceous papules and plaques and also pustular, crusted, or verrucous papules or nodules. Lesions can resemble pyogenic abscesses, cellulitis, or sporotrichosis. Cervical lymphadenitis is reported in children.

Treatment

M. kansasii shows good in vitro susceptibility to rifampicin, rifabutin, ethambutol, ethionamide, amikacin, streptomycin, clarithromycin, sulfamethoxazole, and ciprofloxacin. However, when monotherapy is given, drug resistance is common [152, 153]. Therapy usually consists of isoniazid, rifampicin, and ethambutol or rifampicin, ethambutol, and macrolide [154].

Mycobacterium scrofulaceum Infections

M. scrofulaceum was widely distributed in tap water and soil but has become very rare in the last decades. It is included in the "MAIS group" which consists of *M. avium*, *M. intracellulare*, and *M. scrofulaceum. M. scrofulaceum* causes pulmonary infection and it may be the cause of cervical lymphadenitis in children. Cutaneous infection has been described as multiple subcutaneous abscesses and sporotrichoid infection.

Treatment

For childhood cervical lymphadenitis, surgery is the recommended treatment, in which the lesion is excised without chemotherapy. The success rate for this treatment is 95 %. Drugs which are used in treatment include isoniazid, rifampin, and streptomycin [154]. Good results were described with a combination of isoniazid, ethambutol, rifampin, and ofloxacin [155]. Clarithromycin is a good addition [156].

Mycobacterium haemophilum Infections

M. haemophilum causes skin, joint, bone, and pulmonary infections in immunocompromised persons and of submandibular lymphadenitis in children. Most infections occur in patients with AIDS and in transplant recipients. *M. haemophilum* skin infection has been associated with permanent eyebrow makeup and tattoos [157, 158].

Infections with *M. haemophilum* have been reported in a broad geographical range. The natural habitat and route of infection are unknown.

Treatment

Lymphadenitis in children: excision. *M. haemophilum* appears to be susceptible, in vitro, to ciprofloxacin, clarithromycin, rifabutin, and clofazimine but resistant to isoniazid and ethambutol [159]. In vitro observations may not relate to outcome of treatment in vivo and should be interpreted with extreme caution. Susceptibility test assays have not been properly standardized, because of the fastidious nature of *M. haemophilum* and the need to supplement the media [160].

Mycobacterium fortuitum Infections

M. fortuitum has been isolated from water, soil, and dust. Primary cutaneous disease is seen at all ages. It has been implicated in numerous outbreaks of hospital infections.

The clinical manifestations are localized cellulites, frequently with draining abscesses and nodules. Mostly a history of a penetrating injury with possible soil or water contamination is reported [161]. Postoperative infections, in general, develop 3 weeks to 3 months after surgery or trauma.

Treatment

Ciprofloxacin, amikacin, and cefoxitin are considered as first-line drugs. Alternative drugs are doxycycline, imipenem, ethambutol, and co-trimoxazole. A combined regimen, preferably with three drugs, should be used for 2–4 weeks, followed by ciprofloxacin [162] and a companion drug (e.g., clarithromycin despite the bacteria becomes easily resistant to this drug) for 3 months.

Mycobacterium chelonae and M. abscessus Infections

The two closely related species *M. chelonae* and *M. abscessus* (which consists of two subspecies) cause similar diseases worldwide. The skin disease caused by these opportunistic pathogens can be localized, similar nature to *M. fortuitum*, or may present as a disseminated disease with cellulitis and multiple often draining (sub) cutaneous nodular lesions in "immunocompromised" patients.

The localized infection may occur at all ages, typically after a trauma or a surgical incision. Inoculation may also follow tattooing, implicating contaminated water for the dilution of ink, or cosmetic procedures such as dermal filling, where contaminated ice used to cool the skin may be the cause [163, 164].

Treatment

The only clinical trial performed for this infection type applied clarithromycin monotherapy without distinguishing *M. chelonae* from *M. abscessus*; although just one patient failed on treatment with an acquired drug resistance, the use of monotherapy is no longer recommended [154]. Tobramycin and clarithromycin are drugs of choice for *M. chelonae* [160]. In general, cefoxitin and amikacin are active against both subspecies of *M. abscessus* [165, 166]. Clarithromycin is only active against *M. abscessus* subsp. *bolletii*, as *M. abscessus* subsp. *abscessus* possesses an erm gene that induces resistance in vitro and in vivo [160].

Mycobacterium szulgai

The natural habitat of *M. szulgai* is unknown. It has, however, been isolated from snails and tropical fish. The predominant localization of infections is pulmonary. Cases of skin infection even after minor trauma have been reported: cellulitis, inflamed tender nodules leading to draining abscesses.

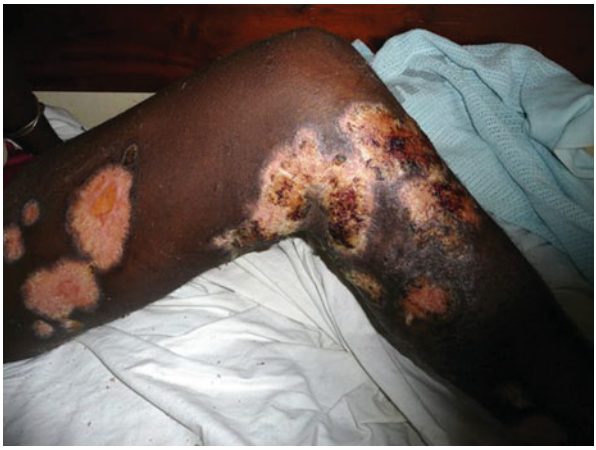

Fig. 6.19 An infection with a NTM (Courtesy of Ayder Hospital, Mekelle, Ethiopia)

Treatment

Triple-drug therapy with rifampicin, ethambutol, and clarithromycin guided by sensitivity testing [167]. It is recommended to use multiple drugs to reduce development of resistance. The treatment may take up to 1 year.

Mycobacterium avium-intracellulare Complex Infections

This group of mycobacterial species (*Mycobacterium avium-intracellulare* complex (MAC)), with several closely related species, occurs worldwide in nature. It is the most common group of NTM infections associated with AIDS in the West. The infection is caused by two closely related and difficult to distinguish bacteria, *M. avium* and *M. intracellulare*. These two bacteria can be found in drinking water, dirt, and household dust. MAC may be isolated in more than 30 % of fecal samples. It primarily causes opportunistic infections in the immunosuppressed, *M. intracellulare* tends to cause lung disease, and *M. avium* causes lung disease and lymphadenitis in children and disseminated disease in the immunocompromised. Symptoms of disseminated *M. avium-intracellulare* infection include fever, night sweats, weight loss, abdominal pain, fatigue, and diarrhea [168].

Skin involvement occurs in the course of disseminated disease, rarely by inoculation. Depending on the degree of immune suppression, widespread skin involvement may present as papules; nodules; plaques, with possible abscess formation; and ulcers (Fig. 6.19). Lymph node involvement can occur.

Treatment

In general, MAC infection is treated with two or three antimicrobials for at least 12 months. Commonly used first-line drugs include macrolides (clarithromycin or azithromycin), ethambutol, and rifamycins (rifampicin, rifabutin). Aminoglycosides, such as streptomycin and amikacin, are also used as additional agents as is ciprofloxacin [169] although supportive evidence for the latter is absent.

Lymphadenitis in Children: Surgery

Treatment of cutaneous atypical mycobacterial infections depends upon the infecting organism, the severity of the infection, and host immunity. In most cases a course of antibiotics is necessary. These include rifampicin, ethambutol, isoniazid, minocycline, ciprofloxacin, clarithromycin, azithromycin, and co-trimoxazole. Treatment of cutaneous localized disease is generally continued until 1 month after clinical cure, for pulmonary and generalized infection even longer: 18–24 months.

Usually treatment consists of a combination of drugs [170, 171].

There are some points to consider when treating atypical mycobacterial infections:

- *M. marinum* bacteria are resistant to isoniazid. Treatment with other antibiotics should be continued for at least 4 weeks after resolution of the skin lesions.
- *M. kansasii* should be treated for at least 18 months.
- *M. chelonae* is best treated by clarithromycin in combination with another agent. Related *M. abscessus* requires intravenous drugs including amikacin and cefoxitin. Sometimes surgical excision is the best approach for both species.
- AIDS patients on HIV protease inhibitors cannot be treated with RMP because RMP significantly increases the breakdown of these drugs. Rifabutin is a suitable alternative.

Treatment

For treatment of cutaneous infections by NTM, it is preferable to select the drugs based on the antimicrobial susceptibility profile. In vitro susceptibility testing is useful for rapidly growing mycobacteria (RPM) but not for slow-growing NTM MAC, *M. haemophilum*, and *M. szulgai* as in vitro results in this group do not correlate with in vivo response to treatment [160]. Empiric therapy is sometimes necessary in case of strong suspicion with negative culture and no identification by means of PCR (Table 6.1).

Duration of treatment is not fixed and is based on clinical judgment and will require in general 6–9 months [149].

For the treatment of rapid growers, it is important to follow the results of in vitro tests. For slow growers that is not the case since in vivo and in vitro results often are not related.

6.10 Drugs Commonly Used in Mycobacterial Infection

6.10.1 Rifampicin and Rifamycin

Rifampin and related rifamycins are the most important drugs for the treatment of mycobacterial infections. The rifamycins are a group of antibiotics that are synthesized either naturally by the bacterium *Amycolatopsis mediterranei* (old name *Streptomyces mediterranei*) or artificially. Rifamycins are particularly effective against mycobacteria and are therefore used in the treatment of tuberculosis, leprosy, Buruli ulcer, and many NTM infections.

The antibacterial activity of rifamycins relies on the inhibition of bacterial DNA-dependent RNA synthesis [171]. This is due to the high affinity of rifamycins to prokaryotic RNA polymerase. Rifampicin and its analogs kill actively multiplying extracellular organisms, intracellular mycobacteria, and semidormant mycobacteria in the tissues.

The addition of rifampicin to treatment regimens for tuberculosis can shorten treatment duration for active disease from 12 to 6 months and for latent infection from 9 months to 2–3 months. In leprosy a single 600 mg dose kills 99 % of all live bacilli, but sadly not the metabolic inactive persisters.

Because of their potencies and sterilizing activities, rifamycins are the cornerstone of modern therapy for most mycobacterial infections and are extremely effective in the treatment of latent infections [172, 173].

6.10.1.1 Dosages

Daily regimen: 10 mg/kg (up to 600 mg/day) orally or IV once a day. For TB there is a discussion, one may consider even about four times this dose, and this could help against persisters [160].

Intermittent regimen: 10 mg/kg (up to 600 mg/dose) orally or IV two or three times a week.

For children 10–20 mg/kg.

6.10.1.2 Adverse Effects

Via lever enzymes P450, CYP, 1A2, 2C9, 2C19, and 3A4, it influences concomitant treatments. Its toxicity is predominantly hepatic and allergic [174]. Hepatic toxicity is dose related and has been observed mainly in patients with underlying liver disease, which then can be fatal.

The “allergic” effects are usually associated with intermittent or prolonged therapy. These allergic effects may be minor (cutaneous, gastrointestinal, or an influenza-like syndrome) or major (hemolytic anemia, shock, or acute renal failure) [175]. Well known is the leprosy flu syndrome due to intermittent rifampicin with less than 3 weeks interval [176], and this can be fatal.

There may be some orange discoloration of urine, tears, and sweat.

6.10.2 Ethionamide

Ethionamide (2-ethylthioisonicotinamide) is an antibiotic used in the treatment of tuberculosis. It was discovered in 1956 [177].

Ethionamide, a prodrug, is activated by the enzyme EthA, a monooxygenase in *Mycobacterium tuberculosis*, and binds nicotinamide adenine dinucleotide to form an adduct which inhibits the 2-trans-enoyl-acyl carrier protein reductase (InhA) in the

same way as isoniazid [178]. Expression of the EthA gene is controlled by EthR, a transcriptional repressor. It is understood that improving EthA expression will increase the efficacy of ethionamide and so EthR inhibitors are of great interest to co-drug developers [179]. The action may be through disruption of mycolic acid [180].

It is a thioamide and used in regimens to treat multidrug-resistant and extensively drug-resistant tuberculosis. It has been proposed for use in combination with gatifloxacin [181].

Dosages: 500 mg to 1 g orally (15–20 mg/kg) in 1 or divided doses per day. Maximum dose: 1 g orally per day. Children: 10–20 mg/kg orally in 2 or 3 divided doses per day or 15 mg/kg orally once per day after meals [171].

6.10.2.1 Adverse Effects

The most common side effects of ethionamide are gastrointestinal. These appear to be dose dependent, with approximately 50 % of patients unable to tolerate 1 g as a single dose. Effects may be minimized by decreasing dosage, by changing the time of drug administration, or by the concurrent administration of an antiemetic agent.

Psychotic disturbances (including mental depression) and postural hypotension have been reported. Concurrent administration of pyridoxine has been recommended to prevent or relieve neurotoxic or pellagra-like effects.

Transient increases in serum bilirubin, SGOT, and SGPT; hepatitis (with or without jaundice) can be seen.

Hypersensitivity reactions including rash, photosensitivity, thrombocytopenia, and purpura have been reported. Hypoglycemia, hypothyroidism, gynecomastia, impotence, and acne also have occurred [182]. The management of patients with diabetes mellitus may become more difficult in those receiving ethionamide.

6.10.3 Ethambutol

Ethambutol is a bacteriostatic antimycobacterial drug prescribed to treat tuberculosis. It is usually given in combination with other tuberculosis drugs, such as isoniazid, rifampicin, and pyrazinamide.

Ethambutol is bacteriostatic against actively growing TB bacilli. It works by obstructing the formation of cell wall. Mycolic acids attach to the 5′-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall [183].

Dosages: Adult 15 mg/kg once a day. Treating relapses: for 2 months 25 mg/kg once a day, followed by 15 mg/kg again. Children the same. For MAIC: 900 mg once a day.

6.10.3.1 Adverse Effects

Ethambutol may induce a decrease in vision due to optic neuritis. This effect is dose related and is generally reversible when administration of the drug is discontinued in time. Irreversible blindness has been reported.

Other reactions are gastrointestinal, allergic skin reactions, and infrequently polyneuritis [184].

6.10.4 Fluoroquinolones

Ofloxacin and moxifloxacin are broad-spectrum fluoroquinolones that are active against both Gram-positive and Gram-negative bacteria but also against mycobacteria: *M. tuberculosis*, *M. leprae*, and several NTM [185, 186].

Fluoroquinolones interfere with DNA replication by inhibiting an enzyme complex called DNA gyrase. This can also affect mammalian cell replication. Some congeners of this drug family display high activity not only against bacterial topoisomerases but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and in vivo tumor models [187].

Although a quinolone is highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. There is debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe and non-abating adverse reactions experienced by some patients following fluoroquinolone therapy.

Dosages: MAI 400 mg orally every 12 h. Tuberculosis: 300–400 mg orally or IV every 12 h. Leprosy: 400 mg OD. For children with leprosy 200 mg OD. Basically the drug is contraindicated for children.

6.10.4.1 Adverse Effects

Quinolones have few direct adverse effects, most notably nausea, headache, dizziness, and confusion. Less common but more serious adverse events include prolongation of the QT interval, phototoxicity, liver enzyme abnormalities, arthropathy, and cartilage and tendon abnormalities, the latter particularly in children [188]. Moxifloxacin is contraindicated in patients with myasthenia gravis.

6.10.5 Isoniazid (INH)

Isoniazid also known as isonicotinylhydrazine (INH) is an organic compound that is the first-line medication in prevention and treatment of tuberculosis. The compound was first synthesized in the early twentieth century, but its activity against

tuberculosis was first reported in the early 1950s. With the introduction of INH, a cure for tuberculosis was for the first time considered conceivable.

INH is available in tablet, syrup, and injectable forms (given intramuscularly or intravenously). It is available worldwide, is inexpensive, and is generally well tolerated.

It is a prodrug and must be activated by a bacterial catalase-peroxidase enzyme that in *M. tuberculosis* is called KatG. KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex. This complex binds tightly to the enoyl-acyl carrier protein reductase known as InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acid, required for the mycobacterial cell wall [189].

INH is bactericidal to rapidly dividing mycobacteria but is bacteriostatic if the mycobacteria are slow growing [190, 191].

Dosage: Adults 300 mg OD; children 5–6 mg/kg OD.

6.10.5.1 Adverse Effects

INH inhibits the P450 system. Severe and sometimes fatal hepatitis may occur within the first 3 months of treatment and many months after treatment. Risk is related to age and increased with daily alcohol consumption. The N-acetylhydrazine metabolite is believed to be responsible for this hepatotoxic effect. The rate of acetylation is genetically determined. Approximately 50 % of Blacks and Caucasians are slow inactivators; the majority of Inuit and Asians are rapid. The half-life in fast acetylators is 1–2 h, while in slow acetylators, it is 2–5 h. Elimination depends on renal function, but the half-life may be prolonged in liver disease. The rate of acetylation has not shown to alter the effectiveness, but there is an increased risk of toxicity. Hepatitis is 250 times more common in slow acetylators.

It may give pellagra-like symptoms, CNS and peripheral neuropathy, gastoenteral and skin problems. Pyridoxine (vit B6) should counteract most. LE is described [192].

6.10.6 Pyrazinamide

Since the discovery of pyrazinamide in 1952 [47], and its routine use to treat TB, the duration of treatment required to achieve acceptable relapse rates has been reduced from 9 to 12 months to the current 6 months [193], although its bactericidal activity is inferior to that of INH and rifampin. It is largely bacteriostatic but can be bactericidal.

Pyrazinamide is a prodrug. It diffuses into *M. tuberculosis*, where the enzyme pyrazinamidase converts pyrazinamide to the active form pyrazinoic acid. Under acidic conditions, the pyrazinoic acid slowly leaks out and converts to the protonated conjugate acid, which is thought to diffuse easily back into the bacilli and

accumulates. Thus, more pyrazinoic acid accumulates inside the bacillus at acid pH than at neutral pH [194].

Pyrazinoic acid was thought to inhibit the enzyme fatty acid synthase (FAS) I, which is required by the bacterium to synthesize fatty acids [195]. It was also suggested that the accumulation of pyrazinoic acid disrupts membrane potential and interferes with energy production, necessary for survival of *M. tuberculosis* at an acidic site of infection. Further studies reproduced the results of FAS I inhibition as the putative mechanism [196]. This study was followed by an in vitro assay of tuberculous FAS I enzyme that tested the activity with pyrazinamide, pyrazinoic acid, and several classes of pyrazinamide analogs. Pyrazinamide and its analogs inhibited the activity of purified FAS I [197]. Pyrazinoic acid binds to the ribosomal protein S1 (RpsA) and inhibits trans-translation. This may explain the ability of the drug to kill dormant mycobacteria [198].

Dosage: 15–30 mg/kg (up to 2 g) orally OD. Children non-HIV: daily therapy, 15–30 mg/kg/dose (maximum, 2 g/dose) OD. HIV: 20–40 mg/kg/dose once daily (maximum, 2 g/day).

6.10.6.1 Adverse Effects

Dermatologic side effects are rare and include rash, urticaria, pruritus, skin pigmentation, desquamation, and photosensitivity. Gastrointestinal side effects include nausea, vomiting, and anorexia. Renal side effects include dysuria and interstitial nephritis. Hepatotoxicity (1%) appears to be dose related and may appear at any time during therapy [199].

6.10.7 Para-Aminosalicylate Sodium (PAS)

The 4-aminosalicylic acid is commonly known as PAS. Since the 1940s it was used for inflammatory bowel diseases (IBDs), where it showed to have great potency in ulcerative colitis and Crohn's disease, both by some thought to be related to mycobacteria.

PAS was introduced for use in tuberculosis in 1948. It was the second antibiotic found to be effective after streptomycin. PAS formed part of the standard treatment for tuberculosis prior to the introduction of rifampicin and pyrazinamide. Its potency is less than that of the current five first-line drugs (isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin), but it is still useful in the treatment of multidrug-resistant tuberculosis.

It is thought to inhibit folic acid biosynthesis and uptake of iron [200]. Mutations in the thyA gene encoding the enzyme thymidylate may lead to resistance. Induction of the folate biosynthesis pathway has been identified in PAS-resistant *M. tuberculosis*, suggesting that PAS may act as a folate antagonist [201].

Dosage: The dose when treating tuberculosis is 150 mg/kg/day divided into two to four daily doses; the usual adult dose is therefore approximately 2–4 g four times a day.

6.10.7.1 Adverse Effect

A joke in the past was that under the windows of a TB ward plants did not grow and that this was due to the terrible taste of PAS. Common side effects are nausea, vomiting, diarrhea, and abdominal pain. Goiter with or without myxedema has been described. Other side effects are fever, skin eruptions, infectious mononucleosis-like syndrome, leukopenia, agranulocytosis, thrombocytopenia, hemolytic anemia, jaundice, hepatitis, encephalopathy, Loffler syndrome, and vasculitis [202].

6.10.8 Streptomycin

Streptomycin was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is bactericidal. Streptomycin cannot be given orally but must be administered by regular deep intramuscular injections.

Streptomycin is a protein synthesis inhibitor. It binds to the small S12 rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formylmethionyl-tRNA to the 30S subunit [203]. This leads to codon misreading, inhibition of protein synthesis, and ultimately death of microbial cells through mechanisms that are still not understood. Human ribosomes differ from bacterial ribosomes structurally and remain intact. At low concentrations, however, streptomycin only inhibits growth of the bacteria by inducing prokaryotic ribosomes to misread mRNA [204].

Dosage: Adults and children, 15 mg/kg daily or two or three times weekly. Patients over 60 years may not be able to tolerate more than 500–750 mg daily. In Buruli ulcer: adults, 1,000 mg daily; children, 20 kg/300 mg; 30 kg/500 mg; 40 kg/740 mg. No patient should be given more than 90 doses of streptomycin (according to weight) in their whole lifetime.

6.10.8.1 Adverse Effects

Fever and rashes result from persistent use. The vestibular portion of cranial nerve VIII (the vestibulocochlear nerve) can be affected, resulting in tinnitus, vertigo, and ataxia. Other side effects are nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis.

6.10.9 Cotrimoxazole

Trimethoprim/sulfamethoxazole or co-trimoxazole is a sulfonamide antibiotic used in the treatment of a variety of bacterial infections. It consists of one part trimethoprim to five parts sulfamethoxazole.

Opinions differ as to whether co-trimoxazole is a bactericidal or a bacteriostatic agent.

The synergy between trimethoprim and sulfamethoxazole was first described in a series of in vitro and in vivo experiments published in the late 1960s [205]. Trimethoprim and sulfamethoxazole have a greater effect when given together than when given separately, because they inhibit successive steps in the folate synthesis pathway.

Sulfamethoxazole acts as a false-substrate inhibitor of dihydropteroate synthetase. Sulfonamides such as are analogs of p-aminobenzoic acid (PABA) and thus are competitive inhibitors of the enzyme, inhibiting the production of dihydropteroic acid.

Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid.

Folic acid is an essential precursor in the de novo synthesis of the DNA/RNA nucleosides thymidine and uridine. Bacteria have to take up folic acid, from the host – if that is not possible, they are dependent on their own de novo synthesis – inhibition of the enzyme starves the bacteria of the two bases.

Dosage: Adults and children over 12 years 960 mg bd orally. Children ≥6–12 years 480 mg bd orally.

6.10.9.1 Adverse Effects

It has been associated frequently with mild allergic reactions and regular with serious adverse effects, including Stevens-Johnson syndrome, myelosuppression, mydriasis, agranulocytosis, and severe liver damage (cholestatic hepatosis, hepatitis, necrosis, and fulminant liver failure).

Due to displacement of bilirubin from albumin, there is an increased risk of kernicterus in the fetus during the last 6 weeks of pregnancy. Renal impairment, up to acute renal failure, and anuria have also been reported. These side effects may be fatal.

Folic acid and folinic acid were found equally effective in reducing the adverse effects of trimethoprim/sulfamethoxazole. The trophoblasts in the early fetus are sensitive to changes in the folate cycle. A recent study has found a doubling in the risk of miscarriage in women exposed to trimethoprim in early pregnancy [206].

Cotrimoxazole is a major cause of severe blistering drug reactions in the HIV-infected patient: Stevens-Johnson syndrome and toxic epidermal necrolysis (TEN).

6.10.10 Cycloserine

Cycloserine is an antibiotic effective against mycobacteria. It is produced by *Streptomyces garyphalus*. For the treatment of tuberculosis, it is only used as a second-line drug.

Cycloserine is an analog of the amino acid D-alanine. It interferes with an early step in bacterial cell wall synthesis in the cytoplasm by competitive inhibition of two enzymes, L-alanine racemase, which forms D-alanine from L-alanine, and D-alanylalanine synthetase, which incorporates D-alanine into the pentapeptide necessary for peptidoglycan formation and bacterial cell wall synthesis [207].

Dosage: 500 mg to 1 g orally per day, in one or two divided doses (10–15 mg/kg/day). Children: 10–15 mg/kg/day in two divided doses. Maximum dose: 1 g/day.

6.10.10.1 Adverse Effects

Most adverse reactions occurring involve the nervous system or are manifestations of drug hypersensitivity. Sudden development of congestive heart failure in patients has been reported.

6.10.11 Minocycline

Minocycline is a broad-spectrum tetracycline; it has a broader spectrum than the other members of the group. It is to date frequently used in mycobacterial infections.

It is a bacteriostatic antibiotic, classified as a long-acting type. Minocycline is the most lipid soluble of the tetracycline-class antibiotics. Minocycline is metabolized by the liver.

Minocycline passes directly through the lipid bilayer or passively diffuses through porin channels in the bacterial membrane. Tetracyclines like minocycline bind to the 30S ribosomal subunit, preventing the binding of tRNA to the mRNA-ribosome complex and interfering with protein synthesis [208].

Dosage: In NTM infections up to 100 mg BD is given. In leprosy 100 mg daily or 100 mg once a month.

6.10.11.1 Adverse Effects

Minocycline inhibits cytochromes P450 as do all tetracyclines; therefore, there are many drug interactions. Because it penetrates into the prostate and brain easily, it also has the greatest number of central nervous system (CNS)-related side effects of all the tetracyclines, such as vertigo and dreams. A common side effect is diarrhea.

In children up to age 9, minocycline may cause permanent staining of the teeth. Uncommon side effects (with prolonged therapy) include skin discoloration and autoimmune disorders that are not seen with other drugs in the class. Photosensitivity, which was expected, is hardly seen.

6.10.12 Doxycycline

Doxycycline, like minocycline, is lipophilic and can pass through the lipid bilayer of bacteria. Doxycycline reversibly binds to the 30S ribosomal subunits and possibly the 50S ribosomal subunit(s), blocking the binding of aminoacyl tRNA to the mRNA and inhibiting bacterial protein synthesis [208]. It is a more bioactive medication than the other tetracycline antibiotics, including minocycline. Conversely, minocycline is a broader spectrum drug than doxycycline and is used against a wider variety of bacteria. It can be considered against some RGM (*M. abscessus*), but most are resistant.

6.10.12.1 Adverse Effects

Doxycycline inhibits cytochromes P450 as do all tetracyclines; therefore, there are many drug interactions. Diarrhea is regularly seen, photosensitivity and dizziness hardly.

6.10.13 Dapsone

4,4'-Diaminodiphenylsulfone (DDS) was the magic drug for leprosy. It was for the first time synthetized by Fromm and Wittmann in 1908. It was used as an antibiotic first for streptococcal udder infections in cattle. For human it was for the high doses used too toxic. It was Faget in 1941, who first used the derivative promin for leprosy, and it was already used for tuberculosis.

As antibacterial, like all sulfonamides, dapsone inhibits bacterial synthesis of dihydrofolic acid, via competition with para-aminobenzoate for the active site of dihydropteroate synthetase [209].

Dosage: For adults 100 mg once a day (1 mg/kg), but sometimes because of hemolysis 50 mg. Children: 0.5–1 mg/kg.

6.10.13.1 Adverse Effects

The most prominent side effects of this drug are dose-related hemolysis (which may lead to hemolytic anemia) and methemoglobinemia. About 20 % of Nordic Caucasian and Celtic patients treated with dapsone suffer hemolysis, and this side

effect is slightly more common in those with glucose-6-phosphate dehydrogenase deficiency. Abnormalities in white blood cell formation, including aplastic anemia, are rare, yet are the cause of the majority of deaths attributable to dapsone therapy.

Toxic hepatitis has been reported. Jaundice may also occur as part of the dapsone reaction or dapsone syndrome. Dapsone is metabolized by the cytochrome P450 system. Dapsone metabolites produced by the cytochrome P450 2C19 isozyme are associated with the methemoglobinemia side effect of the drug.

Other adverse effects include nausea, headache, rash, insomnia, psychosis, and peripheral neuropathy.

Dapsone syndrome: The patient is ill and may have a rash, fever, jaundice, and eosinophilia; these symptoms will occur within the first 6 weeks of therapy or not at all and may be ameliorated by corticosteroid therapy.

6.10.14 Clofazimine

Clofazimine is a fat-soluble riminophenazine dye used in combination with rifampicin and dapsone as multidrug therapy (MDT) for the treatment of leprosy. It has been used for other mycobacterial infections in combination with other antimycobacterial drugs particularly to treat *Mycobacterium avium* infections in AIDS patients and *M. avium* ssp. *paratuberculosis* infection in Crohn's disease patients and in Melkersson-Rosenthal syndrome patients.

Clofazimine, initially known as B663, was first synthesized in 1954 in Dublin as an antituberculosis drug. The drug proved ineffective against tuberculosis, but in 1959 Chang identified its effectiveness against leprosy what later was confirmed by Brown and Hoogerzeil [210, 211].

Clofazimine works by binding to the guanine bases of bacterial DNA, thereby blocking the template function of the DNA and inhibiting bacterial proliferation. It also increases activity of bacterial phospholipase A2, leading to release and accumulation of lysophospholipids, which are toxic and inhibit bacterial proliferation [212].

Clofazimine also acts as FIASMA (functional inhibitor of acid sphingomyelinase) [213]. It may also bind to bacterial potassium transporters, thereby inhibiting their function.

Dosage: For leprosy for adults, 300 mg once a month and 50 mg once daily. For T2R sometimes 300 mg a day is given for a short time. For other mycobacteria 100–200 mg daily. Children according to weight.

6.10.14.1 Adverse Effects

Darkening of the skin is the major side effect. The fat becomes yellowish. There are a few toxic effects: Abdominal symptoms are dose dependent and usually not severe, but they may rarely be fatal. Rare reports have included splenic infarction, bowel obstruction, and gastrointestinal bleeding.

6.10.15 Azithromycin

Azithromycin is a subclass of macrolide antibiotics. It is derived from erythromycin, with a methyl-substituted nitrogen atom incorporated into the lactone ring, thus making the lactone ring 15-membered. It works against quite a number of mycobacteria, particularly the MAC. *M. abscessus* has developed resistance to azithromycin dihydrate to varying degrees.

Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome and thus inhibits translation of mRNA [214].

Dosage: 600 mg orally OD for an adult. Children: 10–12 mg/kg (maximum, 500 mg/dose) orally OD. Some double the dose for severe infections.

6.10.15.1 Adverse Effects

The most frequent reported adverse effects for azithromycin have been nausea, diarrhea, and abdominal pain. Azithromycin can cause abnormal changes in the electrical activity of the heart that may lead to a potentially fatal irregular heart rhythm. It can prolong the QT interval. On the double dose, the side effects are more prominent. Allergic reactions are not common [214].

6.10.16 Amikacin

Amikacin is an aminoglycoside antibiotic. It can be used to treat non-tubercular mycobacterial infections and tuberculosis (if caused by sensitive strains) when first-line drugs fail to control the infection.

It works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth.

Dosage: May be administered once or twice a day but must be given by the intravenous, via nebulization, or intramuscular route. There is no oral form available as amikacin is not absorbed orally.

6.10.16.1 Adverse Effects

Adverse effects of amikacin are similar to that of other aminoglycosides. Kidney damage and hearing loss are the most important effects. In people with kidney failure, dosage must be adjusted according to the creatinine clearance, usually by reducing the dosing frequency [215].

6.10.16.2 Future Developments

The greatest problem at this moment in the treatment of mycobacterial infections is multidrug resistance [216]. Some patients do not respond to treatment and have to receive drugs that are still under research. Some are already approved, like thioridazine [217] and linezolid [218], but also there are new classes of drugs like benzothiazinones, diarylquinolines (bedaquiline), and other compounds such as delamanid and SQ109 [218].

Another problem is the drug interactions resulting into low serum levels of the drugs affecting dosing [219].

In this context, the identification of TREM1 signaling is interesting and promising, providing a new angle for activation of monocytic cells by *M. tuberculosis* antigenic determinants. Potentially, *M. tuberculosis*-derived molecules target this pathway, in synergy with TLRs, to activate innate and adaptive immune responses. Thus, vaccination as treatment may become possible [220].

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References

1. Portaels F. Epidemiology of mycobacterial diseases. Clin Dermatol. 1995;13:207–22.
2. Hautmann G, Katsambas A, Lotti T. Non-tuberculous mycobacterial skin infections. J Eur Acad Dermatol Venereol. 1997;9:1–35.
3. Gangadharam PRJ, Jenkins PA. In: Gangadharam PRJ, Jenkins PA, editors. Mycobacteria. New York: Chapman & Hall; 1998.
4. van Ingen J, Bunnik R, Sturm PDJ. Answer to April 2013 photo quiz. J Clin Microbiol. 2013;51(4):1353.
5. Bhamidi S, Scherman MS, McNeil MR. Mycobacterial cell wall Arabinogalactan, chap 4. In: Ullrich M, editor. Bacterial polysaccharides: current innovations and future trends. Poole, UK: Caister Academic Press; 2009.
6. Faber WR. Mycobacterial infections, chap: 7. In: Faber WR, Hay JR, Naafs B, editors. Imported skin diseases. 2nd ed. Chichester, England: Wiley; 2013. p. 64–78.
7. Trinchieri G. Interleukin-12: a cytokine at the interface of inflammation and immunity. Adv Immunol. 1998;70:83–243.
8. Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanol O, van Dissel JT. Genetic, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae. Nat Genet. 2002;32:97–105.
9. Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. Mayo Clin Proc. 2013;88:38–45.
10. Zufferey C, Germano S, Dutta B, Ritz N, Curtis N. The contribution of non-conventional T cells and NK cells in the mycobacterial-specific IFNγ response in Bacille Calmette-Guérin (BCG)-immunized infants. PLoS One. 2013;8(10):e77334.

11. Basu J, Shin DM, Jo EK. Mycobacterial signaling through toll-like receptors. Front Cell Infect Microbiol. 2012;23:145. doi:10.3389/fcimb.2012.00145. eCollection 2012.
12. Pitt JM, Stavropoulos E, Redford PS, Beebe AM, Bancroft GJ, Young DB, et al. Blockade of IL-10 signaling during bacillus Calmette-Guérin vaccination enhances and sustains Th1, Th17, and innate lymphoid IFN-γ and IL-17 responses and increases protection to Mycobacterium tuberculosis infection. Immunology. 2012;189:4079–87. doi:10.4049/jimmunol.1201061. Epub 2012 Sep 12.
13. Pinheiro RO, de Oliveira EB, Dos Santos G, Sperandio da Silva GM, de Andrade Silva BJ, Teles RM, et al. Different immunosuppressive mechanisms in multi-drug-resistant tuberculosis and non-tuberculous mycobacteria patients. Clin Exp Immunol. 2013;171:210–9. doi:10.1111/cei.12007.
14. Dannenberg Jr AM. Liquefaction and cavity formation in pulmonary TB: a simple method in rabbit skin to test inhibitors. Tuberculosis (Edinb). 2009;89:243–7.
15. WHO. http://www.who.int/topics/tuberculosis/en/
16. Laennec RT. Traite de l'auscultation mediate et des maladies des peumons et du coeur. Paris: Asselin and Cie; 1826. p. 649.
17. Koch R. Die Aetiologie der Tuberculose Berliner Klinische Wochenschrift; 1882.
18. Michelson HE. The history of lupus vulgaris. J Invest Dermatol. 1946;7:261.
19. Sierra X. Historia de la tuberculosis cutanea. Piel. 1995;10:118–26.
20. MacGregor RR. Cutaneous tuberculosis. Clin Dermatol. 1995;13:125–55.
21. Barbagallo J, Tager P, Ingleton R, Hirsch RJ, Weinberg JM. Cutaneous tuberculosis. Diagnosis and treatment. Am J Clin Dermatol. 2002;3:319–28.
22. Traore H, Fissette K, Bastian I, Devleeschouwer M, Portaels F. Detection of rifampicin resistance in Mycobacterium tuberculosis isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance [Technical Note]. Int J Tuberc Lung Dis. 2000;5:481–4.
23. Perez-Velez CM. Pediatric tuberculosis: new guidelines and recommendations. Curr Opin Pediatr. 2012;24:319–28.
24. Bravo FG, Gotuzzo E. Cutaneous tuberculosis. Clin Dermatol. 2007;25:173–80.
25. Ramesh V, Misra RS, Jain RK. Secondary tuberculosis of the skin: clinical features and problems in laboratory diagnosis. Int J Dermatol. 1987;26:578.
26. Marcoval J, Alcaide F. Evolution of cutaneous tuberculosis over the past 30 years in a tertiary hospital on the European Mediterranean coast. Clin Exp Dermatol. 2013;38:131–6.
27. Ho MH, Lee KC, Chong LY. Perianal ulceration in a "Healthy" Chinese man with disseminated tuberculosis. J Dermatol. 2002;29:366–70.
28. Rapini R, Bolognia JL, Jorizzo JL. Dermatology, vol. 2, chap. 74. St. Louis: Mosby; 2007. p. 27.
29. Ko M, Wu C, Chiu H. Tuberculous gumma (cutaneous metastatic tuberculous abscess). Dermatol Sin. 2005;23:27–31.
30. Kalaria VG, Kapila R, Schwartz RA. Tuberculous gumma (cutaneous metastatic tuberculous abscess) with underlying lymphoma. Cutis. 2000;66:277–9.
31. Lessnau K-D. Miliary tuberculosis. 2006. http://www.emedicine.com/med/topic1476.htm.
32. Wünsch Filho V, de Castilho EA, Rodrigues LC, Huttly SR. Effectiveness of BCG vaccination against tuberculous meningitis: a case-control study in São Paulo. Braz Bull World Health Organ. 1990;68:69–74.
33. Darier MJ. Des "tuberculides" cutanees. Arch Dermatol Syph. 1896;7:1431–6.
34. Muto J, Kuroda K, Tajima S. Papular tuberculides post-BCG vaccination: case report and review of the literature in Japan. Clin Exp Dermatol. 2006;31:611–2.
35. Dongre AM, Sanghavi SA, Khopkar US. Papulonecrotic tuberculid at the site of tuberculin test in a patient with concomitant erythema induratum and papulonecrotic tuberculid. Indian J Dermatol Venereol Leprol. 2013;79:248–51.
36. Jordaan HF, Schneider JW, Schaaf HS, Victor TS, Geiger DH, Van Helden P, et al. Papulonecrotic tuberculid in children: a report of eight patients. Am J Dermatopathol. 1996;18:172–85.
37. Yates VM. Mycobacterial infections. In: Burns T, Breatnach S, Cox N, Griffiths C, editors. Rook's textbook of dermatology. 8th ed. Oxford: Blackwell Science; 2010. p. 31.21–2.

38. Singhal P, Patel PH, Marfatia YS. Lichen scrofulosorum: a diagnosis overlooked. Indian Dermatol Online J. 2012;3:190–2.
39. Dogra N, Shah S, Dogra D. Lichen scrofulosorum: an important marker of occult tuberculosis. Indian J Dermatol. 2008;53:91–2.
40. Bazin E. Lecons Theoriques et Cliniques sur la Scrofula. 2nd ed. Paris: Dalahaye; 1861.
41. Wiebels D, Turnbull K, Steinkraus V, Böer A. Erythema induratum Bazin. Hautarzt. 2007;58:237–40.
42. Whitfield A. On the nature of the disease known as erythema induratum scrofulosorum. Br J Dermatol. 1901;13:386–7.
43. Sharon V, Goodarzi H, Chambers CJ, Fung MA, Armstrong AW. Erythema induratum of Bazin. Dermatol Online J. 2010;16:1.
44. Møller KI, Kongshoj B, Philipsen PA, Thomsen VO, Wulf HC. How Finsen's light cured lupus vulgaris. Photodermatol Photoimmunol Photomed. 2005;21:118–24.
45. Smith DG, Waksman SA. Tuberculostatic and tuberculocidal properties of streptomycin. J Bacteriol. 1947;54:253–61.
46. NN. Isoniazid in combination with streptomycin or with P.A.S. in the treatment of pulmonary tuberculosis; fifth report to the Medical Research Council by their Tuberculosis Chemotherapy Trials Committee. Br Med J. 1953;2(4844):1005–14.
47. Yeager RL, Munroe WG, Dessau FI. Pyrazinamide (aldinamide*) in the treatment of pulmonary tuberculosis. Trans Annu Meet Natl Tuberc Assoc. 1952;48:178–201.
48. Shepherd RG, Wilkenson RG. Antituberculous agents. II N, N'Diisopropylethylendiamine and analogs. J Med Pharm Chem. 1962;91:823–35.
49. Wyrzykowska N, Wyrzykowski M, Żaba R, Silny W. Treatment of cutaneous infections caused by Mycobacterium tuberculosis. Postep Derm Alergol. 2012;4:293–8.
50. Timbal MT. Rifomycin. II. Antibacterial activity of rifomycin B. Antibiot Annu. 1959;7:271–6.
51. Nachega JB, Chaisson RE. Tuberculosis drug resistance: a global problem. Clin Infect Dis. 2003;36(Suppl1):24–30.
52. Dooley KE, Obuku EA, Durakovic N, Belitsky V, Mitnick C, Nuermberger EL, Efficacy Subgroup, RESIST-TB, World Health Organization group V. Drugs for the treatment of drug-resistant tuberculosis: unclear efficacy or untapped potential? J Infect Dis. 2013;207:1352–8.
53. Migliori GB, Besozzi G, Girardi E, Kliiman K, Lange C, Toungoussova OS, SMIRA/TBNET Study Group, et al. Clinical and operational value of the extensively drug-resistant tuberculosis definition. Eur Respir J. 2007;30:623–6.
54. Jassal M, Bishai WR. Extensively drug-resistant tuberculosis. Lancet Infect Dis. 2009;9:19–30.
55. WHO Towards universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis. by 2015: WHO progress report 2011. WHO/HTM/TB/2011.3.
56. WHO Multidrug-resistant tuberculosis (MDR-TB) 2013 Update. http://www.who.int/tb/challenges/mdr/MDR_TB_FactSheet.pdf.
57. UK guidelines. http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Tuberculosis/Guidelines/TBGuid01Guidelines/.
58. WHO. Treatment of tuberculosis: guidelines. 4th ed. WHO/HTM/TB/2009.420. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf.
59. CDC. http://www.cdc.gov/tb/topic/globaltb/default.htm.
60. Van Loenhout-Rooyackers JH, Laheij RJ, Richter C, Verbeek AL. Shortening the duration of treatment for cervical tuberculous lymphadenitis. Eur Respir J. 2000;15:192–5.
61. Parthasarathy R, Sriram K, Santha T, Prabhakar R, Somasundaram PR, Sivasubramanian S. Short-course chemotherapy for tuberculosis of the spine. J Bone Joint Surg [Br]. 1999;81-B:464–71.
62. Naafs B, Faber WR. Leprosy. In: Faber WR, Hay JR, Naafs B, editors. Imported skin diseases, chap. 8. 2nd ed. Chichester, England: Wiley; 2013. p. 79–93.
63. Hansen GHA. Undersøgelser Angående Spedalskhedens Årsager. Norsk Mag Laegervidenskaben. 1874;4:1–88.

64. Naafs B, Silva E, Vilani-Moreno F, Marcos EC, Nogueira ME, Opromolla DVA. Factors influencing the development of leprosy: an overview. Int J Lepr. 2001;69:26–33. Editorial.
65. Masaki T, Qu J, Cholewa-Waclaw J, Burr K, Raaum R, Rambukkana A. Reprogramming adult Schwann cells to stem cell-like cells by leprosy bacilli promotes dissemination of infection. Cell. 2013;152(1–2):51–67. doi:10.1016/j.cell.2012.12.014.
66. Naafs B. Current views on reactions in leprosy. Indian J Lepr. 2000;72:97–122.
67. Naafs B. Viewpoint: leprosy after the year 2000. Trop Med Int Health. 2000;5:400–3.
68. WHO. Weekly epidemiological record. No. 35. 2013;88:365–380.
69. Da Costa Nery JA, Schreuder PAM, Teixeira de Matos PC, Veira de Mendonça L, Tebaldi Tardin R, De Melo S, et al. Hansen's disease in a general hospital: uncommon presentations and delay in diagnosis. J Eur Acad Dermatol Venereol. 2009;23:150–6. Epub 2008 Sep 10.
70. Naafs B, Noto S, Schreuder PAM. The diagnosis of leprosy LML. 2011. http://www.aifo.it/english/leprosy/mailing_list/2011/181011.doc.
71. WHO. World Health Organization Expert Committee on Leprosy WHO Tech Rep Series Fifth Report (N°607). 1977.
72. Jain S, Visser LH, Yerasu MR, Raju R, Meena AK, Lokesh B, et al. Use of high resolution ultrasonography as an additional tool in the diagnosis of primary neuritic leprosy: a case report. Lepr Rev. 2013;84:161–5.
73. Chin-A-Lien RAM, Faber WR, v. Rens MM, Leiker DL, Naafs B, Klatser PR. Follow-up of multibacillary leprosy using a phenolic glycoli¬pid-1-based-Elisa. Do increasing Elisa-values after discontinuation of treatment indicate relapse? Lepr Rev. 1992;63:21–7.
74. Rees RJ, Meade TW. Comparison of the modes of spread and the incidence of tuberculosis and leprosy. Lancet. 1974;1(7846):47–8.
75. Naafs B. Leprosy infection and disease. http://www.aifo.it/english/leprosy/mailing_list/2013/180213.htm.
76. Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five-group system. Int J Lepr Other Mycobact Dis. 1966;34:255–73.
77. World Health Organization (WHO). Guide to eliminate leprosy as a public health problem. 1st ed. Geneva: World Health Organization; 1995.
78. Hagge DA, Thapa P, Shrestha IR, Neupane K, Napit IB, Rajan L, et al. Is counting the lesions enough: the significance of slit skin smears and biopsy histopathology in the clinical diagnosis, treatment and classifications of leprosy patients. Paper at the 18th world leprosy congress; 2013.
79. Mouat FJ. Notes on native remedies. No. 1. The Chaulmoogra. Indian Ann Med Sci. 1854;1:646–52.
80. Faget G, Jonhansen FA, Dinah JF, Prejean BM, Eccles C. The Promin treatment of leprosy: a progress report. Public Health Rep. 1943;58:1729–41.
81. WHO. Chemotherapy of leprosy for control programmes. World Health Organ Tech Rep Series. 1982;675:1–33.
82. Jacobson RR, Gatt P. Can leprosy be eradicated with chemotherapy? An evaluation of the Malta Leprosy Eradication Project. Lepr Rev. 2008;79:410–5.
83. Pritze S, Alvarenga AE, Leguizamon O, Haubitz I. Isoprodian and rifampicin in the treatment of leprosy: a descriptive evaluation of therapy durations in Paraguayan leprosy patients. Chemotherapy. 1989;35:373–82.
84. Liu H, Zhang F. Establishment and application of risk prediction test for dapsone hypersensitivity syndrome – preliminary report. Paper at 18th world leprosy congress, Brussels; 2013.
85. Setia MS, Shinde SS, Jerajani HR, Boivin JF. Is there a role for rifampicin, ofloxacin and minocycline (ROM) therapy in the treatment of leprosy? Systematic review and meta-analysis. Trop Med Int Health. 2011;16:1541–51. doi:10.1111/j.1365-3156.2011.02873.x. Epub 2011 Sep 13.
86. Bjune G, Barnetson RS, Ridley DS, Kronvall G. Lymphocyte transformation test in leprosy; correlation of the response with inflammation of lesions. Clin Exp Immunol. 1976; 25(1):85–94.
87. Naafs B. Reactions: new knowledge. Trop Geogr Med. 1994;46:80–4.

88. Naafs B. Treatment duration of reversal reaction: a reappraisal. Back to the past. Lepr Rev. 2003;74:328–36.
89. Barnetson RS, Pearson JM, Rees RJ. Evidence for prevention of borderline leprosy reactions by dapsone. Lancet. 1976;27:1171–2.
90. De Souza Arauyo HC. Inst Oswaldo Cruz, Rio de Janeiro Thesis; 1929.
91. Naafs B. Treatment of reactions. http://www.aifo.it/english/leprosy/documents/treatment_of_reactions_b_naafs.pdf.
92. Schreuder PAM, Naafs B. Chronic recurrent ENL, steroid dependant: long-term treatment with high dose of clofazimine. Lepr Rev. 2003;74:386–9.
93. Shannon E, Noveck R, Sandoval F, Kamath B. Thalidomide suppressed IL-1beta while enhancing TNF-alpha and IL-10, when cells in whole blood were stimulated with lipopolysaccharide. Immunopharmacol Immunotoxicol. 2008;30:447–57. doi:10.1080/08923970802135161 2.
94. Kar BR, Babu R. Methotrexate in resistant ENL. Int J Lepr Other Mycobact Dis. 2004;72:480–2.
95. Noordeen SK. Prophylaxis – scope and limitations. Lepr Rev. 2000;71(Suppl):S16–9; discussion S19–20.
96. Richardus RA, Alam K, Pahan D, Feenstra SG, Geluk A, Richardus JH. The combined effect of chemoprophylaxis with single dose rifampicin and immunoprophylaxis with BCG to prevent leprosy in contacts of newly diagnosed leprosy cases: a cluster randomized controlled trial (MALTALEP study). BMC Infect Dis. 2013;13:456.
97. Walsh DS, Meijers WM, Portaels F. Buruli H9. In: Faber WR, Hay JR, Naafs B, editors. Imported skin diseases. 2nd ed. Chichester, England: Wiley; 2013. p. 94–106.
98. Walsh DS, Portaels F, Meyers WM. Recent advances in leprosy and Buruli ulcer (Mycobacterium ulcerans infection). Curr Opin Infect Dis. 2010;23:445–55.
99. Janssens PG, Pattyn SR, Meyers WM, Portaels F. Buruli ulcer: an historical overview, with updating to 2005. Bull Séances Acad R Sci Outre-Mer. 2005;51:165–99.
100. Stinear TP, Mve-Obiang A, Small PL, Frigui W, Pryor MJ, Brosch R, et al. Giant plasmid-encoded polyketide synthases produce the macrolide toxin of Mycobacterium ulcerans. Proc Natl Acad Sci U S A. 2004;101:1345–9.
101. Adusumilli S, Mve-Obiang A, Sparer T, Meyers W, Hayman J, Small PL. Mycobacterium ulcerans toxic macrolide, mycolactone modulates the host immune response and cellular location of M. ulcerans in vitro and in vivo. Cell Microbiol. 2005;7:1295–304.
102. Walsh DS, Meyers WM, Portaels F, Lane JE, Mongkolsirichaikul D, Hussem K, et al. High rates of apoptosis in human Mycobacterium ulcerans culture-positive buruli ulcer skin lesions. Am J Trop Med Hyg. 2005;73:410–5.
103. Ahorlu CK, Koka E, Yeboah-Manu D, Lamptey I, Ampadu E. Enhancing Buruli ulcer control in Ghana through social interventions: a case study from the Obom sub-district. BMC Public Health. 2013;13:59. http://www.biomedcentral.com/1471-2458/13/59.
104. Walsh DS, Portaels F, Meyers WM. Buruli ulcer: advances in understanding Mycobacterium ulcerans infection. Dermatol Clin. 2011;29:1–8.
105. Debacker M, Aguiar J, Steunou C, Zinsou C, Meyers WM, Scott JT, et al. Mycobacterium ulcerans disease: role of age and gender in incidence and morbidity. Trop Med Int Health. 2004;9:1297–304.
106. World Health Organization. Buruli ulcer progress report, 2004–2008. Wkly Epidemiol Rec. 2008;83:145–54.
107. Walsh DS, Eyase F, Onyango D, Odindo A, Otieno W, Waitumbi JN, et al. Short report: clinical and molecular evidence for a case of Buruli ulcer (Mycobacterium ulcerans infection) in Kenya. Am J Trop Med Hyg. 2009;81:1110–3.
108. Pradinaud R. Buruli ulcer situation in French Guiana. In: Report of the 6th WHO advisory group meeting on Buruli Ulcer, 10–13 Mar 2003.
109. Steffen CM, Smith M, McBride WJ. Mycobacterium ulcerans infection in North Queensland: the 'Daintree ulcer'. ANZ J Surg. 2010;80:732–6.
110. Portaels F, Silva MT, Meyers WM. Buruli ulcer. Clin Dermatol. 2009;27:291–305.

111. Merritt RW, Walker ED, Small PL, Wallace JR, Johnson PD, Benbow ME, Boakye DA. Ecology and transmission of Buruli ulcer disease: a systematic review. PLoS Negl Trop Dis. 2010;4:e911.
112. Portaels F, Meyers WM, Ablordey A, Castro AG, Chemlal K, de Rijk P, et al. First cultivation and characterization of Mycobacterium ulcerans from the environment. PLoS Negl Trop Dis. 2008;2:e178.
113. Marion E, Eyangoh S, Yeramian E, Doannio J, Landier J, Aubry J, et al. Seasonal and regional dynamics of M. ulcerans transmission in environmental context: deciphering the role of water bugs as hosts and vectors. PLoS Negl Trop Dis. 2010;4:e731.
114. Fyfe JA, Lavender CJ, Handasyde KA, Legione AR, O'Brien CR, Stinear TP, et al. A major role for mammals in the ecology of Mycobacterium ulcerans. PLoS Negl Trop Dis. 2010;4:e791.
115. Gryseels S, Amissah D, Durnez L, Vandelannoote K, Leirs H, De Jonckheere J, et al. Amoebae as potential environmental hosts for Mycobacterium ulcerans and other mycobacteria, but doubtful actors in Buruli ulcer epidemiology. PLoS Negl Trop Dis. 2012;6:e1764. doi:10.1371/journal.pntd.0001764. Epub 2012 Aug 7.
116. Walsh DS, Portaels F, Meyers WM. Buruli ulcer: advances in understanding Mycobacterium ulcerans infection. Dermatol Clin. 2011;29:1–8. doi:10.1016/j.det.2010.09.006.
117. World Health Organization. Diagnosis of Mycobacterium ulcerans disease (Buruli ulcer) (WHO/CDS/CPE/GBUI/2001.4). Geneva: 2001.
118. Johnson RC, Nackers F, Glynn JR, De Biurrun Bakedano E, Zinsou C, Aguiar J, et al. Association of HIV infection and Mycobacterium ulcerans disease in Benin. AIDS. 2008;22:901–3.
119. Phanzu MD, Bafende AE, Imposo BB, Meyers WM, Portaels F. Under treated necrotizing fasciitis masquerading as ulcerated edematous Mycobacterium ulcerans infection (Buruli Ulcer). Am J Trop Med Hyg. 2010;82:478–81.
120. Eddyani M, Fraga AG, Schmitt F, Uwizeye C, Fissette K, Johnson C, et al. Fine-needle aspiration, an efficient sampling technique for bacteriological diagnosis of nonulcerative Buruli ulcer. J Clin Microbiol. 2009;47:1700–4.
121. World Health Organization. Guidance on sampling techniques for laboratory-confirmation of Mycobacterium ulcerans infection (Buruli ulcer disease). Available from: http://www.who.int/buruli/Guidance_sampling_techniques_MU_infection.pdf.
122. Stop Buruli Consortium. UBS Optimus Foundation. http://www.stopburuli.org/.
123. Herbinger KH, Adjei O, Awua-Boateng NY, Uwizeye C, Fissette K, Johnson C, et al. Comparative study of the sensitivity of different diagnostic methods for the laboratory diagnosis of Buruli ulcer disease. Clin Infect Dis. 2009;48:1055–64.
124. Sarfo FS, Le Chevalier F, Aka N, Phillips RO, Amoako Y, Boneca IG, et al. Mycolactone diffuses into the peripheral blood of buruli ulcer patients – implications for diagnosis and disease monitoring. PLoS Negl Trop Dis. 2011;5:e1237.
125. Meyers WM. Mycobacterial infections of the skin. In: Doerr W, Seifert G, editors. Tropical pathology. 2nd ed. Berlin: Springer; 1995. p. 291–377.
126. World Health Organization. Provisional guidance on the role of specific antibiotics in the management of Mycobacterium ulcerans disease (Buruli ulcer). (WHO/CDS/CPE/GBUI/2004). Geneva, 2004.
127. Etuaful S, Carbonnelle B, Grosset J, Lucas S, Horsfield C, Phillips R, et al. Efficacy of the combination rifampin-streptomycin in preventing growth of Mycobacterium ulcerans in early lesions of Buruli ulcer in humans. Antimicrob Agents Chemother. 2005;49:3182–6.
128. Nienhuis WA, Stienstra Y, Thompson WA, Awuah PC, Abass KM, Tuah W, et al. Antimicrobial treatment for early, limited Mycobacterium ulcerans infection: a randomised controlled trial. Lancet. 2010;375:664–72.
129. WHO. Treatment of mycobacterium ulcerans disease (Buruli ulcer): guidance for health workers. 2012. http://apps.who.int/iris/bitstream/10665/77771/1/9789241503402_eng.pdf.
130. Sopoh GE, Dossou AD, Brun LV, Barogui YT, Houézo JG, Affolabi D, et al. Severe multifocal form of buruli ulcer after streptomycin and rifampin treatment: comments on possible dissemination mechanisms. Am J Trop Med Hyg. 2010;83:307–13.

131. Portaels F, Aguiar J, Debacker M, Johnson C, Meyer WM. Osteomyelitis in Mycobacterium ulcerans disease: a review of 106 patients treated in Zagnanado (Benin). WHO annual meeting on Buruli ulcer. 2008, 31 March–2 April, p. 21–24; Geneva, Switzerland.
132. Kibadi K, Boelaert M, Fraga AG, Kayinua M, Longatto-Filho A, Minuku JB, et al. Response to treatment in a prospective cohort of patients with large ulcerated lesions suspected to be Buruli ulcer (Mycobacterium ulcerans disease). PLoS Negl Trop Dis. 2010;4:e736.
133. World Health Organization. Prevention of disability in Buruli ulcer: basic rehabilitation. A practical field guide. Geneva; 2008. http://whqlibdoc.who.int/hq/2008/WHO_HTM_NTD_IDM_GBUI_2008.1_eng.pdf.
134. Chauty A, Ardant MF, Marsollier L, Pluschke G, Landier J, Adeye A, et al. Oral treatment for Mycobacterium ulcerans infection: results from a pilot study in Benin. Clin Infect Dis. 2011;52:94–6.
135. Gordon CL, Buntine JA, Hayman JA, Lavender CJ, Fyfe JA, Hosking P, et al. All-oral antibiotic treatment for buruli ulcer: a report of four patients. PLoS Negl Trop Dis. 2010;4:e770.
136. Dossou AD, Sopoh GE, Johnson CR, Barogui YT, Affolabi D, Anagonou SY, et al. Management of Mycobacterium ulcerans infection in a pregnant woman in Benin using rifampicin and clarithromycin. Med J Aust. 2008;189:532–3.
137. Ruslami R, Nijland HM, Adhiarta IG, Kariadi SH, Alisjahbana B, Aarnoutse RE, et al. Pharmacokinetics of antituberculosis drugs in pulmonary tuberculosis patients with type 2 diabetes. Antimicrob Agents Chemother. 2010;54:1068–74.
138. van Ingen J, Egelund EF, Levin A, Totten SE, Boeree MJ, Mouton JW, et al. The pharmacokinetics and pharmacodynamics of pulmonary Mycobacterium avium complex disease treatment. Am J Respir Crit Care Med. 2012;186:559–65.
139. O'Brien DP, Robson ME, Callan PP, McDonald AH. "Paradoxical" immune-mediated reactions to Mycobacterium ulcerans during antibiotic treatment: a result of treatment success, not failure. Med J Aust. 2009;191:564–6.
140. Nienhuis WA, Stienstra Y, Abass KM, Tuah W, Thompson WA, Awuah PC, et al. Paradoxical responses after start of antimicrobial treatment in Mycobacterium ulcerans infection. Clin Infect Dis. 2012;54:519–26.
141. Ruf MT, Chauty A, Adeye A, Ardant MF, Koussemou H, Johnson RC, et al. Secondary buruli ulcer skin lesions emerging several months after completion of chemotherapy: paradoxical reaction or evidence for immune protection? PLoS Negl Trop Dis. 2011;5:e1252.
142. Friedman ND, McDonald AH, Robson ME, O'Brien DP. Corticosteroid use for paradoxical reactions during antibiotic treatment for Mycobacterium ulcerans. PLoS Negl Trop Dis. 2012;6:e1767.
143. Nackers F, Johnson RC, Glynn JR, Zinsou C, Tonglet R, Portaels F. Environmental and health-related risk factors for Mycobacterium ulcerans disease (Buruli ulcer) in Benin. Am J Trop Med Hyg. 2007;77:834–6.
144. Portaels F, Aguiar J, Debacker M, Guédénon A, Steunou C, Zinsou C, et al. Mycobacterium bovis BCG vaccination as prophylaxis against Mycobacterium ulcerans osteomyelitis in Buruli ulcer disease. Infect Immun. 2004;72:62–5.
145. Silva MT, Portaels F, Pedrosa J. Pathogenetic mechanisms of the intracellular parasite Mycobacterium ulcerans leading to Buruli ulcer. Lancet Infect Dis. 2009;9:699–710.
146. Fraga AG, Cruz A, Martins TG, Torrado E, Saraiva M, Pereira DR, et al. Mycobacterium ulcerans triggers T-cell immunity followed by local and regional but not systemic immunosuppression. Infect Immun. 2011;79:421–30.
147. Huygen K, Adjei O, Affolabi D, Bretzel G, Demangel C, Fleischer B, et al. Buruli ulcer disease: prospects for a vaccine. Med Microbiol Immunol. 2009;198:69–77.
148. Sarfo FS, Converse PJ, Almeida DV, Zhang J, Robinson C, Wansbrough-Jones M, et al. Microbiological, histological, immunological, and toxin response to antibiotic treatment in the mouse model of Mycobacterium ulcerans disease. PLoS Negl Trop Dis. 2013;7:e2101.
149. Gentry CA. Reviewed by: Chapman MM, Nix DE. Atypical mycobacteria. Pharmacotherapy self-assessment program, 5th ed. 2005. p. 99–126.

150. Van Ingen J. Nontuberculous mycobacteria from gene sequences to clinical relevance Thesis Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, 21 Sept 2009.
151. Rallis E, Koumantaki-Mathioudaki E. Treatment of Mycobacterium marinum cutaneous infections. Expert Opin Pharmacother. 2007;8:2965–78.
152. Koirala J. Mycobacterium Kansasii treatment & management. http://emedicine.medscape.com/article/223230-treatment.
153. Babalık A, Kuyucu T, Ordu EN, Ernam D, Partal M, Köksalan K. Non-tuberculous mycobacteria infection: 75 cases. Tuberk Toraks. 2012;60:20–31.
154. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F et al., on behalf of the ATS Mycobacterial Diseases Subcommittee American Thoracic Society Documents; An Official ATS/IDSA Statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases adopted by the ATS Board Of Directors, 2006, and by the IDSA Board of Directors, 2007. Am J Respir Crit Care Med. 2007;175:367–416.
155. Po-Ren H, Hsiue T, Jarn J, Ho S, Hsieh W. Mycobacterium scrofulaceum in an immunocompetent host. Clin Infect Dis. 1996;22:159–61.
156. Marazzia MG, Chapgierb A, Defilippia A, Pistoiac V, Manginid S, Saviolie C, et al. Disseminated Mycobacterium scrofulaceum infection in a child with interferon-γ receptor 1 deficiency. Int J Infect Dis. 2010;14:e167–70.
157. Wollina U. Nodular skin reactions in eyebrow permanent makeup: two case reports and an infection by Mycobacterium haemophilum. J Cosmet Dermatol. 2011;10:235–9.
158. Kay MK, Perti TR, Duchin JS. Tattoo-associated Mycobacterium haemophilum skin infection in immunocompetent adult. Emerg Infect Dis. 2011;17:1734–6.
159. Lindeboom JA, Bruijnesteijn van Coppenraet LES, van Soolingen D, Prins JM, Kuijper EJ. Clinical manifestations, diagnosis, and treatment of Mycobacterium haemophilum infections. Clin Microbiol Rev. 2011;24:701–17.
160. van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. Drug Resist Updat. 2012;15:149–61.
161. Patel T, Scroggins-Markle L, Kelly B. A dermal piercing complicated by Mycobacterium fortuitum. Case Rep Dermatol Med. 2013;2013:149829.
162. Nagore E, Ramos P, Bottella-Estrada R, Ramos-Niguez JA, Sanmartin O, Castejon P. Cutaneous infection with Mycobacterium fortuitum after localized microinjections (mesotherapy) treated successfully with a triple drug regimen. Acta Derm Venereol. 2001;81:291–3.
163. Falsey RR, Kinzer MH, Hurst S, Kalus A, Pottinger PS, Duchin JS, et al. Cutaneous inoculation of nontuberculous mycobacteria during professional tattooing: a case series and epidemiologic study. Clin Infect Dis. 2013;57:e143–7.
164. Rodriguez JM, Xie YL, Winthrop KL, Schafer S, Sehdev P, Solomon J, et al. Mycobacterium chelonae facial infections following injection of dermal filler. Aesthet Surg J. 2013;33:265–9.
165. De Groote MA, Huitt G. Infections due to rapidly growing mycobacteria. Clin Infect Dis. 2006;42:1756–63.
166. Simmon KE, Brown-Elliott BA, Ridge PG, Durtschi JD, Bridge Mann L, Slechta ES, et al. Mycobacterium chelonae-abscessus Complex Associated with Sinopulmonary Disease, Northeastern USA Emerging Infectious Diseases 2011;17. www.cdc.gov/eid.
167. Van Ingen J, Boeree M, Janssen M, Ullmann E, de Lange W, de Haas P, et al. Pulmonary Mycobacterium szulgai infection and treatment in a patient receiving anti-tumor necrosis factor therapy. Nat Clin Pract Rheumatol. 2007;3:414–9.
168. Han X, Tarrand JJ, Infante R, Jacobson KL, Truong M. Clinical significance and epidemiologic analyses of mycobacterium avium and mycobacterium intracellulare among patients without AIDS. J Clin Microbiol. 2005;4:4407–12.
169. Kasperbauer SH, Daley CL. Diagnosis and treatment of infections due to Mycobacterium avium complex. Semin Respir Crit Care Med. 2008;29:569–76.
170. Gayathri R, Lily TK, Deepa P, Mangai S, Madhavan HN. Antibiotic susceptibility pattern of rapidly growing mycobacteria. J Post Grad Med. 2010;56:76–8.

171. Masters SB, Trevor AJ, Katzung BG. Katzung & Trevor's pharmacology. New York: Lange Medical Books/McGraw Hill, Medical Pub. Division; 2005.
172. Gao XF, Wang L, Liu G-J, Wen J, Sun X, Xie Y, et al. Rifampicin plus pyrazinamide versus isoniazid for treating latent tuberculosis infection: a meta-analysis. Int J Tuberc Lung Dis. 2006;10:1080–90.
173. Woldehanna S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. Cochrane Database Syst Rev. 2004;1:CD000171.
174. Douglas Collins R. Atlas of drug reactions. New York: Churchill Livingstone; 1985. p. 123.
175. Grosset J, Leventis S. Adverse effects of rifampin. Clin Infect Dis. 1983;5 Suppl 3:S440–6.
176. Naafs B, Matemera BO. A possible "flu" syndrome on once monthly rifampicin. Lepr Rev. 1986;57:271–2.
177. Zang Y. Drug resistance and persistent Tuberculosis: mechanism and drug development. In: Dougherty TJ, Pucci MJ, editors. Antibiotic discovery and development. New York, US: Springer; 2011. p. 720.
178. Baulard AR, Betts JC, Engohang-Ndong J, Quan S, McAdam RA, Brennan PJ, et al. Activation of the pro-drug ethionamide is regulated in mycobacteria. J Biol Chem. 2000;275:28326–31.
179. Willand N, Dirié B, Carette X, Bifani P, Singhal A, Desroses M, et al. Synthetic EthR inhibitors boost antituberculous activity of ethionamide. Nat Med. 2009;15:537–44.
180. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, et al. InhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science. 1994;263:227–30.
181. Cynamon MH, Sklaney M. Gatifloxacin and ethionamide as the foundation for therapy of tuberculosis. Antimicrob Agents Chemother. 2003;47:2442–4.
182. Sharma PK, Bansal R. Gynecomastia caused by ethionamide. Indian J Pharmacol. 2012;44:654–5.
183. Mikusová K, Slayden RA, Besra GS, Brennan PJ. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob Agents Chemother. 1995;39:2484–9.
184. Thee S, Detjen A, Quarcoo D, Wahn U, Magdorf K. Ethambutol in paediatric tuberculosis: aspects of ethambutol serum concentration, efficacy and toxicity in children. Int J Tuberc Lung Dis. 2007;11:965–71.
185. Berning SE. The role of fluoroquinolones in tuberculosis today. Drugs. 2001;61:9–18.
186. Cunha Mda G, Virmond M, Schettini AP, Cruz RC, Ura S, Ghuidella C, et al. OFLOXACIN multicentre trial in MB leprosy FUAM-Manaus and ILSL-Bauru, Brazil. Lepr Rev. 2012;83:261–8.
187. Hooper DC. Mode of action of fluoroquinolones. Drugs. 1999;58 suppl 2:6–10.
188. Adefurin A, Sammons H, Jacqz-Aigrain E, Choonara I. Ciprofloxacin safety in paediatrics: a systematic review. Arch Dis Child. 2011;96:874–80.
189. Bernardes-Génisson V, Deraeve C, Chollet A, Bernadou J, Pratviel G. Isoniazid: an update on the multiple mechanisms for a singular action. Curr Med Chem. 2013;5 [Epub ahead of print].
190. Hoffner SE. Pulmonary infections caused by less frequently encountered slow-growing environmental mycobacteria. Eur J Clin Microbiol Infect Dis. 1994;13:937–41.
191. Vernon A. Treatment of latent tuberculosis infection. Semin Respir Crit Care Med. 2013;34:67–86.
192. Khattri S, Kushawaha A, Dahal K, Lee M, Mobarakai N. Isoniazid (INH)-induced eosinophilic exudative pleural effusion and lupus erythematosus. A clinical reminder of drug side effects. Bull NYU Hosp Jt Dis. 2011;69:181–4.
193. Steele MA, Des Prez RM. The role of pyrazinamide in tuberculosis chemotherapy. Chest. 1988;94:845–50.
194. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis. 2003;7:6–21.
195. Zimhony O, Cox JS, Welch JT, Vilchèze C, Jacobs WR. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of Mycobacterium tuberculosis (abstract). Nat Med. 2000;6:1043–7.

196. Zimhony O, Vilcheze C, Arai M, Welch J, Jacobs Jr WR. Pyrazinoic acid and its n-propyl ester inhibit fatty acid synthase I in replicating tubercle bacilli. Antimicrob Agents Chemother. 2007;51:752–4.
197. Ngo SC, Zimhony O, Chung WJ, Sayahi H, Jacobs Jr WR, Welch JT. Inhibition of isolated Mycobacterium tuberculosis fatty acid synthase I by pyrazinamide analogs. Antimicrob Agents Chemother Antimicrob Agents Chemother. 2007;1:2430–5.
198. Shi W, Zhang X, Jiang X, Yuan H, Lee JS, Barry 3rd CE, et al. Pyrazinamide inhibits trans-translation in Mycobacterium tuberculosis. Science. 2011;333:1630–2.
199. Corbella X, Vadillo M, Cabellos C, Fernandez-Viladrich P, Rufi G. Hypersensitivity hepatitis due to pyrazinamide. Scand J Infect Dis. 1995;27:93–4.
200. Wade MM, Zhang Y. Mechanisms of drug resistance in Mycobacterium tuberculosis. Front Biosci. 2004;9:975–94.
201. Rengarajan J, Sassetti CM, Naroditskaya V, Sloutsky A, Bloom BR, Rubin EJ. The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. Mol Microbiol. 2004;53:275–82.
202. NN. Controlled comparison of oral twice-weekly and oral daily isoniazid plus PAS in newly diagnosed pulmonary tuberculosis. Br Med J. 1973;2:7–11.
203. Sharma D, Cukras AR, Rogers EJ, Southworth DR, Green R. Mutational analysis of S12 protein and implications for the accuracy of decoding by the ribosome. J Mol Biol. 2007;374:1065–76.
204. Voet D, Voet JG. Biochemistry. 3rd ed. New York, US: Wiley; 2004. p. 1341.
205. Bushby SRM, Hitchings GH. Br J Pharmacol. 1968;33(1):72–90.
206. Andersen JT, Petersen M, Jimenez-Solem E, Broedbaek K, Andersen EW, Wreford E, et al. Trimethoprim use in early pregnancy and the risk of miscarriage. Epidemiol Infect. 2012;141:1749–55.
207. Prosser GA, de Carvalho LP. Kinetic mechanism and inhibition of Mycobacterium tuberculosis D-alanine:D-alanine ligase by the antibiotic D-cycloserine. FEBS J. 2013;280:1150–66.
208. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev. 2001;65:232–60.
209. Wozel G, Barth J. Current aspects of modes of action of dapsone. Int J Dermatol. 1988;2:547–52.
210. Chang YT. Chemotherapy of murine leprosy. IV: the effects of amithiozone (TB1/698), p-aminosalicylic acid (PAS), B283 (a phenazine pigment), five antibiotics and three diphenylthiourea compounds on murine leprosy. Int J Lepr Other Mycobact Dis. 1955;23:167–80.
211. Browne SG, Hogerzeil LM. "B 663" in the treatment of leprosy. Lepr Rev. 1962;33:6–10.
212. Degang Y, Akama T, Hara T, Tanigawa K, Ishido Y, Gidoh M, et al. Clofazimine modulates the expression of lipid metabolism proteins in Mycobacterium leprae-infected macrophages. PLoS Negl Trop Dis. 2012;6:e1936.
213. Kornhuber J, Muehlbacher M, Trapp S, Pechmann S, Friedl A, Reichel M, et al. Identification of novel functional inhibitors of acid sphingomyelinase. PLoS One. 2011;6:e23852.
214. Wilms EB, Touw DJ, Heijerman HG, van der Ent CK. Azithromycin maintenance therapy in patients with cystic fibrosis: a dose advice based on a review of pharmacokinetics, efficacy, and side effects. Pediatr Pulmonol. 2012;47:658–65.
215. Hanberger H, Edlund C, Furebring M, Giske GC, Melhus A, Nilsson LE, Swedish Reference Group for Antibiotics, et al. Rational use of aminoglycosides – review and recommendations by the Swedish Reference Group for Antibiotics (SRGA). Scand J Infect Dis. 2013;45:161–75.
216. Kolyva AS, Karakousis PC. Old and new TB drugs: mechanisms of action and resistance. 2012. http://www.intechopen.com/books/understanding-tuberculosis-new-approaches-to--fighting- against-drugresistance/old-and-new-tb-drugs-mechanisms-of-action-and-resistance.
217. Boeree MJ. Global clinical trials for the treatment of TB with thioridazine. Recent Pat Antiinfect Drug Discov. 2011;6:99–103.

218. Park JS. Recent advances in tuberculosis and nontuberculous mycobacteria lung disease. Tuberc Respir Dis (Seoul). 2013;74:251–5.
219. van Ingen J, Ferro BE, Hoefsloot W, Boeree MJ, van Soolingen D. Drug treatment of pulmonary nontuberculous mycobacterial disease in HIV-negative patients: the evidence. Expert Rev Anti Infect Ther. 2013;11:1065–77.
220. Joosten SA, Fletcher HA, Ottenhoff TH. A helicopter perspective on TB biomarkers: pathway and process based analysis of gene expression data provides new insight into TB pathogenesis. PLoS One. 2013;8:e73230.