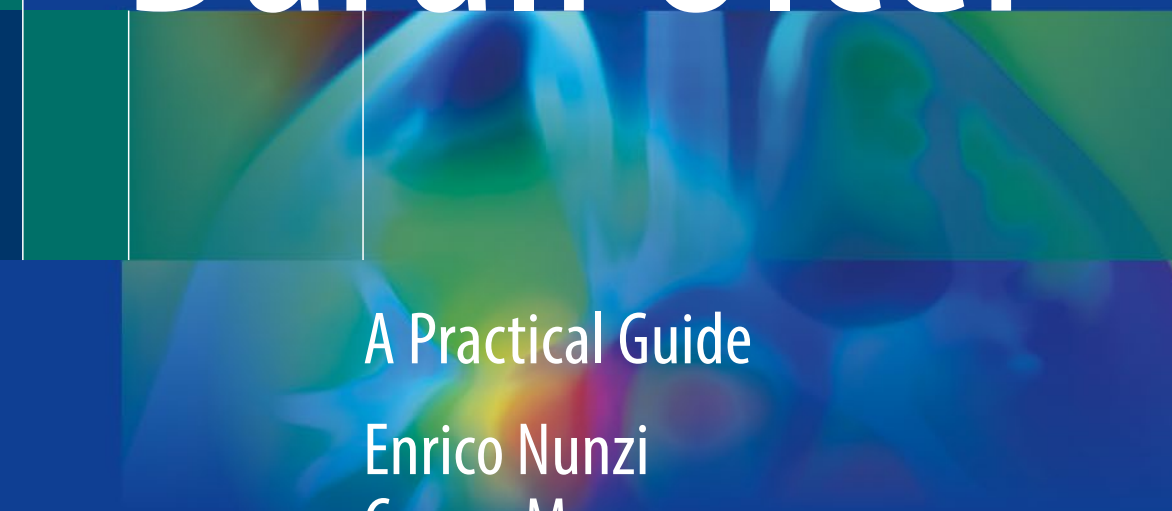
Leprosy and Buruli Ulcer



A Practical Guide

Enrico Nunzi
Cesare Massone
Françoise Portaels
Editors

Second Edition

Springer

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To Elizabeth Duncan
Elisabeth, who dedicated her life to African women affected by leprosy, passed away. These pages, fruit of her intellect, continue her work in the years to come as an expression of her love for the most forgotten people.
This second edition is dedicated to:
P. Bobin, S. G .Browne, F. Gatti,
A. Guédénon, R. C. Hastings,
M. F. Lechat, D. L. Leiker, A. C. McDougall, W. M. Meyers,
D. S. Ridley and Marian J. Ridley
friends and "maestri".

Foreword

Leprosy and Buruli ulcer, respectively the second and third mycobacterial disease in humans after tuberculosis, are currently classified by the World Health Organization as Neglected Tropical Diseases (NTD). The publication of this book almost coincides with the launch of the 2020–2030 Global Neglected Tropical Diseases Roadmap. These diseases are a group of infectious diseases that inflict suffering and chronic disability on one billion people, among the world's poorest populations. NTD affect communities without access to health care services, adequate hygiene and clean water. These tropical diseases, which are among the most serious health problems, cause disfigurement, disability and plunge families into an endless cycle of poverty and disease.

In the 1980s, with the development of multidrug therapy, a turning point was taken in the fight against leprosy. Millions of people have been treated thanks to the combined efforts of all actors involved in the fight against this endemic. It was hoped that by screening and initiating treatment for more patients, this age-old disease could be overcome. Unfortunately, for more than a decade, the number of new cases reported by countries has stagnated at around 200,000 new cases per year. Several million patients who have already received multidrug therapy have to live their entire lives with irreversible disabilities caused by leprosy without receiving adequate care due in particular to the weakness of the country's health systems. Many sick people, in addition to the physical suffering, experience the painful experience of stigma in their communities.

It may seem surprising to note that even today the mode of transmission of this ancient disease is still not fully understood. Recently, new animal reservoirs have been discovered in West Africa in wild chimpanzee. We currently have no vaccine or simple point-of-care diagnostic test that can be used in the communities closest to where patients live.

The situation described above might appear bleak. But there is still hope. The recently set up Global Partnership for Zero Leprosy reflects the desire of all those involved in the fight against this endemic not to give up and to pool their strengths to meet the many challenges that are currently presented. It will be necessary to work on several fronts: research, public health interventions, and strengthening of the health system in endemic countries. All these interventions must be supported by an advocacy for the mobilization of the resources necessary for an effective disease control.

In this perspective, the integration of leprosy control with other neglected tropical diseases, particularly those with skin manifestation, emerges as a promising strategy. In this logic, the pooling of strategies to fight Buruli ulcer is a good opportunity. Indeed, this third, mycobacterial disease has similarities with leprosy in many respects: it affects poor rural populations, it also manifests itself through skin lesions and is a source of stigma and handicap. In many co-endemic countries, these two diseases are managed by the same health staff. The pooling of resources for effective control will therefore be necessary to overcome these endemics.

This book on leprosy and Buruli ulcer edited by Professors Enrico Nunzi, Cesare Massone and Françoise Portaels is therefore timely to remind us of the urgent need not to give up. It will undoubtedly be a valuable document for the use of scientists, health workers and actors engaged in the fight against these endemics. In many countries, up-to-date knowledge on leprosy and Buruli ulcer is lacking. Expertise to diagnose and manage leprosy and Buruli ulcer is increasingly scarce.

The contributors to this book are among the most renowned people in their respective fields of expertise. This is the guarantee of the quality of the information contained in this document. I would therefore like to congratulate the editors of this book and wish it all the success it deserves.

The combined efforts of all actors will be essential to achieve a world free from the burden of leprosy and Buruli ulcer. The publication of this book will be a beautiful stone which will be added to many others in the construction of this world free of the burden of leprosy and Buruli ulcer that we are calling for.

Roch Christian Johnson
President of the International Leprosy Association
Cotonou, Benin
le 1 er décembre 2020

Preface to Second Edition

This second edition is enriched with the inclusion of Buruli Ulcer, another mycobacterial disease which is a part of the "Neglected Tropical Diseases" (NTD). This section of the book has been coordinated by a world expert of this emerging disease, Prof. Françoise Portaels.

NTD, chronic conditions which are closely related to social and environmental situations, especially among poor population groups, are not neglected so much by the health care personnel in the field because they deal with these in their daily work, as by the higher-level decision-makers in terms of health policies and by teaching institutions due to lack of staff with sufficient experience in these pathologies.

The gravity of NTD goes beyond that of individual pathologies as they contribute to a worsening of health of entire populations, facilitating the increase in other acute and fatal diseases.

Today we live in a globalized world where the distances which once served as barriers to diffusion of pathologies have disappeared and where the attention of the Media influences the priority given to interventions against new and emerging acute contagious conditions which affect all population groups.

The control of neglected diseases needs the buildup of adequate knowledge, and this Springer book is a step in that direction.

Loja, Ecuador — Enrico Nunzi
Genova, Italy — Cesare Massone
Antwerpen, Belgium — Françoise Portaels

Acknowledgments

We would also like to seize this occasion to express our thanks and acknowledge Dr. Sunil Deepak, former president of ILEP, for his highly valued advice and technical help.

Contents

Part I

Leprosy: General Section

History and Phylogeography of Leprosy

1

Stewart T. Cole and Pushpendra Singh

1.1 Background

Leprosy, a chronic, dermatological, and neurological disease, results from infection with the unculturable pathogen *Mycobacterium leprae* [1]. The disease is curable yet remains a public health problem even though there is no known ubiquitous reservoir for transmission of *M. leprae* other than human beings. Thanks to the massive implementation in the 1980s of multidrug therapy (MDT) by the World Health Organization, over 14 million patients have been cured and the incidence of leprosy has declined considerably. Nonetheless, an average of 250,000–300,000 new cases have been reported annually during the last 5 years throughout the world [2]. Many of these cases occur in children, thereby indicating that the chain of transmission remains, albeit weakened; this highlights the need for sensitive and reliable epidemiological methods to detect *M. leprae* and to monitor its spread both locally and globally. Genome sequencing has proved a particularly powerful means of understanding the biology and genetics of the leprosy bacillus, and comparative genomics has uncovered polymorphisms that can serve as the basis for developing molecular epidemiological tools. Such tools have started to find application and are helping us to understand how *M. leprae* has evolved.

1.2 History

Leprosy is often described as an "ancient" disease, but what does that really mean? Certainly, in the context of anatomically modern humans and the civilizations they established in the past 5000–10,000 years, the disease may appear ancient, but

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then one should recall that, from an evolutionary standpoint, where millions of years are the norm, the species *Homo sapiens* itself actually appeared quite recently. Various lines of evidence, based on haploid markers from mitochondrial DNA and the Y chromosome, together with the archeological, anthropological, and linguistic records, indicate that the cradle of mankind was East Africa. After initially spreading within Africa, humans migrated to the Near East about 60,000 years ago, before spreading to southern Asia and Australasia, Europe, and Central Asia, until finally reaching the Americas via the Bering Strait about 14,000–20,000 years ago [3]. Now, when we consider that *M. leprae* and the tubercle bacillus *Mycobacterium tuberculosis* are thought to have diverged from their most recent common ancestor 66 million years ago [4], the term "ancient" assumes a different meaning.

There are very old historical reports indicating that leprosy most likely existed in human populations in Egypt [5], India [6], and China [7], although the disease may often have been confused with other dermatological conditions and infections [8]. The oldest written records of leprosy are from the ancient Indian texts in the *Sushruta Samhita* around 600 BCE, which accurately describes the characteristic features and diagnosis of leprosy, and even the traditional treatment with chaulmoogra oil [6]. Skeletal remains provide a more reliable indicator, as the deformities characteristic of leprosy can often be readily identified. For instance, the "clawed hand" and enlarged or degraded maxillofacial regions can often be discerned on well-preserved skeletons [9]. Examples of the latter from 200 BCE are from the Dakhleh Oasis in Egypt [10]. The oldest leprosy skeletal remains found, from Balathal in India, were estimated to be 4000 years old by radiocarbon dating [11]. Such remains are relatively common in Europe, where leprosy was endemic in medieval times, but have not been found in the pre-Colombian Americas [12].

It is thought that the Phoenicians from the eastern Mediterranean region were responsible for the dissemination of leprosy around the Mediterranean Basin from 1500 to 300 BCE and that Alexander the Great's soldiers brought leprosy back from India about 325 BCE. In turn, the expansion of the Roman Empire is thought to have resulted in the introduction of leprosy into France, Germany, and the Iberian Peninsula. After the collapse of the Roman Empire, other invaders such as the Barbarians and the Saracens may have disseminated the disease, which reached the British Isles and Scandinavia, from where the Vikings likely served as carriers [13]. Leprosy was certainly endemic in Europe before the Crusades began in 1095 CE and started to decline gradually from the thirteenth century onward, although the reasons for this are unknown. Norway was one of the last European countries to eliminate leprosy, and it was there, in 1873, that Armauer Hansen made his seminal discovery of the leprosy bacillus in biopsies from fishermen in Bergen (editor note: last autochthonous Italian case is reported in 2010). One way of retracing the history of leprosy is to examine the genomes of strains of *M. leprae*, both ancient and modern, and to identify polymorphisms that have been vertically transmitted for use as molecular epidemiological markers.

1.3 Genomics

Since *M. leprae* has never been successfully cultured in the laboratory and has a generation time of 14 days, one of the longest known for a bacterium, it has proved extremely challenging to perform microbiological or genetic research with this pathogen. An important breakthrough came when it was found that the nine-banded armadillo *Dasypus novemcinctus* was naturally susceptible to infection [14]. Thus, for the first time, it became possible to obtain sufficient bacilli to perform biomedical research, such as vaccine development, with the leprosy bacillus. Once large numbers of bacteria become available, it was a fairly simple matter to extract their DNA in order to carry out whole-genome sequencing. The first *M. leprae* genome to be completely sequenced was that of the TN strain, originally isolated from a patient in Tamil Nadu, India.

The genome sequence of the TN strain of *M. leprae* contains 3,268,212 base pairs (bp) and has average G + C content of 57.8% [15]. The leprosy bacillus thus has the smallest and most A + T-rich genome of any known mycobacterium; for comparison, the close relatives *M. tuberculosis* and *Mycobacterium marinum* have genomes of 4,411,532 and 6,636,827 bp, respectively, and G + C content of 65.9% [16, 17]. Bioinformatic analysis uncovered 1614 genes coding for proteins in the TN genome and a further 50 that encode stable RNAs. These account for a mere 49.5% of the genome, with the remainder occupied by pseudogenes, i.e., inactive reading frames that still have functional counterparts in other mycobacteria. Initially, 1116 pseudogenes were found [15], but this figure rose to 1310 when other mycobacterial genome sequences became available for comparison [4].

The reductive evolution undergone by *M. leprae*, in which DNA was deleted from the genome and pseudogenes accumulated, provides a general explanation for its unusually slow growth, although no specific defect could be identified to account for this. Loss of DNA can be explained by homologous recombination events involving dispersed repeats, of which four families have been named [18]. This proposal is supported by the presence of single copies of these repeats at sites in the genome where synteny with other mycobacterial genomes breaks down as the gene order changes abruptly. None of these repeated elements contain open reading frames, although they do show some properties of transposable elements [18]. The presence of some of these repetitive sequences within pseudogenes suggests that they were once capable of undergoing transposition, but as will emerge below, this is no longer the case.

Analysis of the genome sequence has improved our understanding of the physiology, pathogenesis, and genetics of *M. leprae* and is underpinning the development of better diagnostics and molecular epidemiological tools for monitoring disease transmission.

1.4 Comparative Genomics of *M. leprae* Strains

To identify polymorphic DNA markers that could be used as the basis of a molecular epidemiological test for leprosy, the genomes of three other strains were sequenced, namely, Br4923, from Brazil; Thai-53, from Thailand; and NHDP63,

from the USA. Astonishingly, when the four genome sequences were compared, they were found to share 99.995% identity and to display near-perfect collinearity and size. There was no evidence for gene deletions, insertions, or translocations, with all of the dispersed repeats occupying the same positions in all cases [19, 20]. This four-way comparison revealed only 215 polymorphic sites, including single-nucleotide polymorphisms (SNPs) and small insertion-deletion events (indels). Five new pseudogenes were uncovered, three in strain Thai-53, and one each in strains Br4923 and NHDP63.

In light of the massive gene decay and extensive DNA loss undergone by *M. leprae*, the exceptional conservation of the genomes of these strains was truly unexpected, even more so when their widely different geographical origins are taken into account. Furthermore, it has been estimated from the number of nonsynonymous substitutions per site occurring in the pseudogenes that a single pseudogenization event occurred in the leprosy bacillus in the last 10–20 million years [4]. Given this vast timeframe, it is truly puzzling that so little diversity is observed within either the genes or the pseudogenes. This may indicate that the emergence of *M. leprae* as a human pathogen may have been due to a recent event, such as passing through an evolutionary bottleneck or introduction from another host. The long generation time is another factor that might contribute to genetic homogeneity. The leprosy bacillus is an excellent example of a genetically monomorphic pathogen [21], and in principle, all cases of leprosy stem from the initial infection with a single clone.

1.5 Strain Typing and Molecular Epidemiology

The exact route of transmission of *M. leprae* remains obscure, but early identification of infectious leprosy cases is critical in order to implement MDT rapidly and thus prevent disease progression, disabilities, and further contagion. After diagnosis, molecular typing of strains can help to improve understanding of the transmission and dynamics of the disease. Different molecular epidemiological tools are being developed to trace the possible sources of infection, to differentiate cases of relapse from reinfection, and to probe possible links between human and environmental reservoirs. One should recall that phenotypic methods of drug susceptibility testing (DST) of *M. leprae*, such as the mouse footpad model [22], have now been almost completely replaced by molecular methods [23] that use the polymerase chain reaction (PCR) to amplify regions of the gene encoding the drug target [24, 25]. Ideally, molecular DST and strain typing tests will eventually be combined.

Most genotyping methods take advantage of polymorphic sites in DNA, which exist in several forms. In addition to the SNPs and indels described above, there are variable number tandem repeats (VNTR) that can be exploited. VNTRs are sometimes called short tandem repeats (STRs), or microsatellites (repeat length 2–5 bp) and minisatellites (repeat length 6–50 bp). The paradigm VNTR in mycobacteria is the mycobacterial interspersed repetitive unit (MIRU), a tandemly arranged minisatellite that is a major source of diversity in the genomes of tubercle bacilli and some other mycobacteria. MIRUs serve as the basis of a robust PCR-based typing system that exploits differences in their copy number [26–28]. Unlike in

M. tuberculosis, none of the 20 MIRU loci in *M. leprae* contain tandem repeats [18], so this target sequence could not be used.

Instead, VNTRs consisting of repeat units of 1, 2, 3, 6, 12, 18, 21, and 27 bp have been screened and exploited for typing purposes [29, 30]. While VNTRs provide a better dynamic range for typing, they may be error prone due to the homoplasies frequently associated with such minisatellites [31]. To be successful in molecular epidemiology, the loci targeted must behave in a reproducible, stable, and discriminatory manner. Some of the limitations associated with VNTR typing can be overcome by interrogating multiple loci simultaneously, a technique known as multilocus VNTR analysis.

SNPs can be restricted to a single strain, and are therefore relatively uninformative, or be transmitted vertically and thus present in multiple strains, in which case they are more informative markers. The first SNP typing system was based upon three informative SNPs and used to screen ~400 different strains from 28 different countries across the world. This approach revealed only four combinations (SNP types 1–4) but with strong geographical associations [19]. Subsequently, a further 84 informative markers (78 SNPs and 6 indels) were discovered among the 215 polymorphic sites found by comparative genomics with four strains of the leprosy bacillus. Interrogation of these 84 sites enabled *M. leprae* strains to be classified into 16 different SNP genotypes (1A–4P), again displaying a phylogeographical relationship [20]. Figure 1.1 provides an algorithm of how SNP typing can be performed.

Combination of SNPs at 14676, 1642879, 2935693, = SNP Type	SNPs to determine the Subtype	SNP8453	SNP313361	SNP61425	SNP1642879	SNP Subtype
G,A,C= Type 1	SNP8453 SNP313361 SNP61425 SNP1642879	T				1A
		A	A			1B
			G	A		1C
				G	G	1D

		SNP3102787	SNP1104235	SNP2751790	SNP2935693	Subtype
T,A,C= Type 2	SNP3102787 SNP1104235 SNP2751790 SNP2935693	A				2E
		C	C			2F
			G	A		2G
				G	A	2H

		SNP1295195	SNP2312066	SNP413903	SNP20910	SNP146763	Subtype
C,T,C= Type 3	SNP1295195 SNP2312066 SNP413903 SNP20910 SNP14676	A					3I
		G	C				3J
			G	G			3K
				A	G		3L
					A	C	3M

		INS978589	DEL1476525	Subtype
T,T,C= Type 4	INS978589 DEL1476525	DEL		4N
		T	T	4O
			DEL	4P

Fig. 1.1 SNP typing scheme for *M. leprae*. Using the first three SNPs shown on the *left* (SNP14676, 1642879, and 2935693), a strain may first be typed into one of the four SNP types (1–4) and then subtyped using three or four markers shown at the *right* per SNP type to give the 16 subtypes (A–P). *Yellow cells* denote identity to the base present in the TN reference strain (subtype 1A), while *dark green* indicates identity to the base present in the Brazilian strain Br4923 at this position (subtype 4P). # These SNPs are the same as those used for typing into types 1–4 (*left side of the figure*)

While the SNP-based typing system is both very robust and highly reliable for classifying strains over broader geographical regions, at present, short-range transmission studies, within a district for example, are difficult with this typing system due to too little variation. Combining SNP and multilocus VNTR typing appears to be the method of choice for future typing studies of *M. leprae* by merging the phylogeographic component imparted by SNP subtypes with the dynamic branching provided by VNTRs.

1.6 Phylogeography

In the past decade, it has become increasingly apparent that one can retrace the evolutionary history of peoples and their migrations by studying the genotypes of the pathogens they carry. In this regard, disease-causing bacteria such as *Helicobacter pylori* [21, 32, 33], *Yersinia pestis* [34], and *M. leprae* have served as valuable tools. Conversely, understanding the structure of human populations and their immunogenetics can also provide insight into the evolution of pathogens [35].

Phylogenetic studies of *M. leprae* have allowed us to infer relationships between different strains and to attempt to retrace their history. There is a fairly strict correlation between the geographical origin of the leprosy patient and the SNP profile: types 1A–D occur predominantly in Asia, the Pacific region, and East Africa; types 3I–M in Europe, North Africa, and the Americas; and types 4 N–P in West Africa, the Caribbean region, and South America. *M. leprae* belonging to SNP types 2E–H appears to be the rarest, although this may be due to undersampling, and has only been detected in Ethiopia, Malawi, Nepal/North India, and New Caledonia (Fig. 1.2).

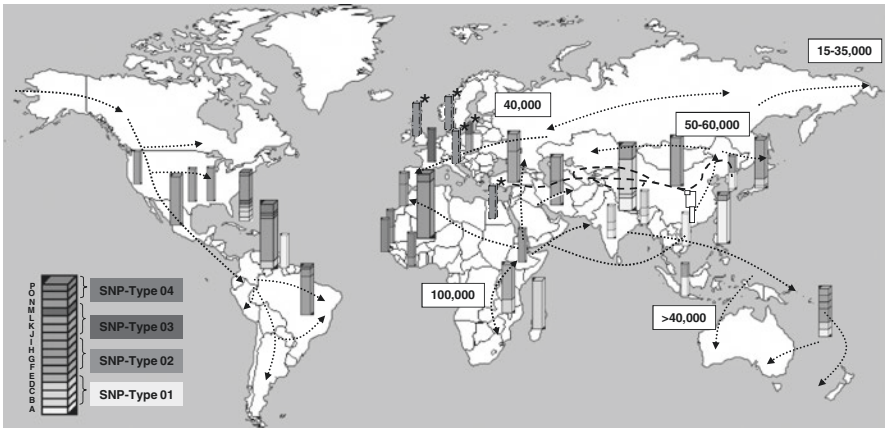

Fig. 1.2 Global dissemination of *M. leprae* from SNP typing. Pillars are located on the country of origin of the *M. leprae* sample and color coded according to the scheme for the 16 SNP subtypes. The thickness of each pillar corresponds to the number of samples tested (1–5, thin; 6–29, intermediate; >30, broad). *Gray arrows* indicate the routes of migration taken by early humans with the estimated time of migration in years [3, 36]. *Dashed line* indicates the location of the Silk Road in the first century, and * denotes result obtained from ancient DNA. (Reproduced with permission from [20])

Applying the principles of maximum parsimony, two plausible evolutionary schemes arise. In the first scheme, which we consider more probable, *M. leprae* of SNP type 2, present in the region of East Africa/Near East, preceded SNP type 1, which migrated eastward, and SNP type 3, which disseminated westward in human populations, before SNP type 3 gave rise to type 4. In the second scenario, type 1 was the progenitor of type 2, with SNP types 3 and 4 following in numerical order. Comparison of what is known of human migrations with the phylogeny of *M. leprae* is highly informative and reveals general agreement with the historical record and several contradictions.

The proposition that leprosy originated in the Indian subcontinent and was introduced into Europe by Greek soldiers returning from the Indian campaign of Alexander the Great [8] is not compatible with the phylogeny of *M. leprae* since SNP type 1 predominates in India, whereas SNP type 3 afflicted European populations. From India, leprosy is thought to have moved to China, in about 500 BC, and then to Japan, reaching Pacific islands such as New Caledonia as recently as the nineteenth century. Once again, this is partly contradicted by the results of *M. leprae* genotyping, as leprosy appears to have been introduced into Asia by two different routes. The first of these, which is historically consistent, is the southern one associated with the SNP type 1 strains present in the Indian subcontinent, Indonesia, and the Philippines. The second is a more northerly route beginning in the eastern Mediterranean region and extending via Turkey and Iran to China, and hence to Korea and Japan. The strains encountered along this route are mainly of SNP subtype 3K [20], and the Silk Road appears to have been a likely means of transport and disease transmission (Fig. 1.2). The Plague or Black Death, caused by *Yersinia pestis* [34], is thought to have reached Europe in the fourteenth century from China via the Silk Road, being carried by humans and their fleas. With respect to leprosy, the opposite route of transmission may have been traveled, with the disease originating in Europe or the Near/Middle East and then spreading to the Far East.

Nothing is known of the history of leprosy in sub-Saharan Africa except that the disease was present prior to the colonial era [8, 13]. The phylogeny of *M. leprae* suggests that the disease was most likely introduced into West Africa by infected explorers, traders, or colonialists of European or north African descent, rather than by migrants from East Africa, as *M. leprae* of SNP type 4 is endemic in West Africa and much closer to type 3 than to type 2. West and southern Africa are believed to have been settled by migrants from the eastern part of the continent before the arrival of humans in the Eurasian regions [3, 37]. It seems unlikely that early humans brought leprosy into West Africa unless clonal replacement has since occurred. From West Africa, leprosy was then introduced by the slave trade from the seventeenth century onward into the Caribbean region, Venezuela, Brazil, and other parts of South America, as isolates of *M. leprae* with the same SNP type, 4N or O, are found there as in West Africa, which is consistent with the history of slavery. Strains of SNP subtype 4P are restricted to South America or the Caribbean, and they must have branched off during the last 400 years, since the introduction of the ancestral strain from West Africa, which was likely of SNP type 4 N or O.

It appears improbable that leprosy was introduced into the Americas by early humans via the Bering Strait; instead, it seems more likely that immigrants from Europe brought the disease, as most of the *M. leprae* strains found in North, Central, and South America have the 3I genotype associated with European leprosy cases. In the eighteenth and nineteenth centuries, when the Mid-Western states of the USA were settled by Scandinavian immigrants, many cases of leprosy were recorded at a time when a major epidemic was underway in Norway [8]. Further support for the "European origin" hypothesis is provided by the finding that wild armadillos from Louisiana, USA, which are naturally infected with *M. leprae*, also harbor the SNP type 3 strain, indicating that they were contaminated from human sources of European origin [19]. Immigrants from France and Spain primarily were the main settlers in the state of Louisiana.

It is also noteworthy that, on islands such as the French West Indies and New Caledonia, there is much greater SNP variety of *M. leprae* (Fig. 1.2), reflecting the passage of, and settlement by, successive human populations. It is known that migrations by sea rather than over land or via coastal routes lead to more diversity in humans through intermixing, and a greater range of diversity is also apparent among their pathogens.

1.7 Paleomicrobiology

Insight into ancient leprosy can be gained from the study of skeletons with the telltale signs of leprosy described above. Although this is a technically challenging approach, fascinating results have been obtained with skeletal remains from a number of settings. Studying these "extinct" cases not only enables comparisons with extant strains of *M. leprae* but also provides information for countries where leprosy has long been eradicated.

In the first report, two archeological cases of leprosy from Medieval England were studied using ancient DNA methods and PCR. The highly characteristic repetitive DNA was used for confirmation of the identity *M. leprae*, and three VNTR loci were also amplified [37]. In subsequent work, SNP typing was undertaken with specimens from the UK, Turkey, Egypt, Denmark, and Croatia. The *M. leprae* present in these samples all exhibited an SNP type 3 profile, and in some cases, further typing was possible, revealing the presence of subtypes 3I, 3K, and 3M [20, 38]. The oldest specimen examined to date, roughly 1500 years old, came from an Egyptian skeleton from Dakhleh Oasis [39], a region close to the proposed origin of *M. leprae* and *Homo sapiens*. The ancient DNA analysis indicated that *M. leprae* of SNP type 3 was involved. Finally, the most recent study was with a sample from Uzbekistan, of a similar age to that from Egypt, and this too revealed that the causative strain of *M. leprae* exhibited an SNP type 3 profile [40]. This is consistent with the fact that extant strains of *M. leprae* from Iran, a country nearby, are often of type 3K [20]. With improvements in ancient DNA methodologies and the massively parallel sequencing capabilities offered by next-generation sequencing technologies, it is not inconceivable that we will be able to use skeletal remains to generate a draft

genome sequence for an "extinct" strain of *M. leprae*, as was done recently for the Neanderthal genome [41].

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Microbiology of *Mycobacterium leprae*

2

Andrea Clapasson and Silvia Canata

2.1 Microbiology

Mycobacterium leprae (*M. leprae*) is an intracellular obligate parasite organism, identified in 1873 by the Norwegian doctor Gerhard Henrik Armauer Hansen. For over a century, it was believed that *M. leprae* was the only etiological agent that caused leprosy, but in 2008 another bacterium was identified in diffuse lepromatous leprosy. This bacterium was called *Mycobacterium lepromatosis* [1].

Taxonomically, this bacterium belongs to genus *Mycobacterium* within the family of *Mycobacteriaceae* in the order *Actinomycetales*. The family includes about 200 species and more than 600 strains are not well characterized. Only 20 species are considered pathogenic to humans and can cause disease. The main pathogenic mycobacteria are *M. africanum*, *M. leprae*, *M. tuberculosis*, *M. avium*, *M. celatum*, *M. genavense*, *M. gordonae*, *M. haemophilum*, *M. simiae*, *M. xenopi*, *M. avium paratuberculosis*, *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. kansasii*, *M. malmoense*, *M. marinum*, *M. scrofulaceum*, *M. szulgai*, and *M. ulcerans*. This list of pathogenic mycobacteria underlines that the Hansen's bacillus is not the only alcohol acid-resistant bacterium that has tropism for humans. There are other alcohol-acid-resistant bacteria that can contaminate or colonize the skin; this should be considered when laboratory tests are interpreted [2].

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2.1.1 Property of *M. leprae*

M. leprae is a slow-growing, acid-fast, Gram-positive bacillus, and is a straight or slightly curved, rod-shaped organism. It is about 1–8 μm long and has a diameter of 0.3–0.6 μm with parallel sides and rounded ends.

It can be found isolated or in clusters (cigar-shaped or globi); it is a non-sporulating organism, with no plasmid and no motility.

When *M. leprae* is stained by Ziehl-Neelsen (ZN) or Fite Faraco (FF) techniques, due to its acid resistance feature, it is called alcohol-acid-resistant or acid-fast bacillus (AFB).

Acid-fast property may be removed by pre-treatment with pyridine, a characteristic that distinguishes *M. leprae* from other mycobacteria.

The ZN and FF techniques have the ability to stain differently the bacillus, according to its vital status (see Chap. 8, "Laboratory Investigations"). This ability, a degeneration symptom, is affected by the immune system of the host organism, by treatment with antibiotics, and it is also due to the normal bacterial cell cycle.

M. leprae is an obligate aerobe and microaerophilic bacterium. It can grow and divide itself inside macrophages and Schwann cells, and it can be found in the cytoplasm of monocytes, in giant cells, in endothelial cells, and in neutrophils. Among mycobacteria, *M. leprae* is the only one which infects peripheral nerves. It has an extremely slow doubling time of 12–14 days, favored by temperatures of around 30 °C, with the best at 32 °C. It is generally susceptible to heat and UV light, but it is resistant to the action of acids and alkalis. The period of time between infections of the disease is difficult to define because it can vary from a few weeks to 30 or more years, but in general the incubation period falls between 2 and 5 years for tuberculoid leprosy and 9 and 11 years for lepromatous leprosy [3].

2.1.2 Envelopments of *M. leprae*

Generally, *M. leprae* has been defined as unencapsulated but is now known to have a capsule-like structure. The layers forming cellular envelopment of *M. leprae* are capsule, cell wall (outer layer and inner layer), and plasma membrane. These elements play a crucial role in intracellular adaptation, immune modulation, or defense versus microbicidal activities of the immune system and as permeability barriers for acquisition of nutrients and drugs.

The composition of the bacterial cell envelope consists of a highly complex array of distinctive lipids, glycolipids, proteins, and polymers, of which the mycolyl-arabinogalactan-peptidoglycan complex (MAPc) is the main structural component [4].

Capsule: to electron microscope, it appears as an electron transparent zone. It consists of a lipid phthiocerol dimycoserosate and a phenolic glycolipid. This glycolipid is made up of three methylated sugars linked by phenol to phthiocerol (fat), known as phenolic glycolipid or PGL; it is chemically unique and the trisaccharide

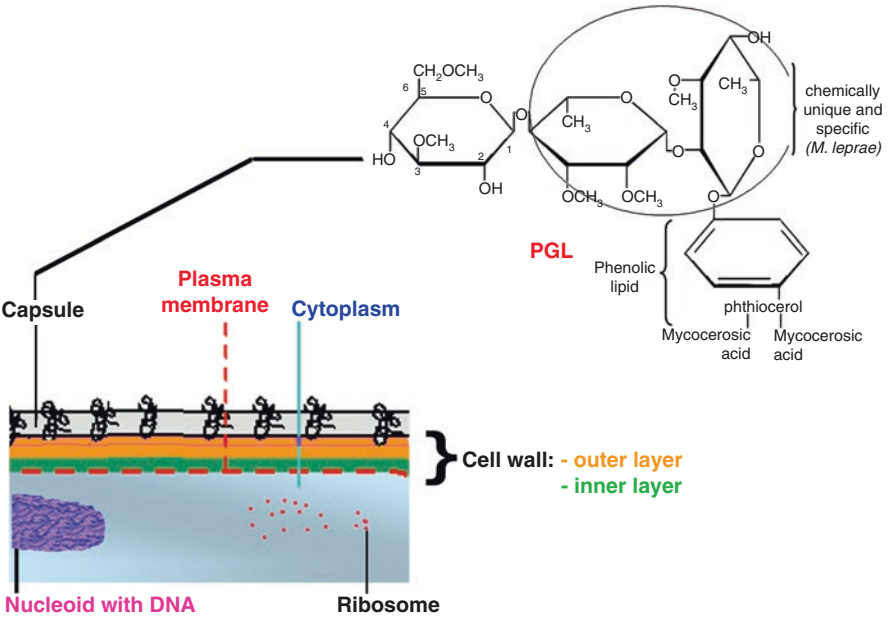

Fig. 2.1 Schematic model of cell envelope of *M. leprae*

is referred to as specific to *M. leprae*. This macromolecule is present in three variants: I, II, III and the main variant is variant I or PGL-I (Fig. 2.1) [4].

Cell wall: it is made up of an inner and an outer layer.

In the inner layer, there is peptidoglycan that is a polymer consisting of sugars and peptides repeated. The peptide has an amino acid sequence specific to *M. leprae*. This sequence specific to *M. leprae* makes the wall more resistant to enzyme attack, providing advantages in pathogenicity (Fig. 2.1). Unfortunately, this peptide is in insufficient quantity to be use it as diagnostic antigen.

The outer layer is a structure made up of chains linked to arabinogalactan esterified with mycolic acids. The scaffold is then consolidated with other molecules and lipoarabinomannans [4].

About 30–40% of total weight of the cell wall consists of lipid, long-chain mycolic acids of high molecular weight. Mycolic acids are ß-keto esters of fatty acids containing 60–90 carbon atoms, and are primarily responsible for special characteristic of acid fatness. The only organisms capable of synthesizing mycolic acids are *Corynebacterium*, *Mycobacterium*, and *Nocardia* (CMN group).

2.1.3 Genome

M. leprae, after it has become an obligate intracellular pathogen, has undergone a reduction in the size of the genome. This is an extreme case of reductive evolution

with deletion of genes and elimination of many important metabolic activities. In order to understand the extent of this gene deletion, we compare the genomes of *M. leprae* and *Mycobacterium tuberculosis*, the closest and best characterized.

The genome of *M. tuberculosis* H37Ra is composed of 4,411,529 base pairs (bp), encoding 3924 genes, while that of *M. leprae* has 3,268,203 (bp) and only half of *M. leprae* genome has functional genes (1604).

In *M. leprae*, there are pseudo-genes (1116) and about 2% of its genome is made up of specific repetitive sequences that can be used for diagnostic purposes in biological molecular assay, for example, the sequence RLEP, repeated for 39 times. This genome and the complex and unique cell wall explain the long doubling time of the bacterium, which is the longest of all known bacteria [4, 5] (see Chap. 1).

2.1.4 Animals and Cultures Where *M. leprae* Can Grow or Survive

M. leprae is not cultivable in vitro using solid media; however, it is possible to keep it alive in liquid media or in macrophage culture, but only for a short time. Its multiplication is possible using animal models like mouse footpad (limited), nine-banded armadillo, and some primates.

2.1.5 Infection and Transmission

The bacterium mainly affects the skin, peripheral nerves, mucosa of the upper respiratory tract, and eyes. It multiplies more easily in the cooler areas of the body, such as the ear lobe, face (forehead, nose, zygomatic regions, chin), hands, feet, buttocks, testes, superficial lymph nodes, and peripheral nerves.

The main points of entry and exit of the pathogen are the same: the mucosa of the upper respiratory tract and the skin lesions.

M. leprae survives outside the human host, with favorable environmental conditions (humid heat and protection from UV rays), for over a month.

2.1.5.1 Risk Group Level of *M. leprae*

There is no unique classification of biohazard level, so for proper handling of pathological or suspect pathological sample, workers need to follow national laws in their country. The Risk Group classification for infectious agents ranges from 1 to 4. For *M. leprae*, it is 3 in European Community (2000), Singapore (2004), and Switzerland, while it is 2 in Australia, Canada, North America, and Japan [6].

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Genetics of Leprosy

3

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3.1 Introduction

Leprosy, like other infectious diseases, was widely accepted as hereditary in the pre-microbiological era. The revolutionary finding of a microorganism—originally named *Bacillus leprae*—in lesions of leprosy-affected individuals led Gerhard H. Armauer Hansen to fiercely refute the belief that leprosy was inherited. Today, scientists have clearly shown that exposure to *M. leprae* is necessary but not sufficient to explain leprosy occurrence, and several genes and genomic regions have been implicated in the complex genetic mechanism controlling host susceptibility to leprosy at different stages of the disease (Fig. 3.1).

3.2 Genetics of *M. leprae* and the Origins of Leprosy

The complete sequence of the *M. leprae* genome was first published in the early 2000s. Compared to *M. tuberculosis*, the *M. leprae* genome shows strong reductive evolution as the bacteria specialized as an obligatory intracellular parasite in humans [1]. Since then, whole genome analysis has provided insights about several aspects of leprosy, including the history of the disease. For example, in 2018, genome sequences of ten *M. leprae* DNA samples obtained from the remains of medieval Europeans produced a snapshot of the last 1500 years of leprosy history in the European continent. *M. leprae* from four distinct phylogenetic branches were found

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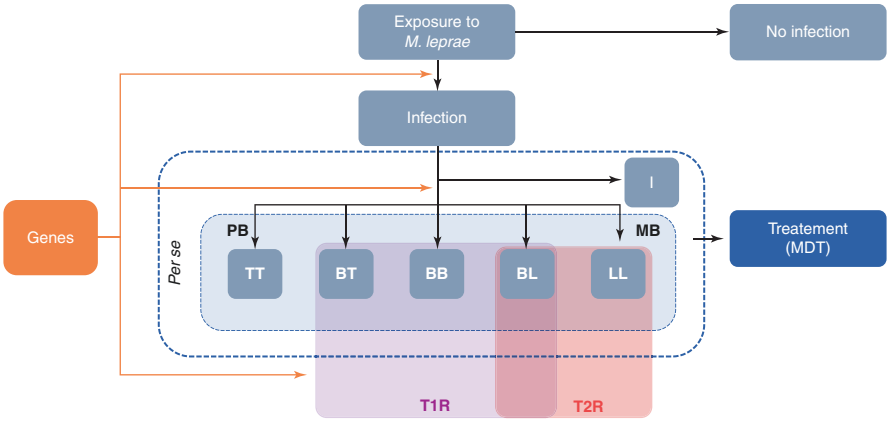

Fig. 3.1 Schematic representation of the clinical classification spectrum of leprosy. *TT* tuberculoid-tuberculoid, *BT* borderline-tuberculoid, *BB* borderline-borderline, *BL* borderline-lepromatous, *LL* lepromatous-lepromatous, *I* indeterminate, *PB* paucibacillary, *MB* multibacillary, *MDT* multidrug therapy, *T1R* type-1 reaction, *T2R* type-2 reaction

among the ten samples, some matching modern strains from different locations around the world. This study highlights the diversity of *M. leprae* strains in medieval Europe, and the authors proposed new models for leprosy dissemination: (1) the introduction of strains from different parts of the world into Europe, which may have happened before the medieval era, or (2) the onset of leprosy occurred in Western Eurasia or in Europe, and not in western Africa, as previously proposed [2].

Regarding leprosy pathogenesis, bacterial genomics also identified a novel mycobacterial species named *M. lepromatosis* [3], a rare mycobacterium that apparently causes a distinct form of leprosy and is mainly found in Central America [4].

Comparative analysis of *M. leprae* isolates from different parts of the world confirmed the conserved nature of its genome. The low variability of the *M. leprae* genome suggests that the wide variety of responses observed upon exposure to the pathogen is largely controlled by host genetic factors. The hypothesis has been reinforced by observations such as familial aggregation of cases, a higher concordance rate of leprosy phenotypes in monozygotic as compared to dizygotic twin pairs [5], and the presence of a strong major gene effect controlling leprosy, as demonstrated by complex segregation analysis [6]. Although powerful to detect the existence of a genetic component controlling a specific trait, these observational studies do not provide any information about the identity of the genes or the nature of the genetic variants underlying the identified effect; for that, molecular studies are necessary.

3.3 Leprosy Genes and Genomic *Loci*

Genetic epidemiology approaches have successfully identified genes and genetic variants impacting upon susceptibility to infectious diseases, including leprosy [7]. The molecular nature of the genetic component controlling host susceptibility to

leprosy has been intensively investigated by candidate gene studies, genome-wide linkage or association searches, and, more recently, genome/exome/target DNA sequencing approaches. A brief description of selected genetic findings in leprosy is presented next.

3.3.1 Major Histocompatibility Complex (MHC) Genes

In leprosy, clinical manifestation of disease depends on the Th1/Th2 balance that is partially controlled by antigen-presentation and cell-cell interactions via MHC genes. The MHC locus located in chromosome 6p21.32-p22.2 harbors the three classes of the human leukocyte antigens (HLA), which include genes that are key mediators of host immune responses. In fact, the first genetic risk factors described for leprosy susceptibility were variants of the MHC complex.

Perhaps the most well-known genetic association with leprosy are alleles of the *HLA-DRB1* gene (rev. in [8]). Variants of *HLA-DRB1* were associated with resistance or susceptibility to leprosy in samples from Brazil, Vietnam [9], and China [10], and the markers near the *HLA-DRB1* locus were the most significant association signal identified in the first genome-wide association (GWA) study in leprosy [11]. A case-control analysis in a New Delhi sample observed consistent association between leprosy and variations of *HLA-DRB1* and *HLA-DQA1*, another well-described HLA class II leprosy susceptibility locus [12].

HLA class I (A, B, and C) has been also intensively studied in leprosy, and HLA-A*2, A*11, B*40, and C*w**7 are some examples of alleles detected more often among leprosy cases as compared to non-affected controls [13]. Class I HLA molecules interact with killer cell immunoglobulin-like receptors (KIR); in a south Brazilian cohort, *KIR* alleles were associated with tuberculoid leprosy [14]. Of note, the HLA-B*13:01 allele was shown associated with dapsone hypersensitivity syndrome [15], an observation that highlights the importance of HLA genes in the control of drug toxicity during treatment and opens the road for pharmacogenomics in leprosy.

Investigation of a cohort of 22 Vietnamese multiplex leprosy families resulted in evidence of linkage between leprosy type and two microsatellite markers of the TNF-α gene (*TNFA*) located in the HLA class III region [16]. This finding is in agreement with evidences of association between promoter polymorphisms of *TNFA* and clinical manifestation of leprosy (rev. in [8]). A study demonstrated that a functional single nucleotide polymorphism (SNP) located at base pair +80 of the *LTA* gene, located immediately upstream *TNFA*, is associated with early-onset leprosy [17]. Finally, variants of additional HLA-linked genes, such as *TAP*, *MICA* [18], and *MICB* have also been described in association with leprosy phenotypes in different populations, the latter two recently replicated in the New Delhi population sample [12].

Nowadays, the challenge is to dissect the exact nature underlying HLA association with leprosy. The MHC/HLA locus is a highly polymorphic gene-rich region presenting long-range linkage disequilibrium (i.e., cross-association between

alleles). The complexity of the MHC/HLA locus makes pinpointing the actual causative variant very difficult; yet, a few studies tackled this challenge. A fine mapping of the HLA complex in the Vietnamese and Indian population narrowed the association to two intergenic SNPs close to *HLA-C* in the HLA class I region [19]. Two studies in 2020 investigated in depth the HLA complex. In the first, a family-based GWAS identified three independent signals, two in the HLA class I region and one in HLA class 2 close to *HLA-DQA1* [20]. The second applied deep sequencing to study 11 HLA class I and II genes at the amino acid level. The authors identified haplotypes of *HLA-DRB1*, *HLA-DQA1*, *HLA-DRB3*, *HLA-B*, and *HLA-C* alleles associated with susceptibility or protection against leprosy. Furthermore, the authors were able to narrow down the association to four independent amino acids (i.e., HLA-DRβ1 57D and 13F, HLA-B 63E and HLA-A 19K), a major advance toward the understanding of the complex pattern of association of HLA genes with leprosy [21].

3.3.2 Non-HLA Genes

To date, numerous non-HLA variants of different genes have been described as leprosy genetic risk factors, with most of the early evidence being produced by hypothesis-driven, candidate gene studies. These types of studies are limited in scope but have been very powerful to detect relevant genetic association between leprosy phenotypes and genes such as *SLC11A1* (an iron transporter across the phagosome membrane), *VDR* (vitamin D receptor), *IL10* (a Th2 cytokine), and *TLR1* (a pattern recognition receptor), among others (rev. in [22, 23]).

More recently, hypothesis-free approaches have been consolidating as an alternative to candidate gene studies, extending the reach of the investigation to the entire genome and allowing the discovery of previously unsuspected genes. In 2001, the first genome-wide linkage analysis for leprosy identified a paucibacillary leprosy susceptibility *locus* at chromosomal region 10p13 [24], but only in 2010 the first candidate gene emerged from that chromosomal region: a non-synonymous SNP located at *MRC1* was associated with leprosy in both Vietnamese and Brazilians [25]. The *MRC1* gene was later associated with paucibacillary leprosy in individuals from southwest China [26]. Two years later, fine mapping of the 10p13 identified the *CUBN* gene associated with multibacillary leprosy in Vietnamese [27].

Interestingly, the linkage signal for paucibacillary leprosy at chromosome 10p13 was replicated in a second genome-wide scan that, most importantly, identified a strong linkage peak for leprosy per se on chromosome 6q25-q27 [28]. Subsequent fine mapping of the 6q25-q27 *locus* led to the first successful positional cloning of genetic variants impacting on risk of an infectious disease: two SNPs located at the shared regulatory region of the *PRKN* and *PACRG* genes were found independently associated with leprosy per se in two population samples from Vietnam and Brazil [29]. These findings triggered an exciting series of subsequent studies aiming to

fully understand the impact of the 6q25-q27 *locus* in general and the *PRKN/PACRG* genes in particular upon leprosy risk and the disease physiopathology. In addition, two studies successfully replicated the *PRKN/PACRG* associations [30, 31], and more sophisticated analyses have revealed interesting nuances of the exact nature of the association signals observed. For example, a study performed in Vietnamese and Indian populations showed that the linkage disequilibrium structure and the age at disease diagnosis are crucial for the association of *PRKN/PACRG* with leprosy per se [31]. Finally, an effort to completely dissect the strong linkage signal identified at the 6q25-q27 *locus* led to the identification of a second association hit with leprosy per se near the *SOD2* gene, coding a superoxide dismutase, in two independent Brazilian population samples [32].

How parkin, an E3 protein-ubiquitin ligase encoded by *PRKN*, is involved in the pathophysiology of an infectious disease is a question that has been generating very exciting results. For example, a remarkable study demonstrated that parkin is a critical player controlling susceptibility to *Mycobacterium tuberculosis* infection in mice, with a particularly important effect upon autophagy; the same authors demonstrated that parkin also modulates susceptibility to other intracellular pathogens—such as *L. monocytogenes*—in different species, indicating a highly conserved evolutionary role in innate immunity for this protein [33]. Interestingly, leprosy patient that experienced excessive inflammatory responses shared *PRKN* mutations observed in Parkinson's disease (PD) cases. This genetic overlap between leprosy and PD highlight the key role of *PRKN* as a mediator of host inflammatory responses [34].

In addition to the HLA-linked variants previously mentioned [11], a GWAS in the Chinese population reported polymorphisms of six non-MHC genes—*TNFSF15*, *NOD2*, *RIPK2*, *LRRK2*, *CCDC122*, and *LACC1*—significantly associated with leprosy. Since then, several studies have validated/replicated the original findings. The *CCDC122* and *LACC1* genes, both located at chromosome 13q14.11, were replicated in population samples from India, Mali [35], Vietnam [36], Brazil [37], and China [38]. The *NOD2* gene was validated in Nepal [39], Vietnam [36], Brazil [37], and China [38]. The *RIPK2* gene was replicated in Indian [40] and Vietnamese individuals.

Several suggestive findings from the original GWAS have been later explored either by expanding the initial population sample or by applying hypothesis-driven approaches. As results, many additional non-HLA genes were identified significantly associated with leprosy, including *IL23R* [41], *BCL10* [42], *CCDC88B* [43], *MED30* [44], and *TYK2* [45], among others (rev. in [46]). While these studies expanded the number of genes and pathways contributing to leprosy susceptibility, one of the most exciting findings has been the overlap of genes associated with both leprosy and inflammatory bowel disease (IBD) [47]; studying the genetic and molecular component shared between these two apparently distinct phenotypes may pave the road to drug repurposing and perhaps the development of alternative therapies for both diseases.

3.4 Leprosy Reactions

Permanent disabilities caused by leprosy reactions are a major disease burden likely to persist even under the unlikely scenario of leprosy elimination as a public health problem. Since leprosy reactions may occur years after completion of leprosy treatment, identifying predictive risk factors—genetic or otherwise—for leprosy reactions is a major research goal.

Genetic epidemiology studies on leprosy reaction are few compared to other leprosy phenotypes. Variants of the *TLR2* and *TLR1* genes were the first associated with leprosy type-1 reaction (T1R) [48–50]. Variants on the *NOD2* gene were associated with both T1R and T2R in Nepal [39]; however, these SNPs were not the same associated with leprosy per se in the leprosy GWAS [11]. In Brazilians, functional *IL6* promoter variants that regulate IL6 plasma levels were associated with leprosy T2R reaction [51]. A subsequent study using survival analysis showed that the same *IL6* variants were associated with the time of leprosy reaction onset [52].

Based on the observation that several studies failed to replicate the association between *TNFSF15/TNFSF8* and *LRRK2* genes and leprosy per se led to investigations of these genes as candidates for T1R. Variants near the *TNFSF15/TNFSF8* genes were associated with risk for T1R [53, 54] in both Vietnamese and Brazilian population samples. In Vietnamese, two *LRRK2* amino acid changes (R1628P and M2397T) and a set of variants regulating gene expression were also preferentially associated with T1R [34, 55]. In 2019, using a targeted resequencing approach, researchers identified additional rare LRRK2 amino acid changes associated with T1R [34]. Remarkably, in the same study, the authors have reported that T1R leprosy cases carried rare *PRKN* damaging mutations, while T1R-free leprosy did not. This was an interesting observation that places parkin as a central mediator of multiple leprosy phenotypes, as noncoding variants near parkin are established risk factors for leprosy per se. A GWAS comparing T1R-affected versus T1R-free leprosy cases identified regulatory variants of a long noncoding RNA (lncRNA) *ENSG00000235140* associated with T1R in Vietnamese and Brazilians. Apart from this novel lncRNA, all other genes reported for T1R had also previously been associated with leprosy per se.

3.5 New Insights

Based on the exposed above, it is difficult to undervalue the contribution of genetics to the advance of the understanding of the molecular basis of leprosy susceptibility. However, it is also true that most of the identified associations provide a small contribution to leprosy risk, thus explaining only part of the large heritability estimated for the disease by observational studies. This may be partially be due to the fact that classic linkage and association studies (candidate gene-based and GWAS) rely on the use of informative, thus polymorphic, markers with a minimum allele frequency

(MAF) higher than 1% [56], which leaves out an entire fraction of the human genetic variation represented by rare variants (MAF < 1%). With the development of novel sequencing techniques, it is now possible to investigate rare or structural variations at a relatively low cost. Thus, analysis of complete genomes/exomes or targeted protein coding regions is likely to find additional genetic factors with an impact on leprosy risk. Using this strategy, a recent study involving whole-exome and target sequencing identified a rare missense in the *HIF1A* gene influencing host susceptibility to leprosy in Han Chinese [57]. Furthermore, susceptibility to leprosy is very likely to depend on other sources of variation such as differential methylation of Cs and Gs, histone modification, and DNA translocations, a field of research yet to be systematically explored.

Finally, new, creative, or better-defined phenotypes are beginning to be explored with exciting results. For example, it is known that continuously exposed patients may suffer from leprosy recurrence, a poorly explored disease phenotype. Recently, a pilot study revealed an enrichment of homozygous genotypes for the risk alleles of genes classically associated with leprosy among two out of three cases of leprosy recurrence when compared to three nonrecurring leprosy patients. The study, although limited to a description of a series of cases, suggests the existence of a genetic profile of particularly high innate leprosy susceptibility among patients that may predispose to disease recurrence [58].

3.6 Perspectives

Genetics and genomics of complex traits in general and of infectious diseases in particular are a vibrant and productive field of medical research. The discovery of functional variants initially identified through genetic approaches and later confirmed in functional studies may lead to better protocols for diagnosis, treatment, and prevention of disease. One possibility is the development of laboratory tests using panels of reliable disease markers coupled with bioinformatics and artificial intelligence tools aiming at producing predictive indicators of prognosis or response to treatment. The description of variants and their impact on protein function can be an initial step toward identifying new therapeutic targets eventually leading to the development of much needed new and more efficient leprosy therapeutic protocols, with fewer side effects and better patient compliance. Moreover, the characterization of leprosy genetic susceptibility markers can lead to important advances in the field of other infectious, inflammatory, or chronic degenerative diseases such as tuberculosis and Parkinson's and Crohn's diseases [36, 46, 59–61].

In summary, our understanding of the genetic mechanisms controlling the classic leprosy phenotypes, such as disease per se and clinical subtypes, is fairly advanced, particularly as compared to other infectious diseases. However, secondary but interesting phenotypes, such as disease recurrence, age of onset, and even leprosy reactions, still need in-depth investigations as they represent the latest frontiers in leprosy genetic research.

3.7 Comments on Human Genetics of Buruli Ulcer

Buruli ulcer (BU), caused by *Mycobacterium ulcerans,* is the third most common mycobacteriosis in the world after tuberculosis and leprosy [62]. BU presents a wide spectrum of clinical manifestations ranging from single, small lesions to severe ulcers, osteomyelitis, osteitis, and joint involvement (see Chaps. 42 and 43).

Similar to T1R in leprosy, BU patients may also develop an abrupt cell-mediated inflammatory reaction, known as a paradoxical reaction [63] (see also Chap. 43).

Host genetic susceptibility to BU is a relatively unexplored field; however, exciting results have been produced through different approaches following the leprosy model. Classic candidate gene studies, usually targeting genes and *loci* associated previously with tuberculosis and leprosy, have revealed association between BU and genes *SLC11A1* [64], *PRKN*, *NOD2*, *ATG16L1* [65], *iNOS*, and *IFNG* [66]. Of note, the *SLC11A1* gene was associated with both BU per se [64] and the paradoxical reaction [67], while *NOD2* has only been associated with the most severe form of the disease [65]. A first BU GWAS led to the description of two *loci* containing the lncRNAs *ENSG00000240095.1* and *LINC01622* associated with the disease [68]. Finally, whole-exome sequencing of a pair of sisters belonging to a consanguineous family and displaying a severe form of the disease revealed a microdeletion on chromosome 8p23.1 as the most likely causative genetic variant [69].

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Host Response to *Mycobacterium leprae*

4

Rodrigo Ribeiro-Rodrigues

4.1 Introduction

Leprosy, caused by *Mycobacterium leprae* (*M. leprae*), is a chronic infectious disease associated with damaging inflammatory lesions in the skin and peripheral nerve. A broad clinical spectrum of pathology determined by the host immune response is the hallmark of leprosy. Tuberculoid patients mount a vigorous cell-mediated immune response in the skin and nerve, displaying delayed-type hypersensitivity response to *M. leprae* antigens. Although limiting the number of bacilli and lesions, this strong response accounts for the prominent impairment of the peripheral nerves [1]. Conversely, lepromatous patients exhibit specific cellular unresponsiveness to *M. leprae* antigens associated with high mycobacterial loads in the skin and nerves. However, most leprosy patients have pathology between the two polar forms and are classified as either borderline tuberculoid (BT) or borderline lepromatous (BL). Leprosy reactions are common in these immunologically unstable borderline groups, and involve an upregulation of the host response to *M. leprae* antigens.

4.2 Innate Immunity

The host defense events activated earlier in infection, during the indeterminate phase, are still the least understood aspects of immune response during leprosy [2]. An effective innate immune response in combination with the low virulence of the leprosy bacillus may underlie resistance to the development of clinical disease.

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4.3 Complement

Lipoarabinomannan, a key molecule from *M. leprae*, has been shown to activate complement and to be associated with nerve damage. Analysis of skin biopsies showed colocalized complement's membrane attack complex (MAC) and LAM deposition along axons in skin lesions from lepromatous patients when compared to tuberculoid patients. Parallelly, an increase in MAC immunoreactivity was observed on skin lesions from reactional patients when compared to non-reactional leprosy patients [3]. C1q deposition was significantly augmented in both reversal reaction and erythema nodosum leprosum lesions when compared to non-reactional matched patients [4]. An association of complement genes with leprosy susceptibility has been also demonstrated [5].

4.4 Antigen-Presenting Cells and Dendritic Cells

Dendritic cells (DC) play a key role in modulating early innate immune response to *M. leprae*. In the absence of an adaptive immune response, DC may be the first cell to encounter the bacilli at the site of *M. leprae* invasion on the host (e.g., the nasal mucosa or skin abrasion). Uptake of the bacilli by DC and subsequent local production of cytokines and chemokines regulate inflammation and influence the course of the adaptive cell-mediated immunity (CMI) into a Th-1 or Th-2 response against *M. leprae*. Although DC are known to be effective presenters of *M. leprae* antigen, MHC class I and II expressions are downregulated in monocyte-derived DC infected with *M. leprae* bacilli. On the other hand, DC stimulated with *M. leprae* membrane antigens upregulate both MHC class II and CD40 ligand-associated IL-12 production [6], suggesting that whole live bacilli may suppress the interaction of DC and T cells. Langerhans cells, a subset of DC, are known to initiate immune responses in the skin. Lepromatous leprosy (LL) patients have significantly fewer Langerhans cells, both in the lesion and in healthy skin, when compared to uninfected controls or tuberculoid leprosy (TT) patients. In contrast, patients with TT lesions have increased numbers of Langerhans cells in the lesions, suggesting an active infiltration of these cells to these sites. The cytokine profile present in the lesion also appeared to be correlated with Toll-like receptor (TLR) function: Th-1-type cytokines were generally associated with TLR1 and TLR2 activation, and Th-2-type cytokines were associated with inhibition of activation. The expression of TLR1 and TLR2 is stronger on monocytes and DC in TT lesions than in the LL counterparts. In addition, in vitro studies showed that the *M. leprae* 19-kDa and 33-kDa lipoproteins could activate monocytes and monocyte-derived dendritic cells through TLR2 [7].

4.5 Macrophages

Macrophages play a pivotal role in the pathogenesis of leprosy, with a different macrophagic population in host tissue associated with each clinical presentation in leprosy. It has been demonstrated that during an inflammatory response, bone marrow-derived monocytes enter the tissue in large numbers and take part in the defense against the pathogens. Macrophages range from pro-inflammatory M1 phenotype, in which vitamin D-dependent antimicrobial pathway predominates, as observed in the paucibacillary lesions and in the onset of reversal reaction, to anti-inflammatory M2 phenotype, in which there is an upregulation of phagocytic pathways as observed in lepromatous skin tissues [2, 8–12]. The role of innate immunity function in the establishment of the polar forms of leprosy seems to be pivotal. Macrophages present in lepromatous skin cells upregulate the production of IL-27, which may contribute to the inhibition of antimicrobial pathways. Although different clinical forms of leprosy display the predominance of a specific macrophage phenotype (M1 or M2), there is a continuum of phenotypes between these ranges with some cells sharing phenotypes of both M1 and M2 macrophages. Nitric oxide, another key microbicidal molecule, secreted by *M. leprae*-carrying macrophages damages nerve fibers directly [2]. Local innate immune mechanisms are crucial to determine the outcome of the different clinical forms and the reactional episodes in leprosy patients, and understanding the function of each cell populations as well as the innate pathways induced by *M. leprae* may contribute to the development of new disease treatments.

4.6 Plasmacytoid Dendritic Cells

Plasmacytoid dendritic cells (PDCs) are bone marrow-derived dendritic cells (DCs) that are unique in their ability to secrete large amounts of IFN-a in vivo after stimulation with a variety of agents including viral DNA. They are thought to play a critical role in early antiviral natural immune responses. PDCs can be accurately identified in routine formalin-fixed, paraffin-embedded sections using antibodies to CD123, which label interleukin (IL)-3a receptor a chain protein expressed at high levels on the surface of these cells. In a retrospective immunohistochemical study on 20 cases of leprosy, CD123 expression was not observed in any of the biopsy specimens evaluated, with the exception of two cases of ENL, in which a focal positivity for CD123 was observed. Our results indicate that plasmacytoid dendritic cells are not involved in the immune response against *M. leprae* [8].

4.7 Pattern Recognition Receptors

Pathogen-associated molecular patterns (PAMPs) present on different microorganisms are recognized, during the innate immune response, by pattern recognition receptors (PRRs) expressed on immune cells at the entry site, and are known to interfere with the onset of the acquired immune response. Three classes of pattern recognition receptor have been identified: (a) C-type lectin receptors, (b) Toll-like receptors, and (c) complement (C′) receptors [1, 2]. (a) *C-type lectin receptors*—The mannose receptor (CD206), a receptor belonging to the C-type lectin superfamily, binds carbohydrate moieties on a variety of pathogens. It is expressed primarily on mature macrophages (MΦ), but not on monocytes, and on some subsets of dendritic cells. The MΦ has been shown to play a pivotal role in uptake of virulent mycobacteria. Mannose-capped lipoarabinomannan, a major mycobacterial ligand, is found on virulent strains of *M. tuberculosis* as well as on *M. leprae*, and can modulate several effector functions of mononuclear phagocytes, including the production of TNF-α, prostaglandin E2, and nitrite, as well as the MΦ microbicidal activity, since mycobacteria uptake via mannose receptor does not elicit a respiratory burst. Another C-type lectin is the dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN; also identified as CD209). DC-SIGN is expressed on dendritic cells and also recognizes pathogens via binding of mannose-containing structures; the major mycobacterial ligand for DC-SIGN is also mannose-capped lipoarabinomannan. It has been demonstrated that DC-SIGN is the major receptor on DC for *M. tuberculosis* uptake, while complement receptors and the mannose receptor play a minor role. It has been proposed that virulent mycobacteria subvert DC function via DC-SIGN by suppressing DC maturation, possibly through the inhibition of IL-12 production and induction of IL-10, which could be possibly achieved through DC-SIGN inhibition of TLR signaling. Langerin (CD207), another C-type pattern recognition receptor; is expressed by Langerhans cells; oligomerizes as trimers at the cell surface; presents a single calcium-dependent, carbohydrate recognition domain specific for mannose, *N*-acetylglucosamine, and fucose; and may play a role in the uptake of nonpeptide mycobacterial antigens. (b) *Toll-like receptors*—Host's ability to rapidly detect invading pathogens is a key feature of innate immunity and is mediated, at least, in part by PRRs capable of recognizing various classes of microbial ligands. TLRs are a set of innate immune receptors that recognize structures common to many different pathogens, and are essential for the optimal induction of innate immunity against microbial infection. TLRs are phylogenetically conserved transmembrane proteins that contain repeated leucine-rich motifs in their extracellular domains. The cytoplasmic signaling domain is linked to the IL-1 receptor-associated kinase, which activates transcription factors such as NF-KB to induce cytokine production. Toll-like receptors are crucial for the recognition of microbial pathogens by both MΦ and DC during innate immunity. Ten TLRs have been identified, of which TLR2-TLR1 heterodimers, TLR2 homodimers, TLR4, and TLR9 appear to be significant in the recognition of mycobacteria. TLRs are necessary for the optimal production of IL-12, a pro-inflammatory cytokine responsible for the induction of Th-1-type immunity, as

well as TNF-α, a cytokine important in cellular activation and granuloma formation but also implicated in the tissue destruction associated with leprosy reactions. A C-to-T substitution at nucleotide 2029 of TLR2, involved in the recognition of mycobacterial lipoproteins, results in the change of Arg to Trp at amino acid residue 667; cells expressing this mutation upon stimulation with either live *M. leprae* or *M. leprae* antigens were associated with a defective activation of NF-kB and a decreased production of IL-12, IL-2, IFN-γ, and TNF-α, but with an increased levels of IL-10 when compared to wild-type cells. (c) *Complement (C′) receptors.* It has been demonstrated that complement receptors 1 and 3 on the surface of monocytes and CR1, CR3, and CR4 on MΦ are key mediators of phagocytosis of *M. leprae*; e.g., complement component C3 promote the uptake of PGL-1, a major surface glycolipid of *M. leprae*. Considering that bacilli uptake via complement receptors does not elicit a respiratory burst, it is possible that this is a mechanism whereby pathogenic mycobacteria use to escape toxic reactive oxygen intermediates (ROIs) generated during phagocytosis [13]. (d) *Nod-like receptors* (*NLRs*)—Nod-like receptors recruit and activate inflammatory caspases into inflammasomes or trigger inflammation via different pathways including the NF-κB mitogen-activated protein kinase and regulatory factor pathways. Polymorphisms in NOD2 are associated with leprosy susceptibility. Phagocytosis blocking inhibits the production of IL-1β and TNF in response to *M. leprae*, suggesting that intracellular signaling after *M. leprae* infection is required for macrophage activation. NLRPs are intracellular receptors that recognize PAMPs and induce the secretion of both caspase-1 and IL-1β in the context of inflammasome. SNPs in NLRP1 and NLRP3 genes were analyzed in Brazilian leprosy patients. The NLRP1 combined haplotype rs2137722/G-rs12150220/T-rs2670660/G was significantly more frequent in patients than in controls as well as in paucibacillary than in multibacillary patients. The NLRP1 combined haplotype rs2137722/G-rs12150220/A-rs2670660/G was associated with paucibacillary leprosy suggesting that NLRP1 might be involved in the susceptibility to leprosy [2]. (e) *Leucine-rich-repeat kinase 2 (LRRK2) Gene*—LRRK2 is highly expressed in immune cells and has been functionally linked to pathways regulating immune cell function, such as cytokine release, autophagy, and phagocytosis. Mutations in the leucine-rich-repeat kinase 2 (LRRK2) gene have been associated with immune-related disorders in leprosy, such as type-1 reactions (T1R), an aggravated inflammatory response leading to peripheral nerve cell damage.

4.8 Adaptive Immunity

It has been shown experimentally that T cells play a fundamental role in the resistance to *M. leprae*, as evidenced by the profuse local multiplication of the bacilli in the footpad of either neonatally thymectomized or congenitally athymic mice. Among humans, it is estimated that >95% of persons are resistant to leprosy; upon exposure, protection probably occurs early, with no overt signs of disease. Currently, no test is available to reliably detect exposure to *M. leprae* or to diagnose preclinical infection. It is acknowledged that individuals with clinical leprosy, even those

diagnosed with paucibacillary disease and exhibiting a strong cell-mediated immunity, harbor living *M. leprae* in their tissues. The protective characteristic of CMI in paucibacillary leprosy is mainly defined as the ability to control mycobacterium multiplication. Although a strong immune response is desired to control *M. leprae* infection, collateral damage to tissues, such as injury to peripheral nerves, caused by CMI-associated granulomatous inflammation may be also observed and have serious, long-term consequences.

4.9 T-Lymphocyte Populations

(a) *MHC-restricted CD4+ and CD8+ cells*. Immunohistochemical staining has shown that TT lesions display mostly CD4+ T-helper cells, and that T-helper/memory phenotype outnumbered the naive phenotype 14-fold. T cytotoxic cells were also numerous in TT lesions, and may play a role in MΦ localization, activation, and maturation, and may play an important role in pathogen restriction or elimination. Interestingly, in TT lesions, CD4+ cells were distributed throughout the lesion, whereas CD8+ cells were stationed at the periphery in the TT lesion. Although the CD4/CD8 ratio in normal peripheral blood is also 2:1, CD4/CD8 ratio in TT lesions was 1.9:1. On the opposing pole, LL lesions displayed a CD4/CD8 ratio of 0.6:1, and unlike TT lesions, the CD8+ T cells were distributed throughout the lesion rather than at the periphery. It has been shown that the CD4+ cells present were primarily of a naive phenotype, and the CD8+ cells were predominantly of a suppressor subset; thus, it has been proposed that these CD8+, suppressor cells may serve to downregulate MΦ activation and suppress cell-mediated immunity [14]. (b) *T regulatory cells*—T regulatory cells (Tregs) constitute 5%–10% of all CD4 T cells in peripheral blood and characteristically express CD25 and the forkhead family transcription factor P3 (FoxP3). In TB, the presence of Tregs at higher frequencies is associated with reduced levels of IFN-γ production and impaired T-cell activation [15]. Recently, we performed a retrospective immunohistochemical study on leprosy cases, including tuberculoid tuberculoid (TT), borderline tuberculoid (BT), borderline lepromatous (BL), lepromatous lepromatous (LL), borderline borderline in reversal reaction (BB-RR), borderline tuberculoid in reversal reaction (BT-RR), and erythema nodosum leprosum (ENL) [8]. FoxP3-positive cells were present in 95% of the cases with an average density of 2.9% of the infiltrate. Their distribution was not related to granulomatous structures or special locations. There was no statistical difference of FoxP3 expression between TT, BT, BL, and LL, whereas a statistical significant increment ($P = 0.042$) was observed in patients affected by reversal leprosy reactions (BT-RR and BB-RR) compared with patients affected by ENL and patients with non-reactional disease forms (BL, LL, BT, TT). Current studies are been conducted by us to further investigate if Tregs have a pathogenetic role in HD as previously demonstrated in *Leishmania major* and *Mycobacterium tuberculosis* [15]. Treg cells mediate their suppressive capacity on inflammatory effector T cells, such as Th1, Th17, and Th9 cells both by contact-dependent and contact-independent manner. From a functional perspective, Treg cells can be

grouped into four basic "modes of action": the various potential suppression mechanisms used by these include suppression by inhibitory cytokines, suppression by cytolysis, suppression by metabolic disruption, and suppression by modulation of dendritic cell maturation or function. Inhibitory cytokines, such as IL-10 and TGF-β, have been the focus of considerable attention as mediators of Treg cell-induced suppression. Differential Treg cell trafficking to disease foci is influenced by tissue chemokine response elicited at the site of lepromatous lesions, and believed to determine the local immunity of BT/TT and BL/LL clinical forms. Accumulation of Treg cells in peripheral compartment or at the pathologic site(s) has been shown to be of critical importance in determining the local immunity, and the outcome of the disease among patients suffering from various forms of tuberculosis. In leprosy as well, Tregs are present in increased numbers in LL patients, and they may have a pathogenic role in leprosy patients harboring uncontrolled bacillary multiplication and have also been shown to play a role in *M. leprae*-induced Th1 unresponsiveness in LL. Conversely, T2R or ENL patients display significantly lower frequency of circulating and in situ Tregs than T1R patients and controls with concomitant increase in pro-inflammatory cytokines such as TNF-α and IFN-γ produced by Th1 lymphocytes [16]. (c) *CD1-restricted T cells*—Human CD1 molecules present non-peptide components (lipid and glycolipid antigens) of mycobacteria to specific CD1-restricted T cells. In vitro and in vivo studies suggest an important role for CD1 molecules on mycobacterial lipid presentation in the immune response to *M. leprae*. In the presence of CD1-expressing antigen-presenting cells, *Mycobacterium*-reactive double-negative T-cell lines derived from the skin lesion of a leprosy patient responded to the mycobacterial subcellular fractions; conversely, lipoarabinomannan-depleted soluble cell wall fraction was not able to induce detectable T-cell proliferation. CD1b restricted recognition of purified lipoarabinomannan from *M. leprae*, and T cells lysed lipoarabinomannan-pulsed monocytes in a CD1b-restricted manner. Lipoarabinomannan also induced these T cells to secrete large amounts of IFN-γ. Interestingly, LL patients present fewer CD1+ cells when compared to TT or reversal reaction patients. A strong upregulation of CD1+ cells in the granulomatous lesions of patients with either TT leprosy or reversal reaction is frequently observed. These cells are also CD83+, a marker for dendritic cells, indicating a strong correlation between CD1 expression and cell-mediated immunity in leprosy. On the other hand, administration of GM-CSF, which can promote dendritic cell activation, induces infiltration of CD1+ cells into the lesions of LL leprosy patients [17]. (d) *TH17 cells*—A third subset of T-helper cells, Th17 cells, produce IL-17A (also referred to as IL-17), IL-17F, and IL-22 cytokines and have been associated with neutrophilia, tissue remodeling and repair, and production of antimicrobial proteins. Although Th17 cells, abundant at mucosal interfaces, are able to contain pathogenic bacteria and fungi infections, they have been also implicated as culprits in inflammatory diseases in both mice and humans. Th17 cells mediate their pro-inflammatory function by recruitment of neutrophils, activation of macrophages, and upregulation of Th1 effector cells. Interestingly, inflammatory damage previously credited to type 1 response is now thought to be dependent on IL-17 and IL-23. CD4+ Th17 cells have been identified in borderline cases of

leprosy, which highlighted their importance in infectious diseases as well [16]. A persistent and very relevant concept is that an imbalance between Th17 and Treg cell function may be critical in the immunopathogenesis. IL-10 produced by Treg cells in BL/LL patients correlates significantly with polarized immunity highlighted by lesser IL-17 by CD4+ T cells in the same group. Blocking of IL-10/TGF-β resulted in the reversal of effector immune response (IL-17) in BL/LL with higher frequency of Th17 cells [18]. Presence of Th17 cytokines (IL-6, IL-17, and IL-23) in vitro results in reduction of FoxP3 expression on Tregs simultaneously, possibly leading to increase in IL-17-producing CD4+ cells in BL/LL, suggesting that the generation of antigen-specific Treg cells is very much dependent on the environment of cytokines they are exposed to. Hence, these cells may be targeted for reversal of effector response in BL/LL patients proving to be an important mode of immune modulation in the immunocompromised hosts to revive the immune response. An imbalance in Treg and Th17 populations has also been observed in patients with leprosy reactions [18, 19]. Studies done in biopsies from T2R patients showed a decrease in Tregs and associated cytokines, TGF-β, and increase in cells producing IL-6, IL-21, and IL-17. On the other hand, T1R patients are showing the opposite trend with increased Tregs and reduced IL-17+ cells. This increase in inflammatory cytokines along with downregulation of Tregs may be responsible for the lesional inflammation characterizing T2R reactions [16].

4.10 Cytotoxic Cells

Cytotoxic cells can be separated in T cells and NK cells. (a) *T cells*—CD8+ and CD4+ T cells can function as class I- and class II-restricted cytotoxic T cells, respectively, and both are capable of lysing *M. leprae*-infected MΦ; lysis of target cells by cytotoxic T lymphocytes is mediated by perforin and cytotoxic granules, such as granzyme B and granulysin [1]. Upon contact with the target cell, perforin is released by cytotoxic T cells forming pores in the target cell membrane, allowing granzyme B to enter the cell, where it activates caspases and leads to target cell death. In leprosy lesions, the presence of granulysin can be correlated with the polar forms of the disease and is observed more often in TT skin lesions than in LL lesions. On the other hand, no correlation is found for perforin. Lysis of *M. leprae*-infected MΦ target cells may contribute to protection in leprosy as an adjunct to the continuing attempts for intracellular killing or growth inhibition mediated by IFN-γ-activated MΦ. Ex vivo and in vitro data from experimental models have demonstrated that the long-term intracellular presence of live *M. leprae* can impair several functions on the infected MΦ, especially those related to its ability to become activated upon stimulation with IFN-γ [20]. (b) *NKT cells*—Leprosy-specific studies on NKT cells (31) have shown mycobacterium-reactive double-negative T-cell lines derived from skin lesion of a leprosy patient responded to subcellular fractions of mycobacteria in the presence of CD1-expressing antigen-presenting cells (APCs). Upon examination of leprosy patients, they found few CD1+ cells in LL leprosy lesions. In contrast, there was a strong upregulation of CD1+ cells in the

granulomatous lesions of patients with TT leprosy or reversal reaction (32). These cells were also CD83+, a marker for dendritic cells, indicating a strong correlation between CD1 expression and cell-mediated immunity in leprosy, strongly suggesting that NKT cells play a determining role in regulating the varied type of immune responses as evidenced in leprosy-affected individuals. (c) *Natural killer cells*—NK cells exert spontaneous non-MHC-restricted cytotoxicity against a variety of neoplastic and pathogen-bearing target cells, and although they are CD3-negative cells, they share many characteristics with cytotoxic CD3+ T cells. Even though the cytotoxicity of NK cells and their more active IL-2-stimulated lymphokine-activated killer (LAK) cell lacks antigen specificity, they are still directed against *M. leprae*-infected macrophages and Schwann cells. Administration of IL-2 into LL lesions appears to recruit NK cells, where they migrate to and may be responsible for the subsequent local clearance of the bacilli [21].

4.11 Macrophages

Macrophages are the primary host cell for *M. leprae*, in the absence of an effective adaptive immune response; these relatively harmless bacilli can multiply in MΦ to over 100 organisms per cell. In addition to harboring bacilli, the MΦ also plays an important role in the host's defense against *M. leprae*, being a key player in both innate and acquired immune responses. Antigen processing and presentation, monokine secretion, and intracellular microbicidal activity are the MΦ's major functions. TLRs have also been shown to be important in monocyte differentiation into either antimicrobial macrophages or antigen-presenting dendritic cells. Upon activation of TLR2 through stimulation with mycobacterial antigens, monocytes isolated from TT patients differentiate into both (DC-SIGN+) MΦ and CD1b+ dendritic cells. On the other hand, when peripheral blood monocytes from LL patients are stimulated in the same fashion, cells differentiated into DC-SIGN+ MΦ but not into CD1b+ dendritic cells, suggesting that stimulation of monocytes from both tuberculoid and lepromatous patients with *M. leprae* antigens may generate, via TLRs, similar innate responses to *M. leprae*; however, lepromatous patients are unable to proceed and elicit an effective adaptive response seen in tuberculoid patients. Although *M. leprae* can survive in normal murine MΦ, IFN-γ-activated MΦ can significantly inhibit or kill *M. leprae* in vitro [1, 20]. In normal MΦ, phagosome-lysosome fusion is blocked by live, but not dead, *M. leprae* and, that in activated MΦ, phagosomes harboring *M. leprae* fuse with secondary lysosomes. MΦ can inhibit or kill invading pathogens through the generation of reactive oxygen intermediates (ROI) and of reactive nitrogen intermediates (RNI). Upon phagocytosis of microorganisms by MΦ, a respiratory burst with a great increase in the consumption of oxygen catalyzed by NADPH oxidase and the production of superoxide occurs, and then other reactive oxygen intermediates, including hydrogen peroxide, hydroxyl radical, and singlet oxygen, are subsequently generated. These toxic oxygen products are key antimicrobial molecules of phagocyte cells, especially against extracellular pathogens. However, leprosy bacilli appear to be well equipped to handle antimicrobial

reactive oxygen intermediates generated by the host MΦ. *M. leprae* is a weak stimulus for the MΦ oxidative burst, possibly due to a downregulation of superoxide generation by PGL-1 or the presence of a superoxide dismutase [22]. On the other hand, RNIs, primarily nitric oxide, which are produced by an inducible form of nitric oxide synthase (iNOS) in activated MΦ are also important to inhibit *M. leprae* metabolic activity in mice. However, the role of RNI as an effector mechanism in human MΦs is somewhat controversial. The isolation of MΦ from granuloma in the footpads of *M. leprae*-infected mice enabled the study of resident MΦ from the actual site of infection in experimental leprosy, allowing determination of both cytokine production and cell surface phenotypic markers of these granuloma-derived cells. Initial footpad granuloma MΦ from *M. leprae*-infected athymic *nu/nu* mice indicated that the MΦs were phenotypically indistinguishable from normal peritoneal MΦ except that they contained enormous numbers of *M. leprae*, and were refractory to activation by IFN-γ for both microbicidal and tumoricidal activities. In addition, there was no IFN-γ-induced augmentation of class II MHC expression, supporting the concept that *M. leprae* is a potent negative modulator of MΦ effector functions and that its influence is largely restricted to the microenvironment of the granuloma.

4.12 Cytokine/Chemokine

Cell-mediated immune response is an important aspect of host resistance to mycobacterial infection and is thought to be tightly regulated by a balance between the type 1 cytokines, including interleukin (IL)-2, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-12, and the type 2 cytokines, such as IL-4, IL-6, and IL-10. It is known that IL-12 induces T-helper 1 (Th1) differentiation and IFN-γ release from Th1 and NK cells. IFN-γ activates MΦ, playing a pivotal role in antimycobacterial immune responses. TNF-α is capable of many pro-inflammatory activities including MΦ activation. In contrast, IL-10 has been shown to be a counter regulatory cytokine that can affect the immunomodulatory effects of IL-12. The production of IL-10 during bacterial infection has been shown to suppress production of inflammatory mediators and aid in the development of Th2 immunity. The Th-1/Th-2 paradigm, based on functional discrimination of T-helper cells according to their pattern of cytokine production, asserts that Th-1 and Th-2 cells promote a cellular and humoral immune response, respectively. This functional differentiation has offered an attractive hypothesis to explain the differences between tuberculoid and lepromatous responses to *M. leprae*. It has been shown that peripheral blood mononuclear cells (PBMCs) and T-cell lines from tuberculoid patients stimulated with *M. leprae* in vitro produce a Th-1 cytokine pattern, while PBMCs and T-cell lines from lepromatous patients generally produce a Th-2 cytokine pattern. Other studies revealed a predominance of IL-2, TNF-α, and IFN-γ mRNA transcripts in tuberculoid lesions, and IL-4 and IFN-γ in lepromatous ones, gene expression profiles consistent with Th-1 and Th-2 patterns, respectively [23, 24]. A similar pattern is observed in CD4+ clones isolated from TT lesions, which secrete primarily

IFN-γ, whereas CD4+ clones from an LL lesion produce predominantly IL-4. Interestingly, CD8+ clones from LL patients likewise generated large amounts of IL-4. Data also indicated that IL-12 and IL-18, highly expressed in tuberculoid lesions, promote resistance to *M. leprae*. However, leukocytes from approximately 40% of all patients in one such study produced a mixed Th-0 cytokine profile, i.e., IFN-γ, IL-2, and IL-4. It is possible that some of the patients whose cells produced the Th-0 pattern were in the borderline portion of the leprosy spectrum (BL or BT); alternatively, that the human immune response to *M. leprae* may not fit perfectly entirely with the murine Th-1/Th-2 model. We have investigated serum levels of cytokines (TNF-α, IFN-γ, IL-2, IL-4, IL-5, and IL-10) and chemokines (CCL2/MCP-1, CCL10/IP-10, CXCL9/MIG, CCL5/RANTES, and CXCL8/IL-8) in patients with relapsing leprosy and controls (without disease but who had leprosy treated and cured). Serum levels of TNF-α and IFN-γ were higher among paucibacillary patients when compared with multibacillary patients with a positive baciloscopic index, whereas similar levels of IL-2, IL-4, IL-5, and IL-10 were observed for these two groups. On the other hand, multibacillary patients presented higher levels of chemokines in serum when compared to paucibacillary patients [*Ribeiro-Rodrigues, unpublished data*]. In summary, data gathered so far support the original concept that TT lesions are manifestations of delayed hypersensitivity and cellular immunity, and that LL lesions occur when immune recognition is observed (as indicated by antibody production) but the host is incapable of developing cellular immunity to *M. leprae*.

4.13 Negative Impact of Helminthic Coinfection in Leprosy

It is estimated that at least one-fourth of world's population harbors at least one species of intestinal parasites and that infection by *Ascaris lumbricoides* affects approximately two billion people with significant morbidity. Intestinal helminth infections are, generally, associated with a strong Th-2-like immune response, characterized by elevated plasmatic IgE titers, peripheral blood and tissue eosinophilia, and tissue mastocytosis, on both human and experimental infection models. Although helminthic infections, in humans, are frequently associated with an immunological hyperactivity state, it is also associated with a reduced or anergic cellular response. It has been suggested that infection with intestinal helminths could facilitate both a subsequent infection by other pathogens and a faster progression to more severe forms of other infectious diseases, such as in tuberculosis and HIV [25]. Our group has reported a significant association between intestinal helminthic infections and either pulmonary tuberculosis, staphylococcal infection, or multibacillary leprosy. Intestinal helminths are known to elicit a strong systemic Th-2-type response, which is normally followed by a reduction on Th-1-type immunity. Considering that resistance to mycobacterial infections is dependent on an effective Th-1-type immune response, it is possible that the presence of intestinal helminths downregulates the required Th-1-type immunity via upregulation of Th-2-type cytokine production, facilitating a subsequent infection by *M. leprae*. We have demonstrated that in vitro

intracellular levels of Th-2-type cytokines, such as IL-4 and IL-10, in PBMC cultures from leprosy patients coinfected with intestinal helminths were elevated when compared to leprosy patients without intestinal worms. Interestingly, intracellular levels of IL-4 and IL-10 were elevated in cultures from lepromatous leprosy patients, whom in turn presented the lowest levels of intracellular IFN-γ [26]. An evidence that Th-1 down-modulation occurs during intestinal helminth infection was given by the fact that intracellular IFN-γ levels in both tuberculoid and lepromatous, helminth-free, leprosy patients were approximately twofold higher than in helminth-infected leprosy patients. Conversely, lepromatous patients harboring intestinal helminths produced close to twofold more IL-4 and IL-10 than helminth-free leprosy patients. It is possible that intestinal helminth infections, in addition to promoting a strong upregulation of Th-2-type immune responses, may stimulate suppressive T cells, known as regulatory T cells (Tregs), which would interfere with effector T-cell activation through the suppression of Th-1-type responses. In a previous work, our group investigated the role of $CD4^+CD25^+$ T cells in pulmonary tuberculosis, and demonstrated that the presence of Tregs is associated with a significant decrease in IFN-γ production by T cells stimulated with *M. tuberculosis* antigens [21].

4.14 Conclusions

A variety of mechanisms of innate and adaptive immunity have been identified and postulated to play a role in the development of cellular immunity in leprosy. However, none of these can yet explain the remarkable spectrum of cellular immune responses to this organism in humans. Genetic influences on immunity to *M. leprae* in humans appear to operate at two levels: some mechanisms act at the level of the overall susceptibility, and others function at the level of the acquired immunity. It is possible that the presence of intestinal helminths, downregulating the required Th-1-type immunity via upregulation of Th-2-type cytokine production and/or Treg elicitation, may facilitate a subsequent infection by *M. leprae*. Leprosy susceptibility gene has been identified, and evidences suggest that other genes are capable of influencing adaptive immunity. These findings are not fully understood, and no information thus far indicates what triggers such diverse reactions or why they affect some patients but not others.

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Pathogenesis of Leprosy

5

Cesare Massone and Enrico Nunzi

Leprosy pathogenesis has not been definitively understood. However, three points are indisputable: the etiological agent is *Mycobacterium leprae* (*M. leprae*), the disease develops in susceptible individuals, and in endemic countries the environment (low socioeconomic status and overcrowding) plays a role in the transmission of the infection.

Leprosy disease and clinical manifestations are the result of a dynamic interactive process between *M. leprae* and the cell-mediated immunity (CMI) of genetically predisposed subjects. The vast majority (95%) of the exposed population is not susceptible to the disease; of the remaining 5%, the larger part successfully eliminates *M. leprae* through an efficacious immune response, while only a relatively small percentage (1%) develops leprosy [1–3].

M. leprae has some peculiarities (Chap. 2): it is the only bacterium with neurotropism that is more appropriate for peripheral nerves, and it is not cultivable in any known artificial media. There are no suitable animal models for experimental studies. Leprosy patients are the only reservoir of significance, despite the fact that leprosy-like infection has been reported in a few wild armadillos in the south of Texas and Louisiana. Although the exact mode of transmission is not known, untreated multibacillary patients are the main source of infection as they can discharge up to 10^7 bacilli/day by droplets from the nose, from the mouth, or from ulcerated nodules (portal of exit). Protected from ultraviolet radiation and in a hot wet climate, *M. leprae* can also survive for 6 weeks in soil [4–6].

Overcrowding and poor socioeconomic conditions favor leprosy transmission. The portal of entry of organisms into the body is still debated. The mucosa of the

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upper airways is considered the main route of entry. Published reports of initial leprosy lesion developing locally following accidental inoculation, tattooing, vaccination, and dog bites in humans suggest that *M. leprae* can enter through the skin. The exact incubation time is unknown and can vary from a few months until 20 years or more [1–3]. The portal of entry of the bacillus is one of the factors that influence the position of the patient in the leprosy spectrum. Transcutaneous inoculation is correlated to the indeterminate form (I) and to the hyperergic forms (TT, BT) with a shorter incubation period. Entry of the bacillus via the mucosa of the upper respiratory tract is correlated to hypoergic disseminated forms (BB, BL, LL) with longer incubation periods [7].

Once *M. leprae* is inside the subject, it enters lymph and blood vessels to reach its target: the Schwann cells. *M. leprae* enters Schwann cells by binding the G domain of the a2 chain of laminin 2, a component of their basal lamina. This form of laminin is restricted to peripheral nerves, which explains the specific tropism of *M. leprae*. The Schwann cells engulf *M. leprae* within their phagosomes, but cannot destroy *M. leprae* because Schwann cells lack lysosomal enzymes. Schwann cells are sanctuaries where the bacilli are protected from macrophages and can replicate slowly over years. *M. leprae* seems to have abandoned genes normally required for replication ex vivo and assumed a unique ecological niche with a very limited host range and the need for growth within cells. Only genes essential for the formation of a mycobacterial cell wall have been retained. The leprosy bacillus might therefore be dependent on host metabolic products, which could explain its long generation time and inability to grow in culture [1, 6].

Host genetic factors influence the CMI and have a partial effect on both the development of leprosy and the pattern of disease (Chap. 3). The nature of the adaptive T cell response is determined in part also by the instruction of the innate immune response (Chap. 4). Moreover, besides typical TH1/TH2 responses, also natural killer T cells (NKT), FOXP3+ regulatory T (Treg) cells, and T helper 17 cells (TH17) and even B cells might be implicated in leprosy pathogenesis (Chap. 4) [8, 9].

The CMI determines either the elimination of the bacillus or the development of the disease. In fact, at some stage, infected Schwann cells process and present antigenic determinants of *M. leprae* to antigen-specific T lymphocytes that initiate a chronic inflammatory granulomatous reaction (Chap. 4). *M. leprae* may migrate outside the nerves to endothelial cells or may be phagocyted by macrophages that act as antigen-presenting cells [10]. At this exact point, the CMI plays a pivotal role. Subjects with a predominant Th1 immune response will develop a high degree of CMI with epithelioid granuloma formation that will destroy all the bacilli with either healing or development of localized disease (tuberculoid leprosy, TT) [11]. In TT M1 macrophages produce tumor necrosis factor-alpha (TNF-a) and interferon-gamma (IFN-γ) and generate free radicals that destroy *M. leprae*. LL shows a predominance of two populations of macrophages: M2 macrophages that induce the production of interleukin (IL)-10, transforming growth factor (TGF)-ß, and fibroblast growth factor (FGF)-ß, which contribute to the immunosuppressive response as well as tissue repair. M4 macrophages produce IL-6, TNF-a, MRP8, matrix

metalloproteinase (MMP)7, and MMP12; this subpopulation is less effective in the elimination of *M. leprae*, and M4 macrophages can induce the establishment of a regenerative environment and remodeling of the extracellular matrix, which are important for the pathogen–host interaction during infection by *M. leprae* [12, 13].

TT has a short incubation time (2–3 years) and remains circumscribed to the skin and nerves of a limited area of the body. In fact, macrophages of TT patients are able to annihilate bacilli, to completely process the mycobacterial antigens, and to obtain normal complete antigenic information and CMI immune response. On the contrary, individuals with a predominant Th2 response will develop a weak CMI without forming an efficacious granulomatous response and an increased humoral immunity: bacilli will survive and replicate, developing systemic disease (lepromatous leprosy, LL) [2]. Macrophages of LL patients engulf bacilli but are only able to partially destroy *M. leprae*, probably because of a deficit of lysosomal phospholipases, resulting in incomplete antigenic information and accumulation of mycobacterial phospholipids as cytoplasmic droplets (lepra cells, described by Virchow in 1863) [14]. Bacilli age and replicate over the years (incubation time 10–20 years) outside the nerves (in the dermis around the superficial vascular plexus) in the cooler areas of the skin and disseminate through the blood to the lymph nodes, liver, and spleen. Skin lesions derive from progressive accumulation of *M. leprae* and macrophages in the skin. In contrast to patients who present a vigorous CMI response, patients with anergy against *M. leprae* can be infected also after short contact with an infected subject [1, 3].

As seen, the CMI determines the clinical form of the disease, which varies along a spectrum (Chap. 6) that starts with a tuberculoid pole, goes through borderline cases, and ends with a lepromatous pole (Chap. 6). The spectral manifestations of leprosy are continuous, and there is a gradation in the clinical manifestations of the disease (Chap. 10). Patients with tuberculoid leprosy (TT) have a high degree of CMI, having one or two skin lesions with monolateral asymmetrical distribution, with no or few bacilli and epithelioid granuloma on histopathology (Chap. 12). Moving in the spectrum toward the lepromatous pole, the CMI decreases progressively; borderline tuberculoid (BT) patients have few lesions, asymmetrically distributed, with no or few bacilli and epithelioid granulomas on histopathology. In mid-borderline (BB) patients, the lesions become symmetric, there is a discrete number of bacilli, and granulomas show both epithelioid and macrophage features. CMI progressively decreases, so that borderline lepromatous (BL) and lepromatous leprosy (LL) patients show many symmetrically distributed lesions with many bacilli and macrophage granuloma on histopathology. BL and LL have a low CMI and increased humoral immunity. In each of the five forms, the clinicobacteriological and histopathological parameters have to agree with each other (Chaps. 10 and 26) [15–17]. Different sophisticated immunological studies on lymphocytes, cytokines, and molecular receptors in patients have confirmed that the immune response determines the clinical and histological manifestations of leprosy in all its different forms (Chap. 4) [6].

In short, the spectrum is determined by the balance between CMI and bacilli: high CMI response means low number of bacilli (paucibacillary leprosy: TT and

part of BT); low CMI response means high number of bacilli (multibacillary leprosy: BB, BL, LL, and part of BT). In the Ridley–Jopling spectrum, clinical, bacteriological, and histopathological parameters always have to correlate [2].

Patients of the two poles (TT and LL) have immunologically stable disease, while borderline patients (BT, BB, and BL) can shift from one form to another in the presence of trigger factors (immunosuppressive drugs, concomitant diseases, stress, and pregnancy) and can frequently manifest acute nerve damage related to type 1 reaction. Nerve damage during type 1 reaction is associated with an abrupt increase in CMI against *M. leprae* antigenic determinants released by Schwann cells. The nerve is damaged as an innocent bystander during the immune response [2].

Indeterminate leprosy represents an early stage of the disease in which the degree of CMI is still not clear. Patients with indeterminate leprosy can either heal or might develop leprosy and move on the spectrum (Chap. 6) [2, 18].

M. tuberculosis infection and bacillus Calmette–Guérin (BCG) vaccination protect against leprosy [1–3, 6].

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Classification of Leprosy

6

Cesare Massone and Alexandra M. G. Brunasso

Diagnosis and classification are two essential points for correct patient management. Correct classification allows proper treatment and alerts of the risk of leprosy reaction and nerve damage. A generic diagnosis of "leprosy" must be avoided.

6.1 Classifications of Leprosy Before Ridley and Jopling

Leprosy classification has been a matter of debate for many years. The first classifications were based only upon clinical parameters, generating confusion and controversies. Moreover, different countries and schools applied different classifications, making communication between leprologists at international meetings almost impossible.

Since the beginning of the twentieth century, there has been the need for a unitary international classification; different systems were proposed at international meetings in Manila (1931), Cairo (1938), Rio de Janeiro (1946), and Havana (1948), until the Madrid Congress held in 1953, where a classification based on four main disease groups was formulated [1]:

- Lepromatous leprosy (L) (macular, nodular, diffuse infiltrate, pure neuritic).
- Tuberculoid leprosy (T) (minor, major, and reactional).
- Indeterminate leprosy (I) (macular, pure neuritic).
- Borderline or dimorphous leprosy.

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6.2 Ridley–Jopling Classification

In 1962 and 1966, Ridley and Jopling (R&J) [2, 3] proposed a new classification based not only on the clinical features but also on histopathology, the degree of cell-mediated immune response (CMI) against *M. leprae*, and bacterial load [4]. This classification recognizes the complex pathogenesis of leprosy and is based on a five-group spectrum (Fig. 6.1) that extends from tuberculoid leprosy (TT) with heightened CMI (hyperergic pole; Chap. 5), through borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), to the poorly resistant (anergic) lepromatous type (LL) characterized by increased humoral immunity. TT and LL patients are immunologically stable (meaning that they do not usually shift to another type), while borderline patients (BT, BB, and BL) are immunologically unstable (i.e., they may abruptly shift from one form to another).

Indeterminate leprosy (I) does not fall into this spectrum because there is lack of correlation between the clinico- and histopathological features. Indeterminate leprosy represents an early stage of the disease in which the degree of CMI is still not clear. Patients with indeterminate leprosy either can heal or might develop leprosy and move on borderline part of the spectrum [1].

In each of the five forms of the spectrum, the clinicobacteriological and histopathological parameters have to agree with each other (Table 26.1). Along the

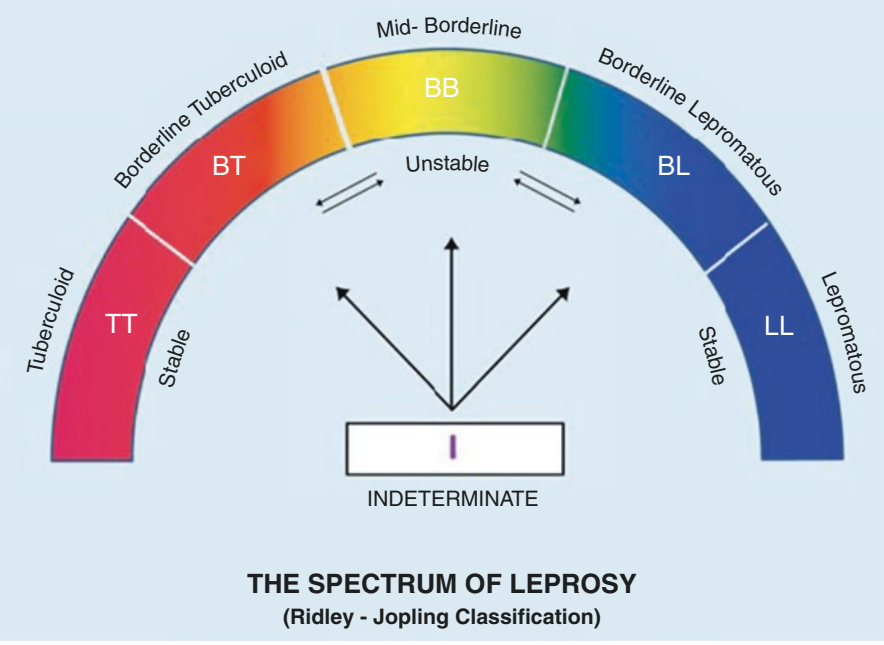

Fig. 6.1 The spectrum of leprosy (Ridley–Jopling classification). (Redrawn from Leiker DL, Nunzi E (1986) Leprosy in light skin. An Illustrated Manual. AIFO-Italia, Bologna, with permission)

leprosy spectrum, between the two polar forms, there is a graduation of the clinical manifestations (Table 26.2). Of course, not all patients may fit neatly into one of the five forms. As Ridley himself noted, "The spectrum is uninterrupted and there may be patients with an intermediate position among two groups" [4, 5]. Patients with TT leprosy have a high degree of CMI, having one or two skin lesions with monolateral distribution, with no or few bacilli and epithelioid granuloma on histopathology (Chap. 12). Moving in the spectrum toward the lepromatous pole, the CMI decreases progressively; BT patients have few lesions, asymmetrically distributed, with no or few bacilli and epithelioid granulomas on histopathology. In BB patients, the lesions become symmetric, there are some bacilli, and granulomas show both epithelioid and macrophage features. CMI progressively decreases, so that BL and LL show many symmetrically distributed lesions (see Chap. 12) with many bacilli and macrophage granuloma on histopathology.

BT and BL are the most frequent forms of presentation of leprosy. TT is uncommon, as it can also heal spontaneously. The BB form is rare because it is immunologically highly unstable and BB patients frequently shift to BT or BL. LL shows different prevalence among continents, probably due to genetic factors: in South America it represents more than 20% of cases, in Asia 5–20%, and in sub-Saharan Africa less than 5%.

The Ridley–Jopling classification was proposed over 40 years ago, and it has never been standardized. Moreover, discrepancies between clinical pictures and histopathological features have been described (Chap. 12). The Ridley–Jopling spectral concept cannot explain single variations encountered in leprosy, which represent however only a minority of cases. Interestingly, the spectral concept based on CMI has been expanded to explain the divergent manifestation seen in other diseases such as leishmaniasis, tuberculosis, and even lupus erythematosus.

6.3 The WHO Classifications

In 1982, the WHO introduced the multidrug therapy (MDT) with two different treatment schemes correlated to the result of the slit-skin smear examination. Patients were classified as paucibacillary (PB) if the bacterial index (BI) was 2+ or as multibacillary (MB) if the BI was C2+ [6].

In 1988, all cases with positive slit-skin smear at any site were grouped as MB and all cases with negative slit-skin smear as PB [7].

In 1998, the WHO repealed the use of slit-skin smear examination for the classification and recommended a new classification based only on the number of the lesions, with patients having up to five lesions in total being PB and those with six or more skin lesions being MB. If skin smear is done and is positive, the patient is classified as MB irrespective of the skin lesions. This lesion counting system is currently published in the WHO Guidelines for the diagnosis, treatment and prevention of leprosy (2018) [8, 9]. According to the WHO, "The diagnosis of leprosy remains based on the presence of at least one of three cardinal signs: (1) definite loss of sensation in a pale (hypopigmented) or reddish skin patch; (2) thickened or enlarged

peripheral nerve with loss of sensation and/or weakness of the muscles supplied by that nerve; or (3) presence of acid-fast bacilli in a slit-skin smear."

6.3.1 The Issue of the Two Classification Systems

Treatment schedules are determined by classification. Both the R&J and WHO classifications are well established but also have shortcomings.

With the lesion counting system, classifying and treating patients has become simpler. The WHO system is appropriate and has had great success, especially in highly endemic, low-resource settings. It is easy to use and teach, and general healthcare workers can easily allocate patients to the appropriate treatment regimen. Moreover, some countries such as Brazil apply a modified WHO classification [10, 11].

Nevertheless, this system has some weakness. Assessing skin lesions might not always be easy. It depends on the amount of skin examined, the quality of the light, and of course the expertise of the leprosy workers, who still have to recognize the wide range of manifestations of leprosy. Moreover, the number and appearance of skin lesions may change over time; for example, during reaction, lesions may become more evident. In early BL/LL cases, skin lesions are often few and difficult to see or even invisible. Furthermore, the size of the lesion also matters but is not considered in the WHO classification [12].

As demonstrated in various studies, underestimation of the number of lesions and misclassification (particularly for MB patients) may happen, leaving patients at risk of under- or overtreatment [13]. For example, MB patients presenting few lesions will be incorrectly classified as PB and will receive insufficient chemotherapy, becoming exposed to the possibility of drug resistance and relapses. Of course determination of bacterial load in skin smears considerably improves the reliability of the classification, but this service is often not available in areas where leprosy is most common. Most importantly, contrary to the R&J classification, the WHO system does not identify the categories of patients at high risk for leprosy reaction and in need of accurate follow-up.

The classification influences also research and epidemiological studies. The PB category comprises patients with I, TT, and part of BT leprosy. In fact, the line dividing PB from MB cases crosses the BT form. The MB category is equally heterogeneous and comprises part of BT, BB, BL, and LL patients, rendering comparison among studies performed with the two systems impossible. Moreover, due to the changes in the WHO classification in the last 25 years, it is hard to compare works done 20 years ago with those done more recently. Also, data among countries and even within a country itself are at risk of misclassification [9, 13].

We completely agree with Lockwood et al. that the two classifications, R&J and WHO, should be seen as being complementary rather than exclusive [9]. The WHO classification is useful for allocating patients to treatment groups and should be used in peripheral centers where skin smears and histopathology are not available. The R&J classification has to be used in referral centers and in the research context,

because it permits better understanding of disease pathology, prognosis, and risk factors for complications, and provides standardization and comparability of studies over time and location [14, 15].

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Part II

Leprosy: Patient's Examination

Leprosy Patient History

7

Enrico Nunzi, Cesare Massone, and Salvatore Noto

7.1 Patient History

Mycobacterium leprae (*M. leprae*) is the conditio sine qua non to develop leprosy infection and then the disease.

Anamnesis regarding the source of *M. leprae* is less important in countries where leprosy is considered to have been recently "eliminated" or where there is an important presence of new autochthonous cases [1].

On the contrary, in those countries where leprosy is an "imported" disease, searching for the source of the infection is very important. It is necessary to investigate if the patient has lived in countries in tropical or subtropical areas in the last 10–15 years, or if the patient comes from one of those countries. Short periods spent in these areas should also be considered, even if only on holiday [2]. Investigation must be carried out on cohabitants of the patient if they come from or have lived in these countries.

To identify countries in which it is possible to come into contact with *M. leprae*, it is important to critically consider official epidemiologic information. Several countries tend to underestimate or deny the presence of leprosy in their regions.

It is necessary to be very careful before diagnosing leprosy in those countries where the disease is rare and "imported." Histopathology is necessary to confirm the

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diagnosis in those patients who show clinical and microbiologic parameters in favor of leprosy but in whom anamnesis is negative for possible contact with leprosy patients. If acid-fast bacilli (AFB) are present, they should be tested with polymerase chain reaction (PCR) to identify the bacteria.

Of 150 new cases observed in the referral center of Genoa (Italy), only one patient presented negative for contact with *M. leprae*. In the Netherlands, out of 1600 patients identified between 1945 and 1990, only a single case had never traveled outside the country [3].

7.2 Prodromal Symptoms

During anamnesis in patients suffering from the multibacillary form of leprosy, it is possible to discover the aspecific symptoms that characterize the prodromal stage. In the lepromatous form, the prodromal symptoms can persist over years with aspecific manifestations of the upper respiratory tract (numerous episodes of epistaxis, dryness of nostrils with formation of crusts) or bilateral edema of the malleolus and foot.

During anamnesis, symptoms such as localized paresthesia, plantar hyperalgesia, and neuralgia along peripheral nerves such as the ulnar nerve, trigeminal nerve, and sciatic nerve are referred by the patients.

7.3 First Symptoms

To understand the natural history of the disease in a patient, it is important to determine when and how the first lesion appeared, i.e., whether the lesions appeared abruptly at onset or slowly.

In anamnesis, to understand the first leprosy symptoms, it is important to search for the initial arrangement of the lesions on the body, the number of lesions, and if there is presence or absence of anesthesia.

These anamnestic data give important information on the "stability" of the disease, and it must be used in association with the clinical features to formulate therapeutic strategies.

Leprosy, a disease with chronic course, may begin with an "overture" with cutaneous and systemic symptoms (fever, neuralgia, arthralgia) of leprosy type 2 reaction. It is also possible to find an acute onset with cutaneous and/or nervous symptoms of leprosy type 1 reactions during pregnancy, after delivery, or in patients suffering from acquired immune deficiency syndrome (AIDS) under treatment such as the immune reconstitution inflammatory syndrome (IRIS) phenomenon.

Fever with cutaneous eruptive lesions and "red eye" (which appears between the first lesions and the diagnosis) are compatible with leprosy reactions.

7.4 Subjective Symptoms

An important subjective symptom in dermatology is itching.

A common opinion underlines the lack of itching in leprosy, but this is not always true. In fact, in this disease, there can be the presence of localized itching in the skin before the appearance of leprosy type 1 reaction lesions. Treatment regimen with clofazimine leads to cutaneous xerosis with widespread itching in connection with changes of environmental hygrometry, which happens in multibacillary patients under MDT treatment.

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Laboratory Investigations in Leprosy

8

Andrea Clapasson and Silvia Canata

8.1 Laboratory Investigations

The most relevant problem in the fight against leprosy in its various forms is the delay in clinical recognition. This leads to the transmission of *M. leprae* or *M. lepromatosis*.

Therefore, it is very important to know the most suitable combination of laboratory investigations.

Until a few years ago, the diagnosis of leprosy was based on clinical evidence (presence of skin lesions with loss of sensitivity and/or thickened peripheral nerves) and on laboratory investigations such as search for acid-fast bacilli (AFB) in slit-skin smear (SSS) examination and histopathology. There has been an evolution in recent years, and different types of molecular biology analyses can help doctors during the differential diagnosis stage.

8.1.1 Tools for Laboratory Investigations

The tools for laboratory investigations are used for diagnosis, classification, and monitoring response to treatment. They may be divided into classical and modern assays. However, the "modern" assays must be used cum grano salis; they cannot

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replace clinical examination and they are very important in the context of differential diagnosis.

The classical techniques are:

- Ziehl-Neelsen staining (ZN), auramine staining, and Kinyoun staining for the research of mycobacteria in SSS or in nasal swabs (NS).
- Fite-Faraco staining (FF) for research of mycobacteria in biopsy.
- Serological tests for research antibodies anti-PGLI and anti-35 kDa of *M. leprae*. These assays have a sensitivity which is in 90–100% of lepromatous patients (BL/LL) but only 40–60% in tuberculoid leprosy patients (BT/TT) [1].

The modern techniques are of molecular biology, based on polymerase chain reaction (PCR). Some of these are as follows:

- 16S rRNA real-time polymerase chain reaction.
- PCR targeting RLEP sequences [2].
- Microarray analysis and PCR-restriction fragment length polymorphism analysis (PCR-RFLP) for species typing of mycobacteria [3]. This typology of assay is very useful for the discrimination among *M. leprae*, *M. lepromatosis*, and other mycobacteria.
- Real-time PCR or limiting dilution PCR (LD-PCR) to monitor drug therapy [4].
- Reverse transcriptase PCR (RT-PCR) or real-time PCR to research viable bacteria [5].
- Single-strand conformation polymorphisms (SSCP) or sequencing techniques to identify relevant mutations in drug therapy [6, 7] or GenoType LepraeDR *test*. The last assay is commercial test and it is not certificated for *M. lepromatosis*, but only for MB patient.
- Sequencing techniques.

It is very important to remember that "the bacteria load of biological sample and previous drug assumption can lead a not true result." To minimize these falls it is necessary to choose the right *combination of techniques* of both molecular biology and histopathology. Each of these modern techniques has disadvantages and advantages. The main disadvantages are the need of expensive instrumentation, cold chain requirement, and qualified laboratory staff, while the advantages are sensibility and rapidity. An example of advantage is the rapidity in the research of resistant strain of *M. leprae*. If you use the mouse footpad technique, the result is obtained after 6–12 months, while the same result, by inverse hybridization or sequencing of specific gene, is obtained in 2 days [7].

The techniques based on molecular biology are used for the purpose of research, while ZN and FF techniques are used routinely for diagnosis of leprosy. However, the molecular biological methods are gaining importance and are indispensable for rapid determination of the species of mycobacteria, and for an accurate determination of the vitality of *M. leprae*.

8.1.2 Nasal Swabs and Slit-Skin Smear Examination

Rule not to be forgotten: *an accurate sampling produces a reliable result*.

The NS is a test where a sample of biological material, obtained by a dry swab of cotton, is smeared over the slide in a circular area. Looking for AFB in NS has only the purpose of determining the end of the contagiousness of multibacillary patients in treatment; it is not a diagnostic or classification criterion.

The SSS examination is a test where a sample of tissue fluid and pulp, obtained by a scalpel, is spread in onto the slide.

There is a difference between the morphology of bacteria in the nasal mucosa and the skin of the same patient.

In the nasal mucosa of untreated lepromatous leprosy patients, there is a higher percentage of solid-staining bacilli than that present in the skin.

The next step is ZN staining. It is performed for the diagnosis of new cases and classification of leprosy, for monitoring of therapy, or for identification case of relapsed of Hansen's disease.

The test is invasive and health personnel must wash his/her hands, wear gloves, and use sterilized equipment and a new blade for each patient.

The best sites for taking SSS are active edges of skin lesions and the cooler regions of the body (see Chap. 2, "Microbiology"). Samples should be obtained from three to six different sites (in BL-LL from both ear lobes and from the edge or just within the edge of four active lesions).

The SSS is useful for diagnosis, classification, choice, and monitoring of therapy and identification of relapse. The SSS test should be taken from:

- Patients suspected to have leprosy.
- Leprosy patients suspected of relapse.

8.1.3 Nasal Smear Technique [8]

We describe two phases in the technique of the nasal swab; these are sampling and staining.

The patient should sit at ease in good light with his head backward and his chin up; in this way, the nasal septum is easier to reach. The best time to collect the nasal secretion is the early morning. The specimen is obtained by rubbing the upper part of the septum using a small cotton swab mounted on a stick.

The biological sample is spread onto a slide. Leave the smears to dry in the open air; therefore, fix and stain as described for SSS.

8.1.4 Slit-Skin Smear Examination Technique [8]

The sites selected for SSS are cleaned with alcohol. Squeeze the skin between the thumb and forefinger, and maintain pressure to expel blood (Fig. 8.1a). Make an

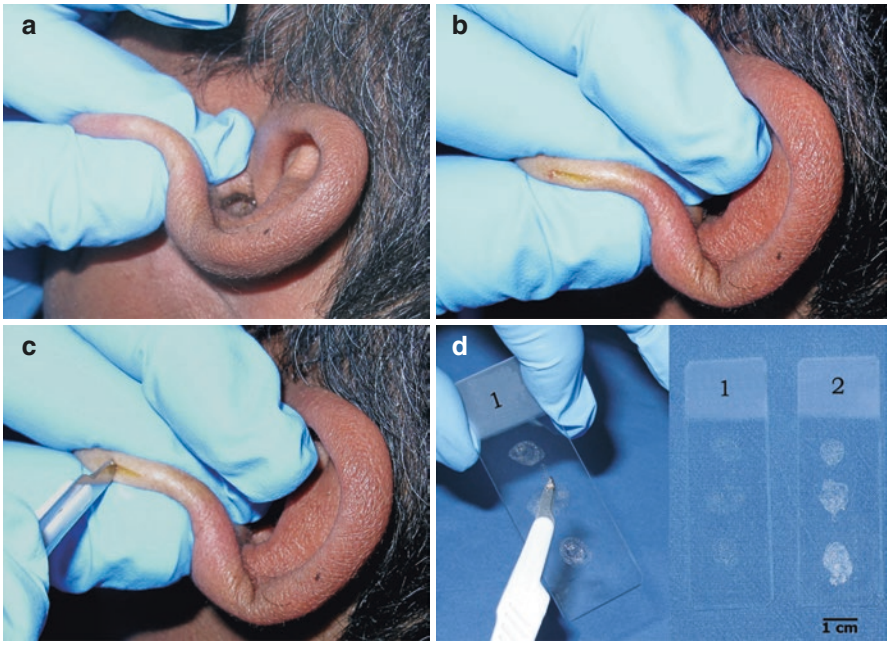

Fig. 8.1 (**a**–**c**) Slit-skin smear technique. (**d**) Slides with sample

incision in the skin about 5 mm wide and 2 mm deep. Continue pressing until the smear has been taken. If the wound continues bleeding, clean with a sterile gauze (Fig. 8.1b).

Turn the blade until it is at right angle to the incision and scrape, once or twice, some tissue material from the sides and bottom of the cut (Fig. 8.1c). Keep the sample bloodless because blood could interfere with the slide reading. Smear onto a slide in single layer (Fig. 8.1d), to cover an area of 5–10 mm diameter, by making circular movements with the flat side of the end of the blade. Slides are left in the open air until they are completely dry. Now they are fixed quickly passing the slides uppermost over the top of a Bunsen burner for three times. Heat fixation is critical because overheating affects the property of acid-fast staining and may crack the slide. Otherwise, a heating cabinet or a hot plate with temperature controlled is recommended. If this solution is used, an exposition for 5 min at 40–50 °C is necessary. Another method of fixation is exposure of the smear for 10 min in formaline fumes.

Limit of SSS technique is low sensitivity (it detects about a third of the AFB); the reading of the slide must be performed by trained personnel, using a microscope with 100× immersion objective.

The slides must not to be exposed to sunlight or dust [9]. After examination, all slides must to be kept for 3 months in a box closed to be re-examined, if the need comes.

The lack of sensitivity of ZN's method can be partly improved by using the auramine technique, but being a fluorescent method, the slides are only readable with a fluorescence microscope [10]. Smears that have been examined by fluorescence microscopy may be re-stained by Ziehl-Neelsen staining to confirm observations, but it is not possible and vice versa.

8.1.5 Cold Ziehl-Neelsen Technique for SSS and NS

1. Cover the sample (skin smear or nasal smear) with primary stain (*), for 20 min.
2. Rinse gently with indirect stream of tap water, until the water flows off clear.
3. Decolorize each slide separately with 2.5 mL of solution of hydrochloric acid and ethanol or sulfuric acid and alcohol (**). This step is more critical of all procedure, because *M. leprae* is more easily decolorized than other mycobacteria, for example, of *M. tuberculosis*. If duration of destaining is too long, there are false negatives, while if it is too short, there are false positives.
4. Rinse with indirect stream of tap water.
5. Counterstain with methylene blue 1% (***), for 30 s.
6. Rinse the stain with indirect stream of tap water until the water flows off clear.
7. Allow slides to dry, away from sunlight.
8. Observe the slides under oil immersion.

AFB appear red, while non-AFB organisms and cellular materials appear blue.
(*) Primary stain (1%):

(a) In a beaker previously weighed, dissolve 6.75 g of basic fuchsin in 67.5 g absolute alcohol.
(b) Add 37.5 g of 5% aqueous phenol [phenol solution: weight 5 g of phenol crystal and dissolve them in 100 mL distilled water (heating gently)].
(c) Add deionized water up to 675 g.
(d) Mix well and filter before use.

Prepare the solution with all components under the fume hood, using appropriate safety equipment (gloves, mask for dust and fumes). The prepared solution is transferred in dark glass bottle with screw cap (capacity 1 L). Label bottle with name of reagent as well as preparation and expiry dates. Store at room temperature for 6–12 months.

(**) Destaining reagent: 95 mL ethanol 96° and 1 mL hydrochloric acid 37% (fuming). *Important:* you must always add acid, drop by drop, to solvent, *not* vice versa.

In countries where the acquisition of alcohol may be problematic, an aqueous solution of 23.75% sulfuric acid and 3% alcohol may be used as decolorizing agent. This is prepared as follows: add 25 mL of 95% sulfuric acid *slowly* (*not* vice versa) to solution of 71.5 mL of distilled water and 3.3 mL of 90% denaturized alcohol.

(***) Counterstain: dissolve 1 g methylene blue in 100 mL distilled water.

Many mycobacteria can survive and grow in nutritionally poor environments such as water puddles and even chlorinated tap water. Environmental mycobacteria might be present in the tap water; boiled water does not solve the problem; you will kill them but they will appear again as AFB after staining. The water is a reagent and its quality is the most important thing; *use purified or distilled water for your solution, not tap water, rain water, or boiled water*.

If it is possible, include one positive and one negative control among the slides when you are staining, for the quality control of Ziehl-Neelsen reagents.

Wear personal protective equipment (respiratory, hand, eye, skin, and body protection) during preparation of solutions and during staining method, and you make the solution in a fume hood.

The *M. leprae* is more easy decolorized than other mycobacteria and its acid resistance is removed by treatment with pyridine. The AFB can be observed only if they are present at equal or higher concentration than 10^4/g of the skin [10].

M. leprae is not the only AFB; there are other microorganisms stained with ZN, namely, other mycobacteria, *Cyclospora*, *Cryptosporidium*, *Isospora*, *Nocardia*, *Rhodococcus* (partially acid fast), and some yeasts. Moreover, some substances are also stained by ZN like inclusions of lead and waxy substance.

8.1.6 Bacteriological Index (BI) and Morphological Index (MI)

Bacteriological index (BI) and morphological index (MI) provide complementary information.

The BI is a parameter directly related to the bacterial load; it is the estimated number of all bacteria (independently of their shape) present in the smear. It is obtained counting the bacilli in a number of oil immersion fields. The value is calibrated using the logarithmic scale of Ridley.

Grading of the BI of each smear

0→ = 0 AFB in any of 100 immersion fields (it is defined as negative)
1 + → = 1–10 AFB on average in 100 immersion fields
2 + → = 1–10 AFB on average in 10 immersion fields
3 + → = 1–10 AFB on average in each field
4 + → = 10–100 AFB on average in each field
5 + → = 100–1000 AFB on average in each field
6 + → = >1000 AFB on average in each field

The average score of the smears is the BI of patient.

The MI studies the shape of the bacilli. It is given by the percentage of the uniformly or solidly (S) stained bacilli. MI is the correlation between shape and vitality. The S-AFB are live bacilli. BI and MI should decrease during therapy.

8.1.7 Bacteriological Follow-Up

The assessment of BI suffers from a number of variables: depth of scrape, amount of tissue fluid removed, and size and thinness of the smear. These issues should not be underestimated, and in the follow-up, successive SSS are better performed by the same operator and samples should be taken at the same sites as previous ones. Finally fixation, destaining time, presence of blood, dust, dirty microscope slides, and direct sunlight are other parameters that may alter the final result. It would be useful to include one positive and one negative control among the slides when you are staining. Proper anti-leprosy therapy decreases patient's BI of about "1+" per year; therefore, bacteriological follow-up in multibacillary patients is carried out annually.

8.1.8 How to Read the Slide?

To check systematically every sample, run the slide with a movement similar to "zigzag" (Fig. 8.2). In this way, using a 100× oil immersion objective, examine 100 adjacent fields.

Depending on the type of stain taken from each AFB, you will have:

- "Solid" form (S). AFB have a homogeneous staining.
- "Fragmented" form (F). AFB have small gaps in the stain.
- "Granular" form (G). AFB are formed most degraded and they are formed by rosary beads. G-form are bacilli which show two or more unstained zones across the whole width of the bacillus [8].

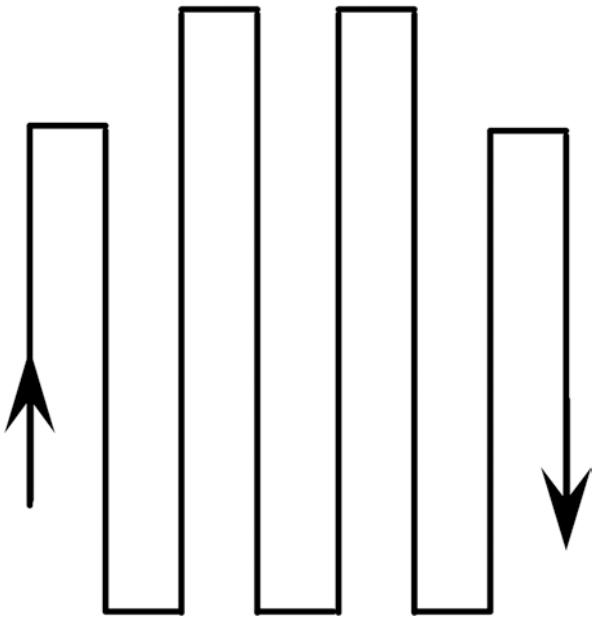

Fig. 8.2 Zigzag observation to scan smear

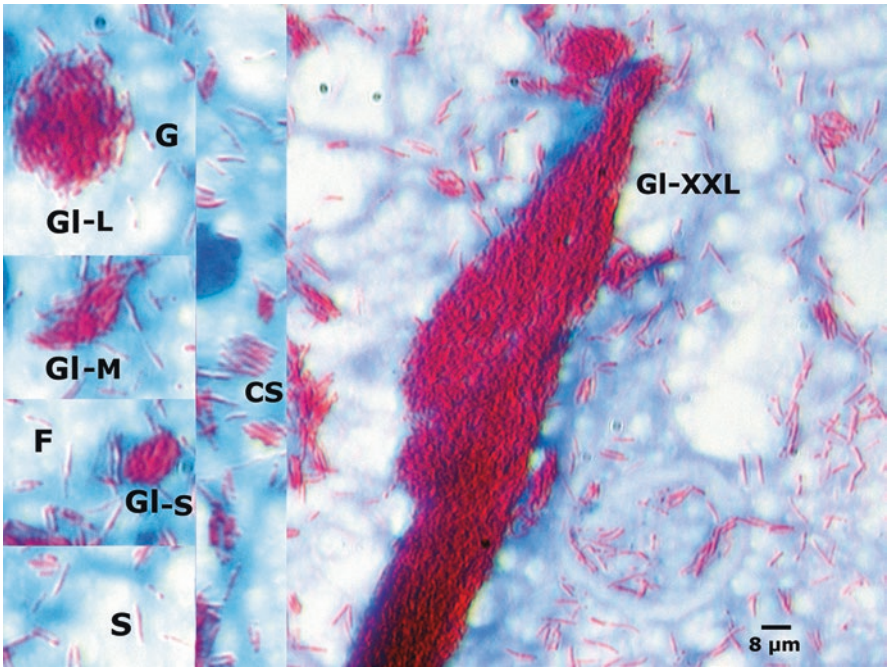

Fig. 8.3 Comparison of different globus: *Gl-L* large globus, *Gl-M* medium globus, *Gl-S* small globus, *Gl-XXL* extra-large globus, *CS* cigar-shaped cluster, *S* solid, *F* fragment, *G* granular

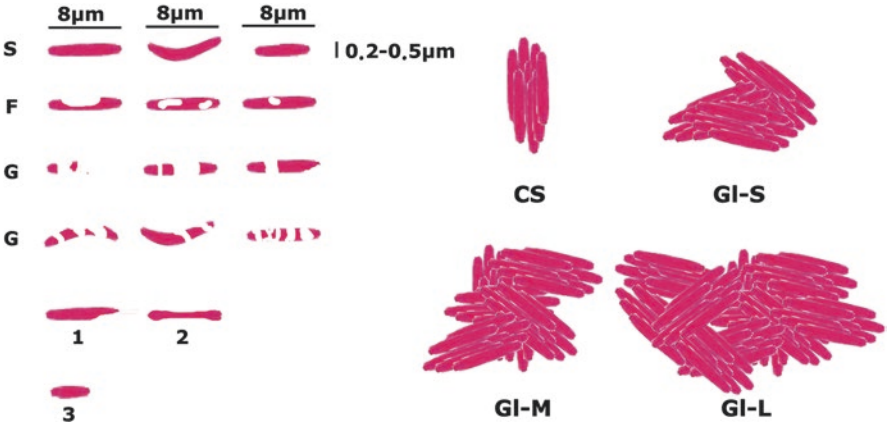

Fig. 8.4 Interpretation table of AFB: solid (S), fragment (F), and granular (G). (1, 2) Likely they are artifacts. These AFB should not be counted. (3) AFB is generally classified as F. Clusters in cigar-shaped (CS); globi: small (Gl-S), medium (Gl-M), and large (Gl-L)

It is also possible to find clusters of bacteria called "globi." During the therapy, each globus shows different affinity for the dye; it switches from a hyperchromic to a hypochromic staining.

The density of the bacteria decreases in the hypochromic globus; they are mainly F- and G-forms. The F and G are bacteria irregularly stained showing heavy degenerative changes.

The bacteria present in the globi cannot be counted accurately and must be estimated. A small globus may contain about 30 AFB, a medium 60, and a large about 100. Rarely in some lepromatous cases, it is possible to find extra-large globi which can contain more than 1000 AFB (Figs. 8.3 and 8.4).

The staining property of the AFB changes during the normal life cycle of the bacterium and during therapy.

In multibacillary cases at diagnosis, the "solids" are the minority, but during therapy they disappear first. Contemporarily, the percentage of fragmented and granular forms increases, and then remains only granular; these are the last to disappear (from the skin). This modification in the stain is an indirect parameter of viability of AFB and a measure of the patient's response to treatment.

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Part III

Skin

Physical Examination in Leprosy: Skin

9

Enrico Nunzi, Cesare Massone, and Salvatore Noto

Leprosy is a disease characterized mainly by involvement of peripheral nerves and the skin. The first signs that lead leprosy patients to medical examination are most often dermatological.

Skin examination has three fundamental steps, namely, general overview of the skin, detailed observation of lesions, and paraclinical tests [1].

9.1 General Overview: Distribution and Shape of Lesions

In leprosy, the spread of bacilli and skin lesion arrangement depend on cell-mediated immunity (CMI).

Lower CMI corresponds to increased bacterial load and number of lesions; at the same time, lesion distribution becomes increasingly symmetric.

In the lepromatous part of the spectrum, the bacilli and lesion distribution also depend on skin temperature. Warmer regions (scalp with hair, axilla, the middle part of back, groin, and inner part of thighs) present no lesions, while cooler parts of the body, such as the nose and nostrils, cheekbones, eyebrows, chin, and earlobes, host many bacilli and lesions.

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In leprosy, according to the CMI, two main patterns of lesion arrangement can be observed (see also Chap. 26).

(a) Asymmetric arrangement (Figs. 9.1 and 9.2)
(b) Symmetric arrangement (Fig. 9.3)

The asymmetric arrangement pattern is peculiar to hyperergic paucibacillary forms. Rarely, a single lesion of hyperergic tuberculoid polar form can appear on the centerline of the body, having a symmetric aspect (Fig. 9.4), so that in such cases, the diagnostic pathway for patients with asymmetric pattern is utilized (see Chap. 26).

The symmetric arrangement pattern is always bilateral by definition. This is peculiar of hypo-anergic multibacillary forms of the leprosy spectrum; decreasing

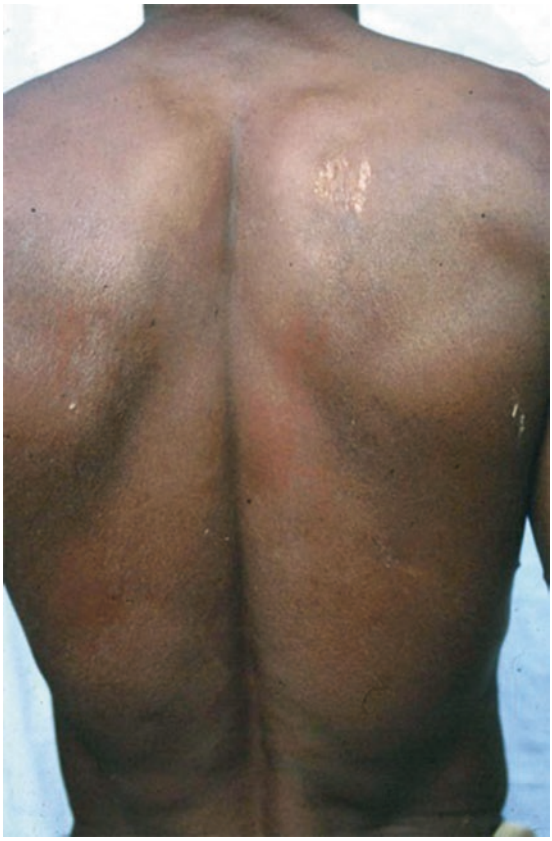

Fig. 9.1 Asymmetric arrangement: monolateral lesions

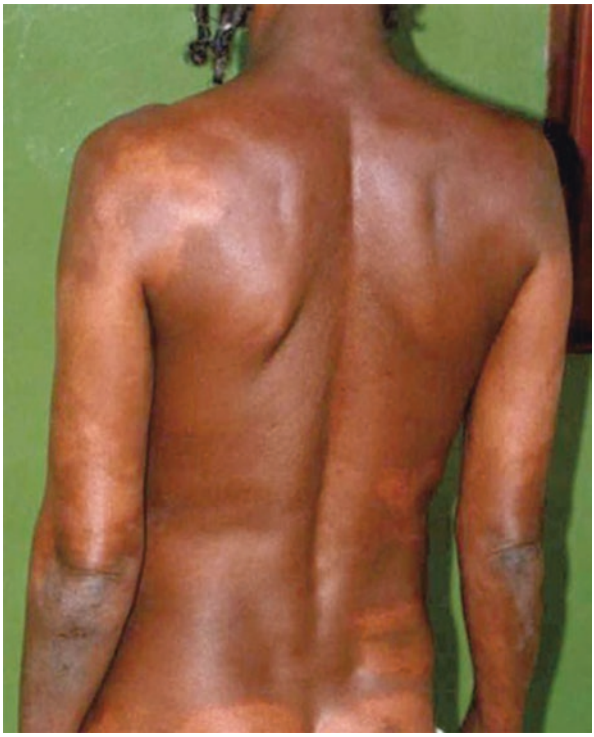

Fig. 9.2 Asymmetric arrangement: bilateral lesions

CMI corresponds to an increasing number of lesions. Their distribution on the body will appear even more symmetric, reaching the greatest symmetry at the anergic lepromatous pole.

In leprosy the shape of the skin lesion can be:

(a) Annular
(b) Targetoid
(c) "Swiss cheese," i.e., macules and plaques with punched-out surface
(d) Linear
(e) Polycyclic

Annular lesions can be seen in the asymmetric pattern due to central immunological healing process, and in the symmetric pattern only in the mid-borderline form of leprosy (BB), in which targetoid or Swiss cheese-shaped skin lesions can also appear. Polycyclic lesions with asymmetrical distribution and elevated papular edges are typical of BT forms.

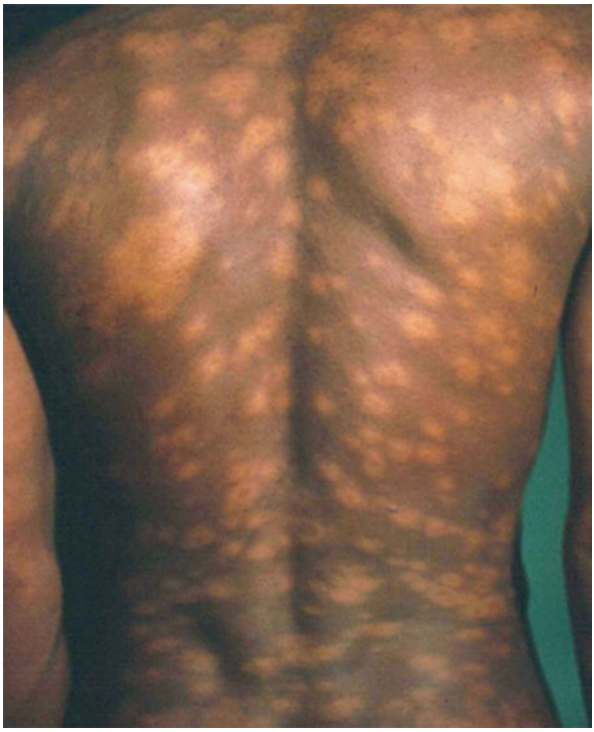

Fig. 9.3 Symmetric arrangement: bilateral lesions

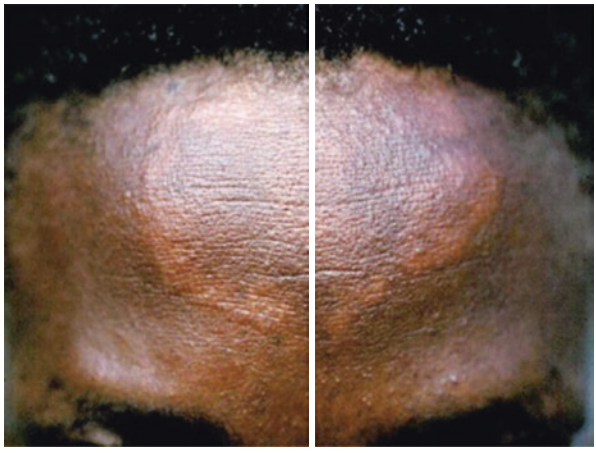

Fig. 9.4 Tuberculoid leprosy: single lesion on the centerline of the body. In such cases, the diagnostic process for the asymmetric arrangement pattern must be used

9.2 The Elementary Skin Lesions in Leprosy

The elementary skin lesions in leprosy are:

(a) Flat lesions (on the cutaneous surface):
- In active leprosy: erythematous, coppery, or hypopigmented macule
- In cured leprosy: hyperpigmented macule

(b) Elevated lesions (on cutaneous surface):
- In active leprosy: papule, nodule, plaque, and diffuse infiltration. This last lesion characterizes the polar lepromatous anergic form
- In leprosy reactions: plaque, nodule, wheal, scaling, vesicle, and bulla

(c) Depressed lesions (on cutaneous surface):
- In active leprosy: ulcer
- In leprosy reactions: erosion and ulcer
- In cured leprosy: atrophy, sclerosis, ulcer, and scar

9.2.1 Papules and Nodules in Active Leprosy

In dermatology, the difference between papules and nodules depends on the diameter, with papules having diameter smaller than 1 cm. In active leprosy (not in leprosy reactions), the differences between these two lesions cannot be assessed just by measuring size. In leprosy, papules are observed in hyperergic paucibacillary forms, and nodules are present in the hypo-anergic multibacillary form.

Papules show asymmetric arrangement and do not contain acid-fast bacilli (AFB), whereas nodules show presence of AFB and exhibit symmetric arrangement on the body (except in some anecdotal cases). Papules and nodules cannot be present together in the same patient [2, 3].

9.2.2 Morphological Features of Skin Lesions

(a) Edges: well- or ill-defined
(b) Surface: dry and rough or smooth

These features are also conditioned by CMI; well-defined edges depend on hyperergy which contains the infection, while vague edges show hypo-anergy with CMI that cannot repress bacillary spread. A wrinkled surface is caused by anhidrosis and is a sign of strong CMI. In this case, hyperergy provokes early destruction of autonomic nerve bundles that innervate sweat glands.

Lesions disappear when using multidrug therapy (MDT). In hyperergic forms, there can be a restitution of sensitivity. The color of the skin returns to normal; in late-treated multibacillary patients, lesions heal with atrophy.

9.3 Paraclinical Tests in Leprosy

(a) Tests for sensitivity and autonomic nerve function (see Chap. 15) on single lesion or lesions with asymmetric arrangement.

Sensory nerves

- Tactile sensitivity
- Pain sensitivity
- Thermal sensitivity
- Thermal sensitivity is the earliest to be lost. For convenience, in the course of routine tests, tactile sensitivity on lesions is assessed.

Autonomic nerves

- Function of sweat glands
- Integrity of the axon reflex
- It is more complex to test for sweat gland functionality with pilocarpine or the acetylcholine test, or to assess axonic reflex integrity using the triple response of Lewis provoked by histamines (use of which can also provoke some problems in atopic subjects). These tests are useful in children, where sensitivity testing is usually unreliable [4].

(b) Slit-skin smear can be used in lesions with symmetric arrangement or on cooler skin which is apparently normal. The slit-skin smear is carried out on edges of skin lesions (see Chap. 8).

Nasal smear (nasal swab) examination for AFB is performed for epidemiological reasons only; it has no diagnostic importance. The nasal mucosa is the main route of exit of *M. leprae* to the external environment, but bacilli are found in this region only in some BL and LL patients.

9.4 Clinical Examples

Clinical aspect must be examined from a dynamic point of view: each lesion must be assessed according to the full clinical aspect and patient history. This gives information which helps in understanding the natural progression and history of the disease. This is extremely important in leprosy because it helps to evaluate the immunological instability of the patient and the tendency to develop leprosy reactions.

The patient in Fig. 9.3 shows macules with symmetric distributional pattern. On the back, there is a large macule and other macules which are smaller, with vague edges. There are also small, annular lesions. According to the anamnesis, the patient initially developed BT leprosy revealing larger lesions on the left scapular region with well-defined and vague edges. Diagnosis was not achieved, CMI decreased, and bacterial load increased, with the appearance of smaller lesions in symmetric arrangement. The early hyperergic asymmetric arrangement turned into anergic symmetric arrangement. Some lesions have annular shape, meaning that the disease

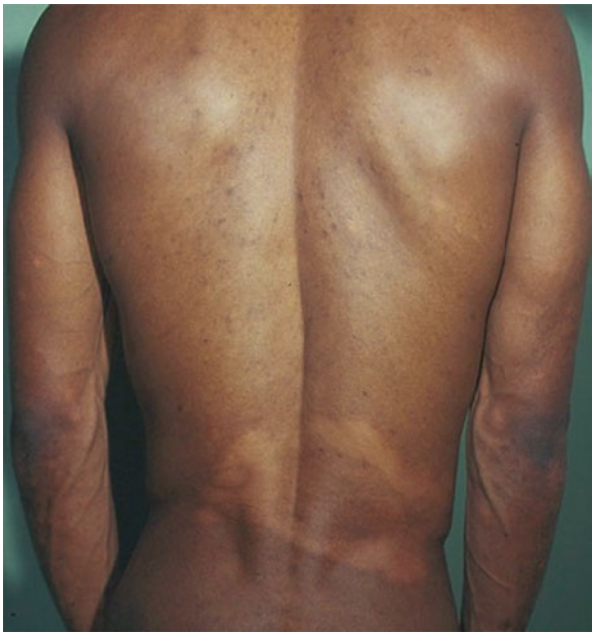

Fig. 9.5 Borderline leprosy (BL). Symmetric arrangement of BL form coexisting with an early asymmetric annular macule with BT aspect

passed through the mid-borderline form to reach the LL pole. This patient is not stable along the disease spectrum. MDT decreased the bacterial load and caused the appearance of leprosy type 1 reaction (edema in lesions).

The patient in Fig. 9.5 shows a large annular BT lesion on the back coexisting with nodules in symmetric arrangement that appeared later. The first lesion that appeared still shows annular shape with well-defined and vague edges of BT leprosy. This large BT lesion remained unchanged in time, while subsequently nodules with symmetric arrangement appeared. This enabled us to presume regional immunity of different levels [5].

This dynamic approach to dermatologic diagnosis of leprosy enables the patient's picture and evolution to be considered from past to present, and estimation of the possible future clinical evolution. This is necessary in order to establish a therapeutic strategy and to prevent leprosy reactions, which are acute inflammatory phenomena that strongly condition prognosis.

9.5 Regional Physical Examination

Localizations on specific body areas are important for diagnosis because they can reveal typical aspects of the disease. Physical examination must be done methodically, including the skin, peripheral nerves, eyes, upper respiratory system, and oral cavity in order to identify all early and late disease symptoms (Figs. 9.6 and 9.7).

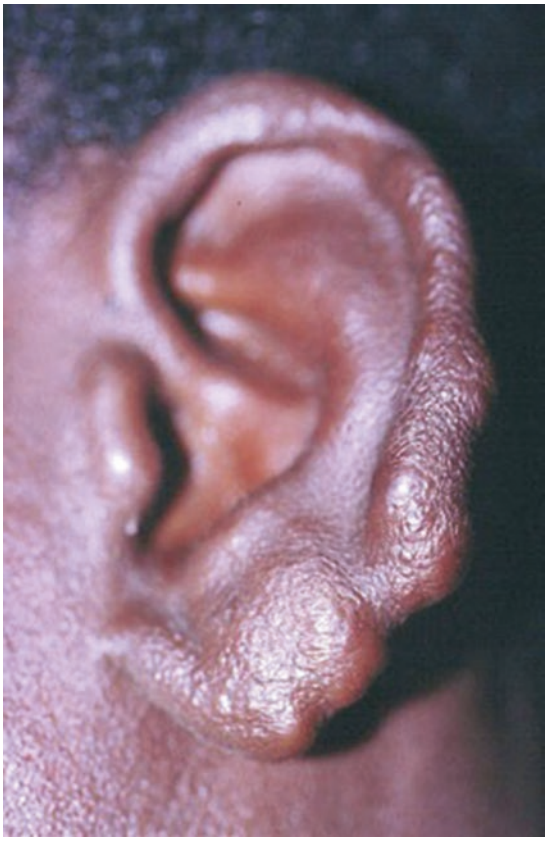

Fig. 9.6 Lepromatous leprosy (LL). Ear infiltration

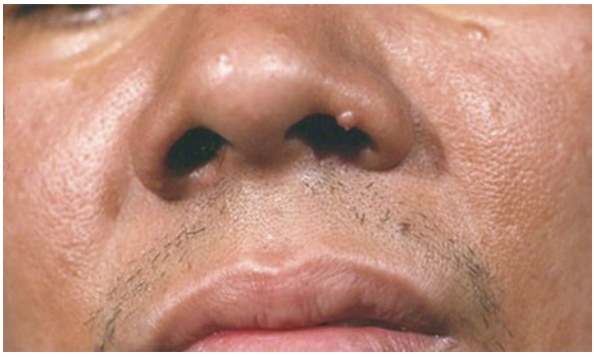

Fig. 9.7 Lepromatous leprosy (LL). Nodule at the nostril edge

Head Forehead – Specific skin lesions – Swelling of upper orbital nerve	Eye – Lagophthalmos – Ectropion, entropion – Conjunctival nodule – Corneal macule – Corneal ulcer – Hypopyon – Synechia – Corneal hypoesthesia – Loss of eyelashes
Eyebrow – Specific skin lesions – Alopecia – Swelling of upper orbital nerve	
Ear – Specific skin lesions – Cutaneous atrophy – Pigmentation	Nose – Nodular lesions on nostril edge – Perforated nasal septum – Nasal pyramid collapse

Oral cavity – Infiltration or perforation of palate – Tongue infiltration – Anterior angulation of the maxillary incisors	Neck – Specific skin lesions – Swollen great auricular nerve
Chin and cheek – Specific skin lesions.	

Upper limbs Arm – Specific skin lesions – Swelling of radial nerve Elbow – Swelling of ulnar nerve Forearm – Specific skin lesions	Trunk and abdomen – Specific skin lesions – Gynecomastia
	Buttock – Specific skin lesions
	Scrotum – Specific skin lesions tumefaction/ testicle atrophy
Wrist – Specific skin lesions – Swelling of the cutaneous branch of radial nerve – Swelling of median nerve	Lower limbs – Specific skin lesions
	Popliteal fossa – Swelling of common popliteal nerve
Hand – Specific skin lesions – Muscular atrophy – Swelling of cutaneous branch of radial nerve – Finger contracture – Ulcers – Scars – Phalanx reabsorption	Internal malleolus – Swelling of posterior tibial nerve
	Foot – Specific skin lesions – Muscular atrophy – Finger contracture – Ulcers – Bone reabsorption

It is extremely important to check eyes, hands, and feet systematically in order to identify onset of invalidity (see “Physical Examination: Nerves”).

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Clinical Features of Leprosy

10

Enrico Nunzi, Cesare Massone, and Salvatore Noto

Each "determinate form" of leprosy (TT, BT, BB, BL, LL) (see Chap. 6) in the disease spectrum (Fig. 10.1) has an immunological, histopathological, microbiological, and clinical individuality, as opposed to the "indeterminate form," which does not lie on the spectrum because of its uncertain cell-mediated immunity (CMI). In the determinate forms, the arrangement of the lesions on the body (Fig. 10.2) and their type (Fig. 10.3), features (surface, edges, and presence or absence of sensitivity), and shape (annular) are conditioned by the CMI of the patient and therefore by the position of the patient in the disease spectrum.

Macules are lesions present in each type of leprosy, including the indeterminate form, while plaques may characterize the clinical aspect of the forms of the leprosy spectrum. These two lesions have clinical differences passing from hyperergic paucibacillary forms (TT, BT) to hypo-anergic multibacillary forms of leprosy (BB, BL, LL) (see Chap. 6).

Papules characterize the hyperergic forms (TT, BT), while nodules occur in the hypo-anergic multibacillary forms of leprosy (BL, LLs). Diffuse infiltration of the body is present in LLp (Fig. 10.3).

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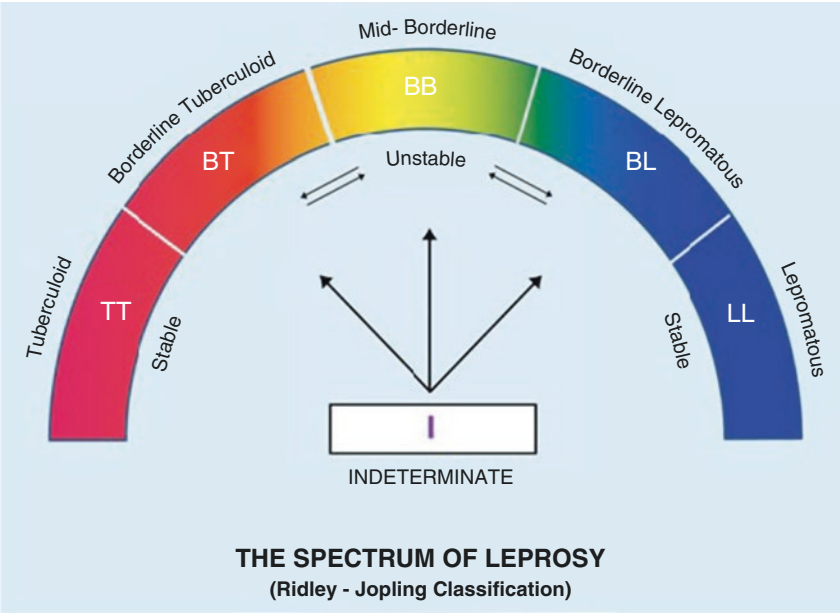

Fig. 10.1 The spectrum of leprosy (Ridley–Jopling classification). [Redrawn from Leiker and Nunzi (1986) Leprosy in light skin. An Illustrated Manual. AIFO-Italia, Bologna, with permission]

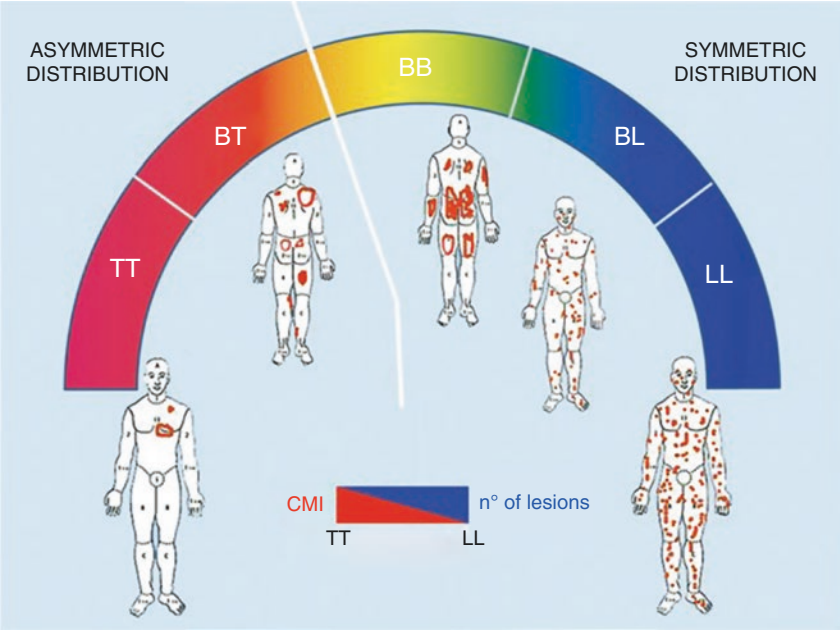

Fig. 10.2 Arrangement of skin lesions according to the leprosy spectrum. [Redrawn from Nunzi E, Leiker DL (1990) Manuale di leprologia. AIFO-Italia, Bologna, with permission]

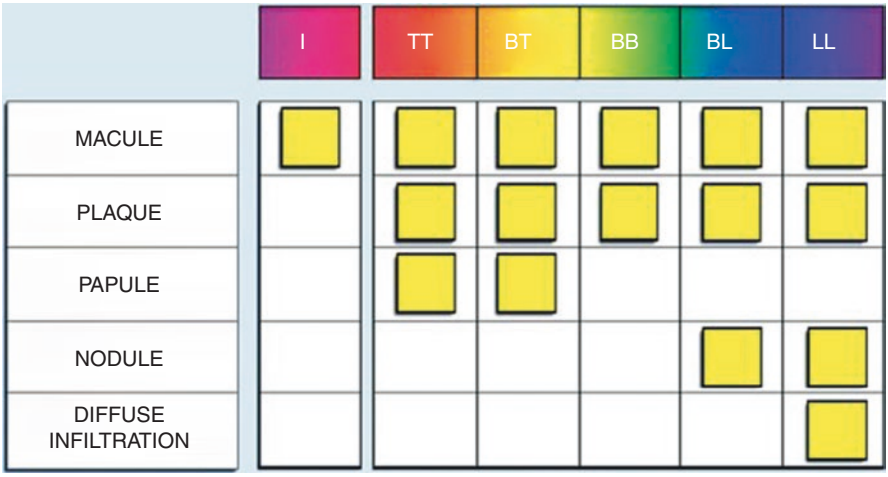

Fig. 10.3 Type of skin lesions in leprosy. [Redrawn from Nunzi E, Leiker DL (1990) Manuale di leprologia. AIFO-Italia, Bologna, with permission]

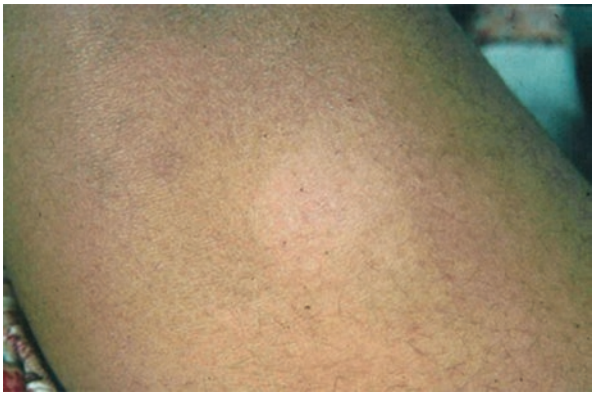

Fig. 10.4 Indeterminate leprosy (I). Hypopigmented macule with normal sensitivity, smooth surface, and ill-defined edges; from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

10.1 Indeterminate Leprosy (I)

An early, temporary form of the disease is indeterminate leprosy. The indeterminate form appears in subjects who have not developed CMI against *Mycobacterium leprae* (*M. leprae*) yet. It can be seen in children in endemic areas or in adults moving from countries without leprosy to endemic areas.

The macule is the only skin lesion in indeterminate leprosy (Figs. 10.4 and 10.5):

- A single macule or multiple macules (in which case there are no more than three lesions grouped together).
- No more than 3–4 cm wide.
- Red in light-skinned patients, or coppery in dark-skinned patients. They may also be hypopigmented with a homogeneous decrease in color.
- Permanent.

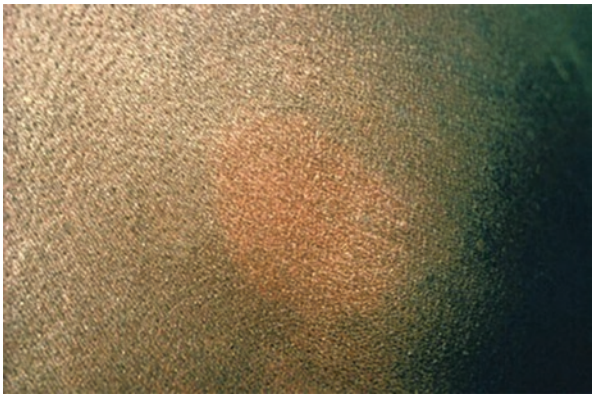

Fig. 10.5 Indeterminate leprosy (I). Coppery macule with normal sensitivity, smooth surface, and ill-defined edges; from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

- Smooth surfaced, no scaling.
- With vague edges.
- Non-pruriginous.
- With normal sweating.
- With normal or slightly decreased thermal and/or tactile sensitivity.
- With normal body hairs.
- Resistant to topical therapies.

In children, cutaneous manifestation is frequently localized on the face, buttocks, and upper or lower limbs. Peripheral nerves are not clinically affected. Acid-fast bacilli (AFB) are absent from skin smear.

Most patients affected by indeterminate leprosy heal spontaneously, while others evolve to a borderline form of the spectrum.

Careful clinical examination can identify changes in the indeterminate macule which can predict evolution onto the disease spectrum [1].

Moving from I to BT leprosy, the macule presents a series of differences, namely, anesthesia and prominent edges. There is also the presence of anhidrosis, which causes rough and dry surface (Fig. 10.6).

The evolution from I to BL leprosy is characterized by central infiltration of the macule.

10.2 Leprosy Spectrum: The Determinate Forms

The determinate forms are characterized by a clinical, bacteriological, and histopathological dichotomy [2]. These enable us to understand the distinction made by the World Health Organization (WHO) into paucibacillary and multibacillary forms of leprosy.

10.2.1 Paucibacillary Forms (Hyperergic) (TT and Skin Smear Negative BT)

These forms show anesthetic macules and plaques with well-defined edges and dry surface, plus papules. Hyperergic forms have a short incubation period compared

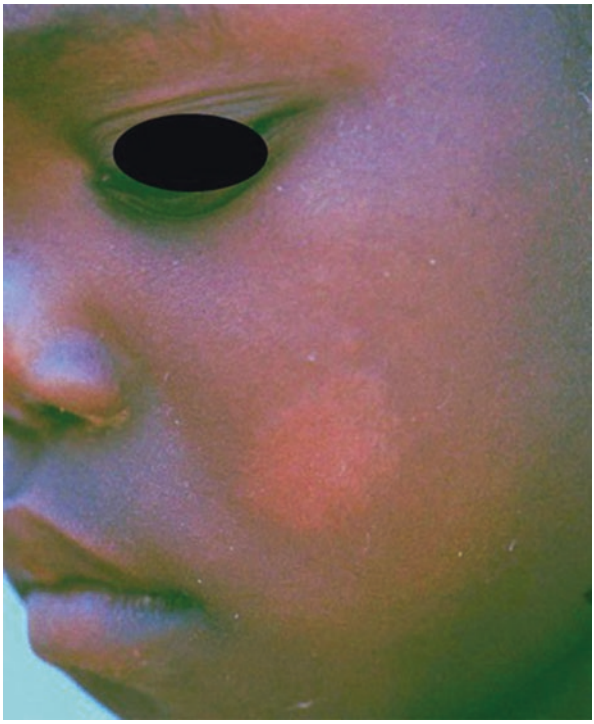

Fig. 10.6 From indeterminate to borderline tuberculoid macule: prominent edges, rough and dry surface, and small satellite lesions

with anergic forms. In fact, in hyperergic patients, the symptoms are caused by early immunological aggression to peripheral nerves and the skin.

10.2.2 Multibacillary Forms (Hypo-anergic) (Smear Positive BT, BB, BL, LLs, LLp)

These forms show macules and plaques with vague edges and smooth surface, nodules, and diffuse infiltration. Sensitivity in lesions is preserved. AFB in lesions are identified on slit-skin smear examination.

10.3 Particular Clinical Features in the Disease Spectrum

10.3.1 Immune Zones

Bacilli survive and multiply best in cooler parts of the body. Axilla, the middle part of the back, groin, inner part of thighs, perineum, and scalp with hair are called "immune zones" because they usually do not present lesions (being warmer parts of the body) (Figs. 10.7 and 10.8).

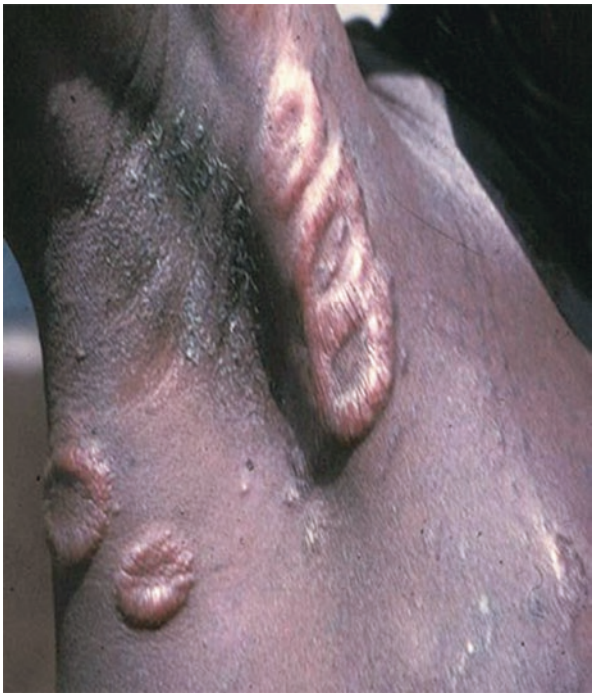

Fig. 10.7 Immune zone (axilla) in BB leprosy. Bacilli survive and multiply best in prominent parts of the body because they are colder. Warmer parts of the body do not present lesions; from Donini P in Nunzi and Leiker (1990) Manuale di Leprologia AIFO-Italia, Bologna

10.3.2 Annular Lesions

In the hyperergic forms (TT, BT), macules or plaques with central healing can be observed. They have a vague inner edge and a well-defined outer edge (TT), or there is an alternation between ill- and well-defined parts (BT) (Fig. 10.9). In the hypoanergic multibacillary forms of the spectrum of leprosy, there are annular lesions which can be seen only in the BB form and have different features, namely, exhibiting a well-defined inner margin, while the external edge is vague as in multibacillary forms (Fig. 10.10).

At diagnosis, most leprosy patients are classified as BT or BL/LL.

Clinical aspects on the spectrum of leprosy may be mono- or polymorphous, according to the type of lesions.

10.4 Tuberculoid Leprosy (TT)

In TT leprosy, the CMI is able to control *M. leprae* dissemination. In some cases, TT may heal spontaneously.

Typical TT cutaneous lesions are macules, papules, and plaques with chronic development.

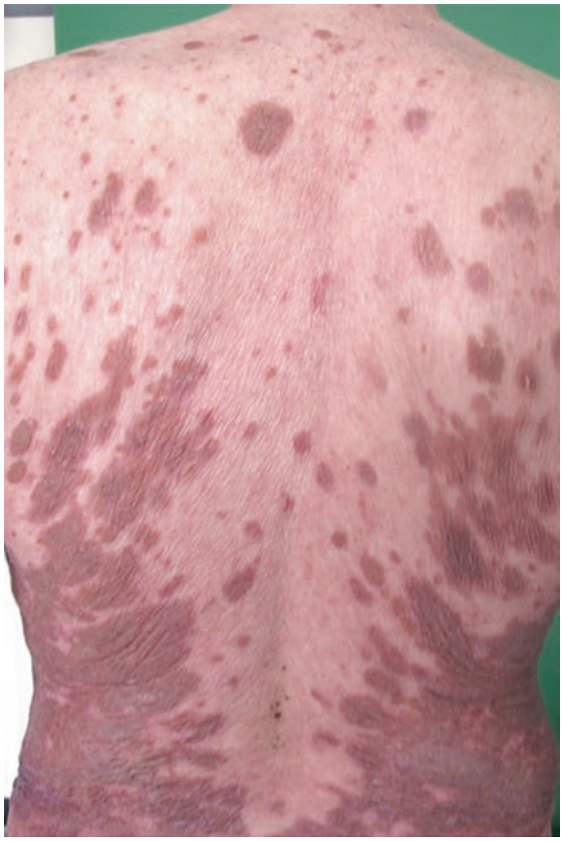

Fig. 10.8 Immune zone in LL leprosy. Distribution of lesions on the back: most of them are localized in the cooler, lateral areas with partial sparing of the middle part

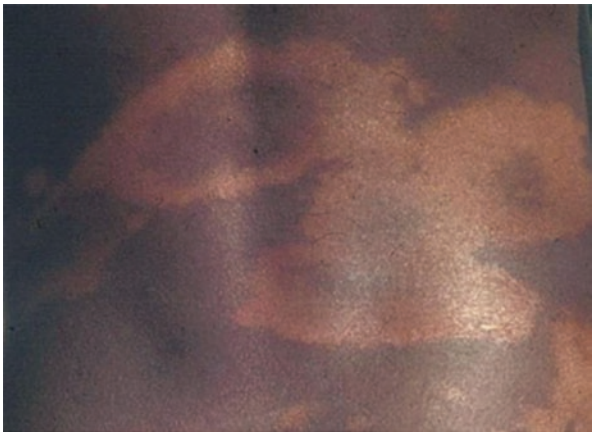

Fig. 10.9 Annular lesions in BT leprosy. Area surrounded by damaged skin

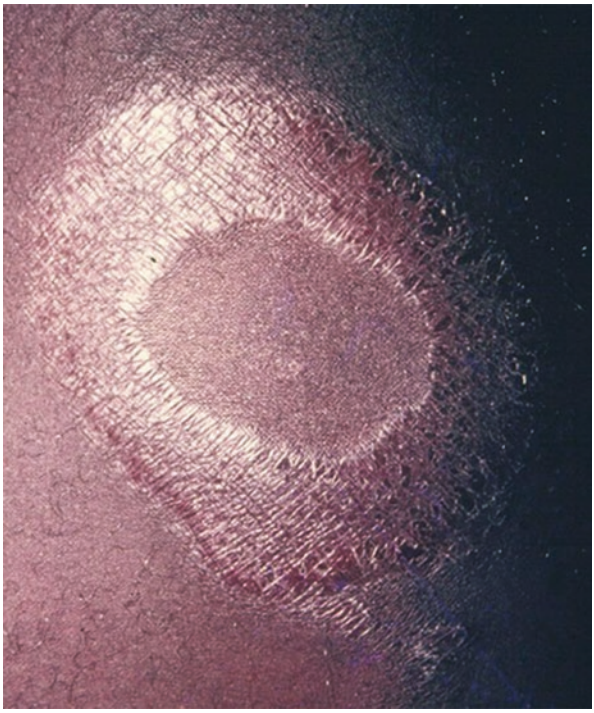

Fig. 10.10 Annular lesion in BB leprosy: "saucer" lesion; from Nunzi and Leiker (1990) Manuale di Leprologia, AIFO-Italia, Bologna

- Skin lesions are few and have asymmetric monolateral distribution (Fig. 10.11).
- Lesions are anesthetic: first thermal and then tactile and pain sensitivity are lost.
- There are no AFB on slit-skin smear or histological examination.
- Peripheral nerves on the same side can be affected (Fig. 10.12).

The macule is the most frequent early lesion in TT (Figs. 10.13, 10.14, 10.15, 10.16) and has the following features:

- Red in light-skinned patients, or coppery in dark-skinned patients; it may also be homogeneously hypopigmented.
- Dry surface and rough to the touch (caused by anhidrosis).
- Well-defined edges.
- Variable in size.
- Decreased sweating.
- Rarefied body hair.

Due to the strong cell-mediated immunity, macular tuberculoid leprosy lesions can:

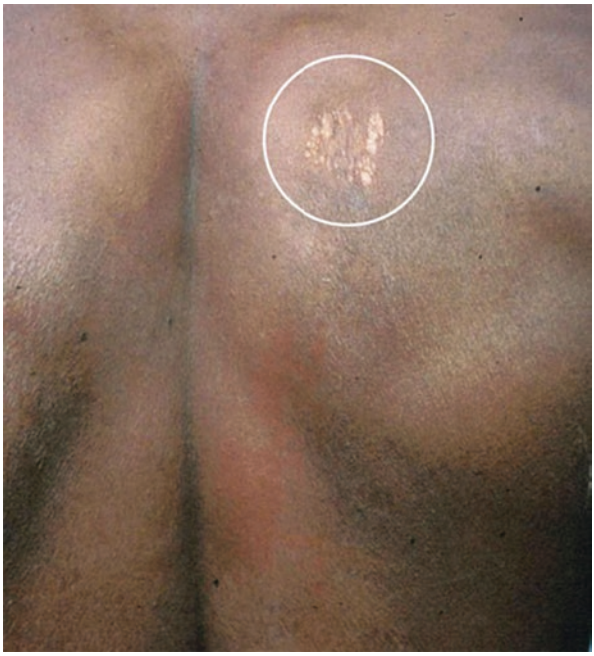

Fig. 10.11 TT leprosy. Single plaque formed by coalescence of papules. Lesion is healing spontaneously from the center. See details in Fig. 10.12

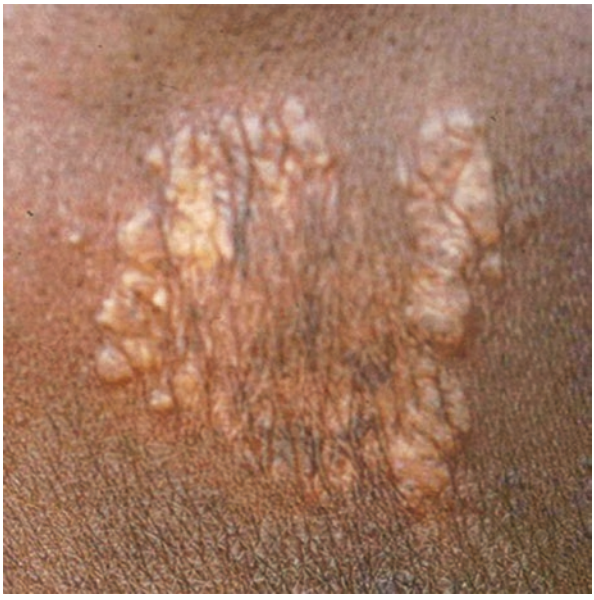

Fig. 10.12 TT leprosy. Plaque and papules. Detail of Fig. 10.11

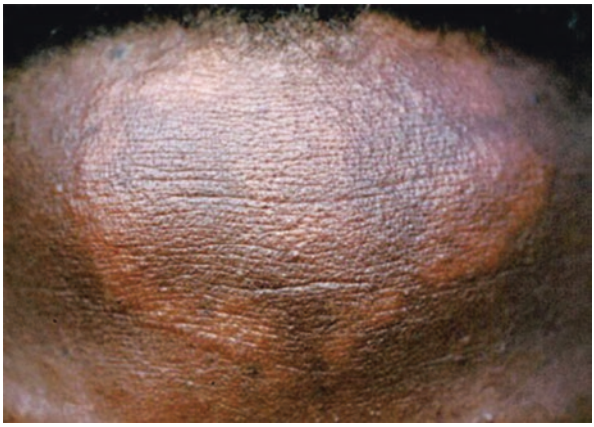

Fig. 10.13 TT leprosy. Annular lesion: the center tends to heal spontaneously, and the skin exhibits a degree of return to normal coloration. Edges are raised, coppery, and well defined

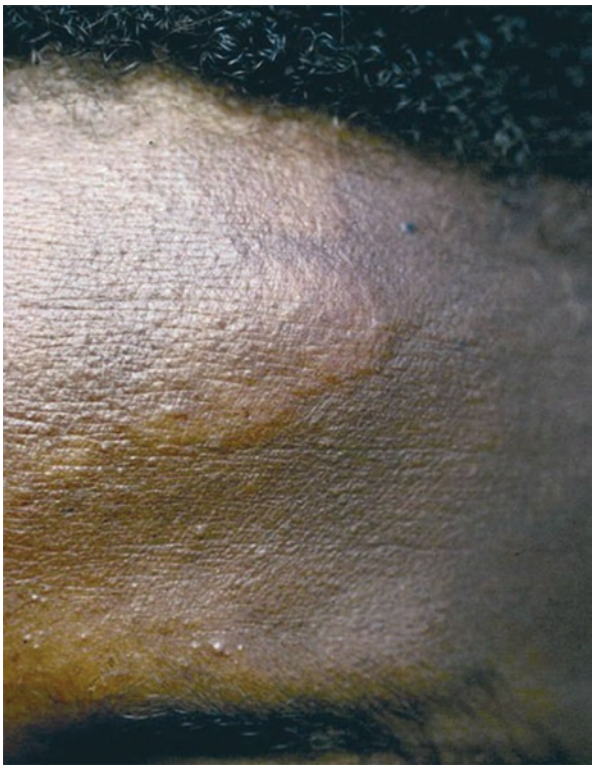

Fig. 10.14 TT leprosy. Detail of Fig. 10.13: raised, well-defined edges

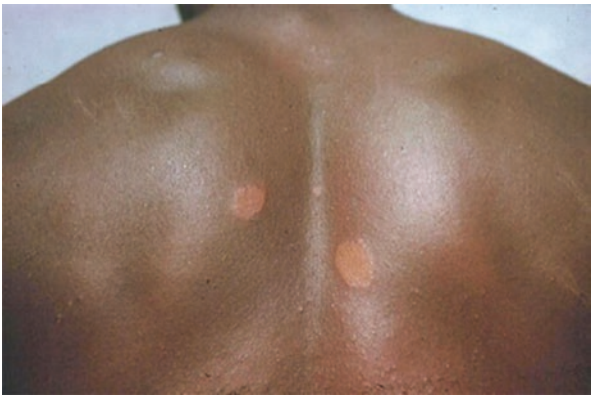

Fig. 10.15 TT leprosy. Anesthetic macules which are near one another, with asymmetric arrangement and well-defined raised edges; from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

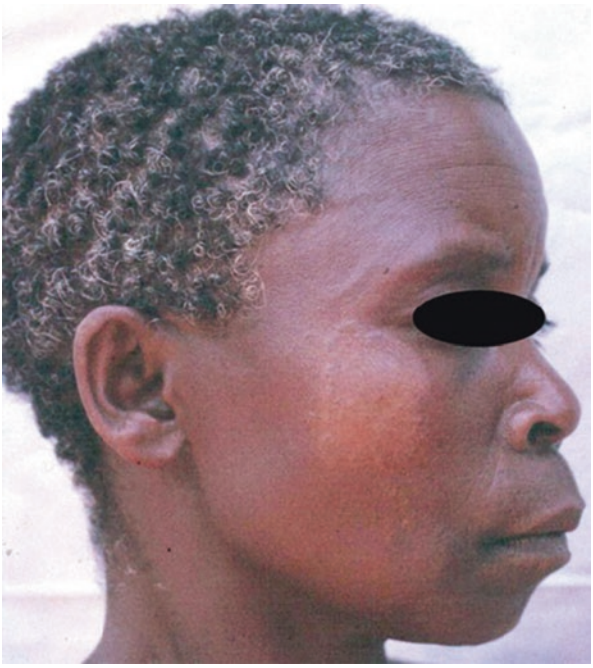

Fig. 10.16 TT leprosy. Single, anesthetic, coppery-colored macule; well-defined edge raised in the upper part of the lesion

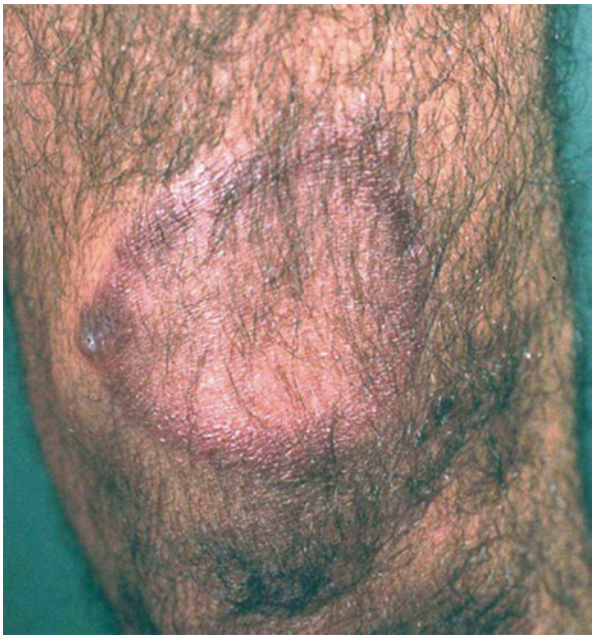

Fig. 10.17 TT reactional leprosy. An anesthetic lesion with features of TT (single lesion, well-defined edges) but also raised edges due to edema and violet color (inflammation), typical of BT leprosy in type 1 reaction. These two aspects classify the patient as having the rare reactional TT or low-resistant TT leprosy, lying between TT and BT in the disease spectrum [3]

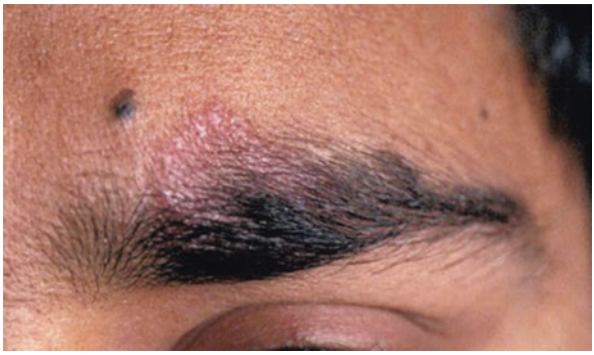

Fig. 10.18 TT leprosy. Erythematous, anesthetic plaque; well-defined margin

- Heal spontaneously from the center, assuming annular shape
- Have a raised edge due to papule development (Figs. 10.13, 10.16, 10.17)

Plaque in TT leprosy (Figs. 10.18, 10.19, and 10.20) is characterized by:

- Red, coppery color, or hypopigmentation.
- Dry surface and rough to the touch (caused by anhidrosis)
- Well-defined margins
- Tactile hypo-anesthesia, which may be difficult to appreciate because of skin thickening
- Absent or rarefied body hair

Healing from the center of the lesion transforms plaques into an annular lesion with raised edges.

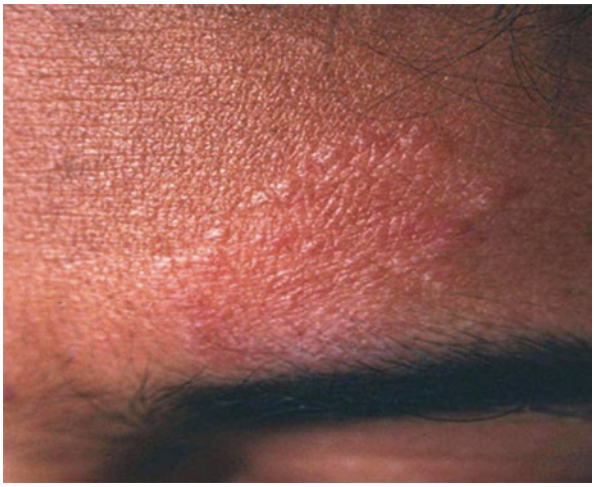

Fig. 10.19 TT leprosy. Single, anesthetic plaque with well-defined edges and rough surface

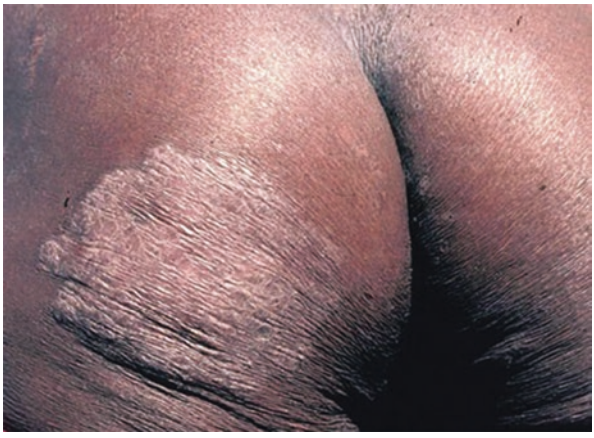

Fig. 10.20 TT leprosy. Large plaque and rough surface

The papule appears either on macule edges due to CMI strengthening (Fig. 10.19), or in groups (Figs. 10.21 and 10.22) which can coalesce into small plaques (Fig. 10.18).

If there are more than three lesions with tuberculoid features and they show bilateral asymmetric distribution, borderline tuberculoid leprosy must be considered.

One nerve can be damaged on the same side as the cutaneous lesion. The involved nerve can show one or more nodules (similar to prayer beads). These can lead to a necrotic process of the nerve that can open up the skin (fistulae), healing with small cutaneous scars.

Search for AFB with skin smear is negative [bacterial index (BI) = 0].

TT lesions heal spontaneously or after treatment, with a restitution of pigmentation, sweating, and sensitivity.

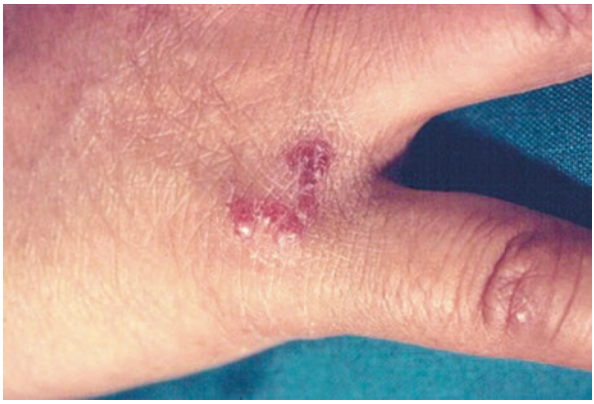

Fig. 10.21 TT leprosy. Group of large papules on the dorsum of the hand; the patient has a nodule in the ulnar nerve of the same upper limb

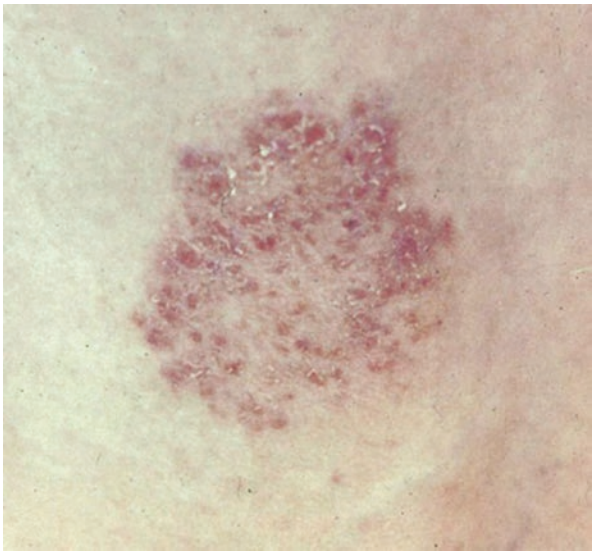

Fig. 10.22 TT leprosy. Multiple erythematous papules, single and with coalescence (Courtesy of DL Leiker)

10.5 Borderline Group

Most of the patients affected by leprosy are classified into the borderline group. According to the Ridley–Jopling spectrum, in the borderline group, we can distinguish three forms.

10.5.1 Borderline Tuberculoid Leprosy (BT)

The BT form includes paucibacillary and multibacillary patients.

Since 1982, the WHO has sequentially adopted various criteria for classification of patients as multibacillary or paucibacillary (see Chap. 6) [4]:

(a) Bacterial index:
Paucibacillary for BI <2+ at any site in initial skin smears
Multibacillary for BI ≥ 2+ at any site in initial skin smears

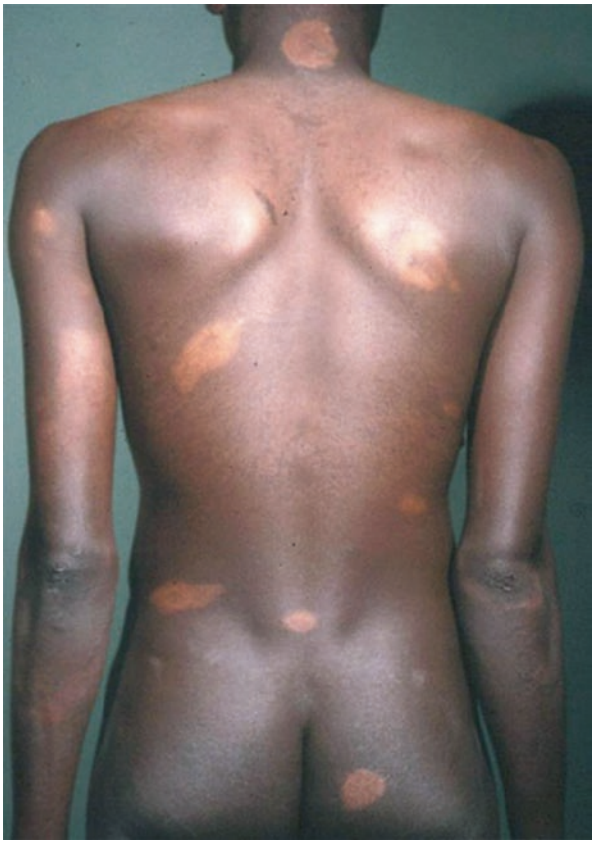

Fig. 10.23 BT leprosy in dark-skinned patient. Asymmetric (bilateral) arrangement in multibacillary BT leprosy (more than five lesions on the body). The patient was treated with paucibacillary regimen and presented relapse 1 year after end of treatment

(b) AFB presence:
All patients showing smear positivity classified as having multibacillary leprosy
(c) Number of lesions on the body:
Paucibacillary cases have up to five skin lesions in total. Multibacillary cases have six or more skin lesions

Correct distinction between those patients (pauci- vs multibacillary) in this form of the disease has important consequences. In fact, if BT multibacillary patients are treated with the treatment regimen of paucibacillary forms, they could relapse.

Clinically, the BT form of leprosy is characterized by:

- Asymmetric (bilateral) arrangement (Figs. 10.23 and 10.24)
- Macules, plaques, and papules

Macules and plaques are characterized by:

- Red/coppery color or hypopigmentation (Figs. 10.23, 10.24, 10.25, and 10.26).

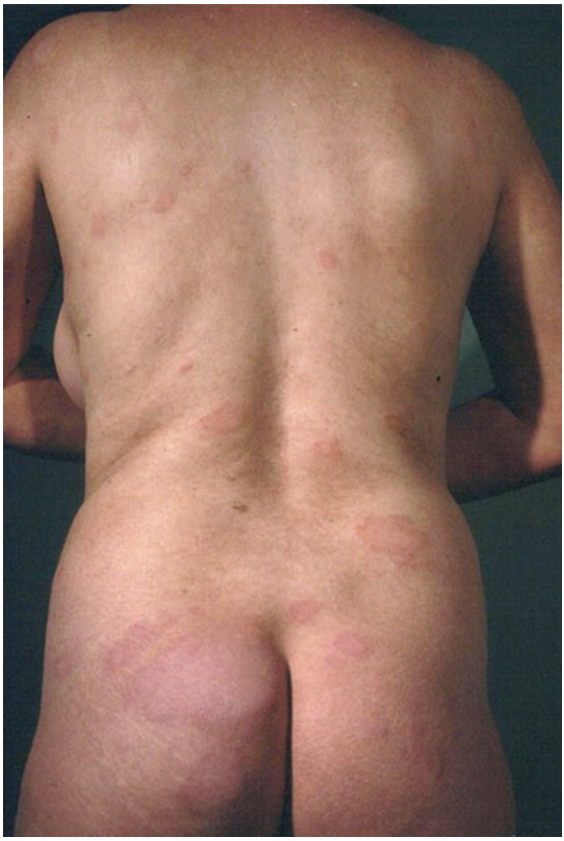

Fig. 10.24 BT leprosy in light-skinned patient. Asymmetric (bilateral) arrangement in multibacillary BT leprosy (more than five lesions on the body)

- Dry surface and rough to the touch (caused by anhidrosis) (Fig. 10.26).
- Edges which are partly vague and partly well defined (Figs. 10.25, 10.26, and 10.27).
- Hyperergy can lead to healing from the center outward, with formation of annular lesions (Fig. 10.27).
- Macules and plaques can be characterized by "finger-shaped" lesions which extend in healthy skin.
- Rarefied body hair.
- Near the larger lesions, there could be small "satellite lesions" (Fig. 10.28, 10.29, 10.30) which show that CMI is not able to limit *M. leprae* spreading. BT macules can reach larger dimensions than the tuberculoid form. These can involve a complete body area (Figs. 10.26, 10.31).

Papules can appear on the borders of the macule as a sign of hyperergic activity. A series of papules with archiform arrangement (Fig. 10.31) sometimes represent what remains of a macule which underwent a self-healing process.

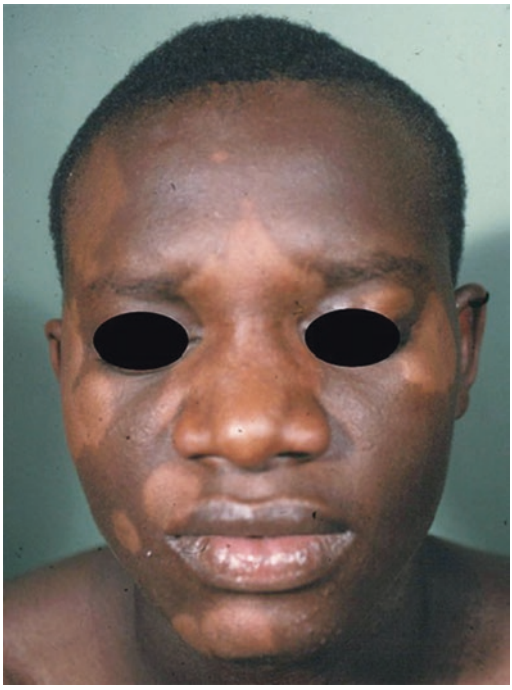

Fig. 10.25 BT leprosy. Bilateral asymmetric macules. Well-defined and vague edges. Satellite lesions

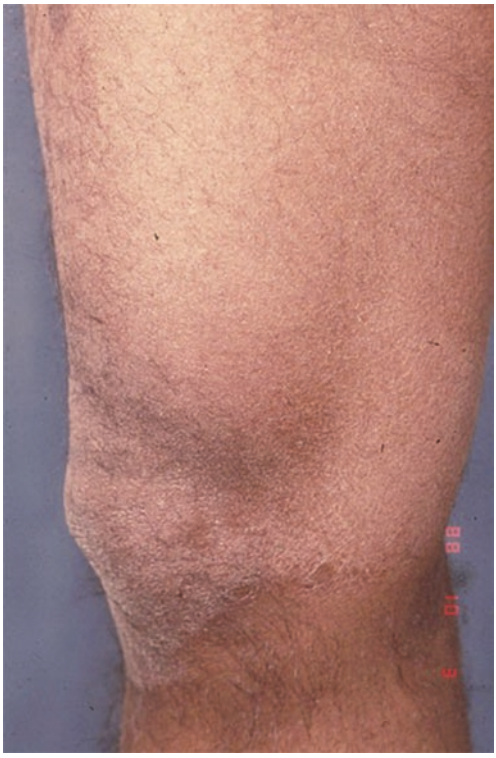

Fig. 10.26 BT leprosy. Anesthetic lesion which involves the thigh. Dry surface and well-defined edges

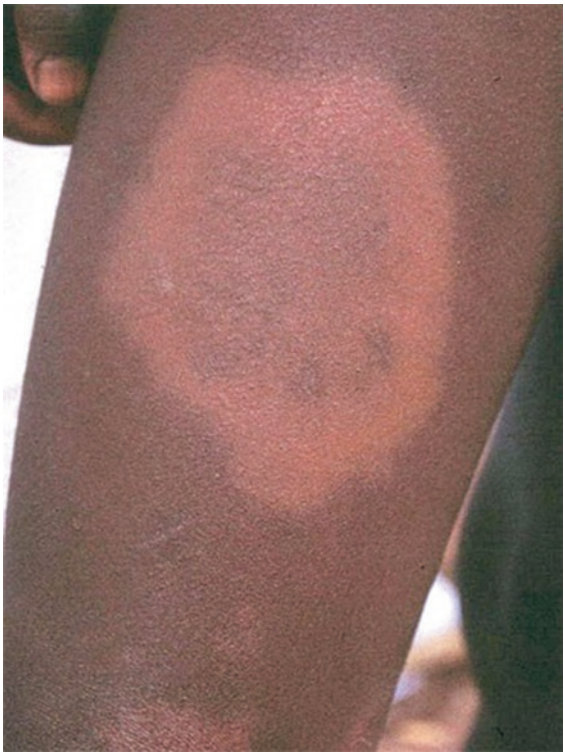

Fig. 10.27 BT leprosy. Annular lesion characterized by central healing (normal skin color) and vague edges

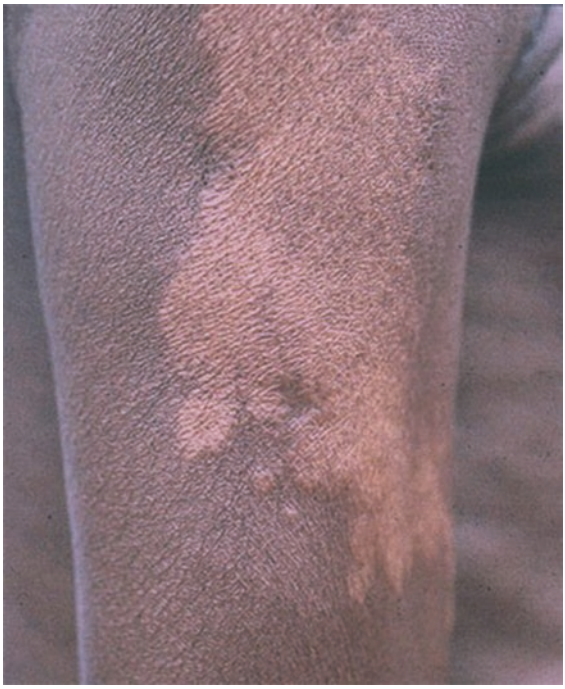

Fig. 10.28 BT leprosy. Satellite lesions near the irregular edges of the main macule

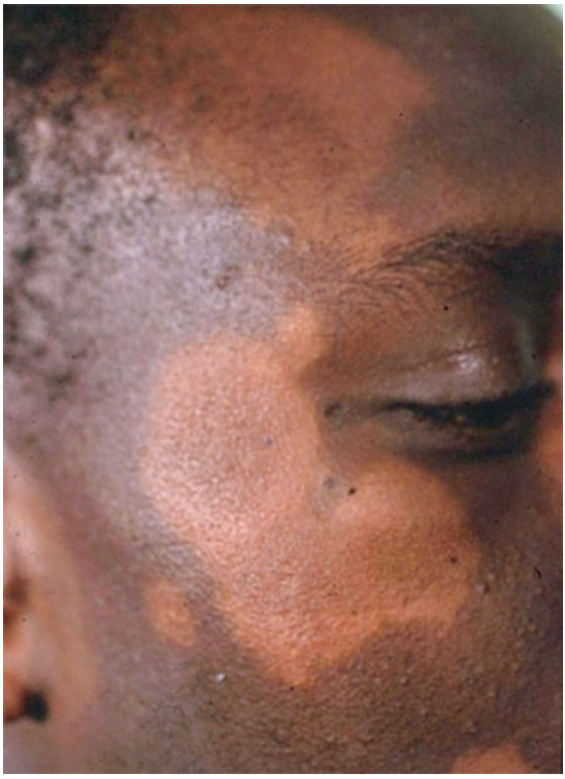

Fig. 10.29 BT leprosy. Note satellite lesions. Well-defined, irregularly shaped hypopigmented lesions with satellites. The distribution of the lesions around the eye carries high risk of damage to the branches of the facial nerve serving the eyelids

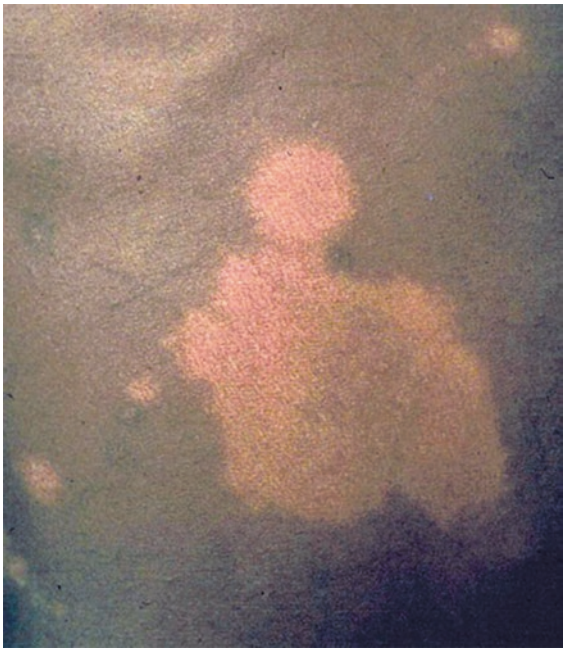

Fig. 10.30 BT leprosy. Satellite lesion along vague edge; from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

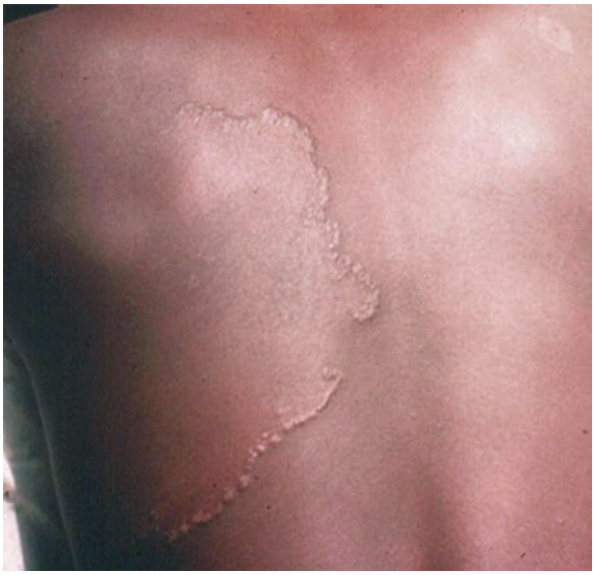

Fig. 10.31 BT leprosy. Series of aligned papules with archiform tendency; Donini P. from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

In BT leprosy, the relationship between CMI and bacterial load can easily change due to killing of bacilli (caused by the patient's immune system or by therapy) with development of type 1 leprosy reaction and corresponding high risk of damage to peripheral nerves. In this form, nerves are damaged with asymmetric bilateral distribution; neuropathies cause swollen fusiform nerves at sites of predilection. There are few reports of BT leprosy cases characterized by skin lesions without affecting peripheral nerves.

Search for AFB on skin smear may be positive with bacterial index (BI) from 0 to 2+.

10.5.2 BT and Spontaneous Movement Along the Leprosy Spectrum

If the BT form goes untreated, it may convert, after a decrease in CMI, toward BL or LLs. Edges of the lesions become vague, and small-sized lesions appear on the body. The initial asymmetric lesion distribution becomes symmetric (Fig. 10.32).

10.5.3 Mid-borderline Leprosy (BB)

In the spectrum, the mid-borderline leprosy form (BB) has the most polymorphic clinical aspect (Figs. 10.33 and 10.34). It shows characteristic lesions with quite symmetric bilateral arrangement: saucer lesions (Figs. 10.10 and 10.35), "Swiss cheese" lesions (Fig. 10.36), and targetoid lesions (Figs. 10.37 and 10.38). The center of the saucer lesions is apparently undamaged and is defined by an inner, well-defined edge, whereas the outer edge is vague. Swiss cheese lesions present

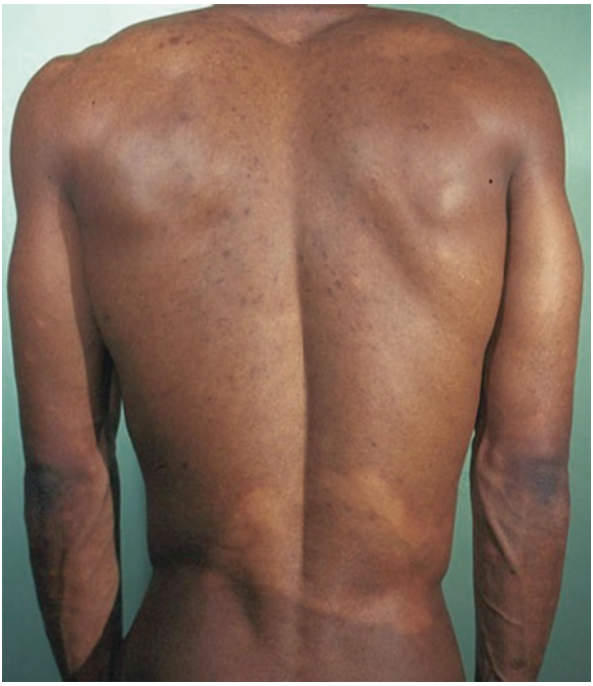

Fig. 10.32 Multibacillary leprosy (BL). Asymmetric large annular macule on the lumbar region (BT). Scattered on the trunk, there are nodules with symmetric bilateral diffusion of BL. The disease began as BT and was downgraded to BL

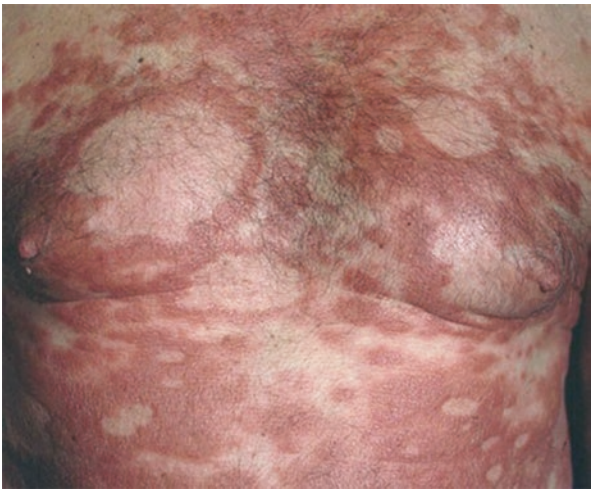

Fig. 10.33 BB leprosy. Multiform, symmetric lesions in BB form in type 1 reaction

edematous plaque, punched out from small undamaged areas, like a small mold with well-defined edges (Figs. 10.34 and 10.36).

BB leprosy is less frequently diagnosed because of its CMI instability. This makes BB leprosy to move rapidly toward one of the two poles of the spectrum (TT or LL). Immunological instability provokes edematous lesions (type 1 reaction) (Figs. 10.34 and 10.36). BB lesions are macules and plaques with symmetric bilateral distribution.

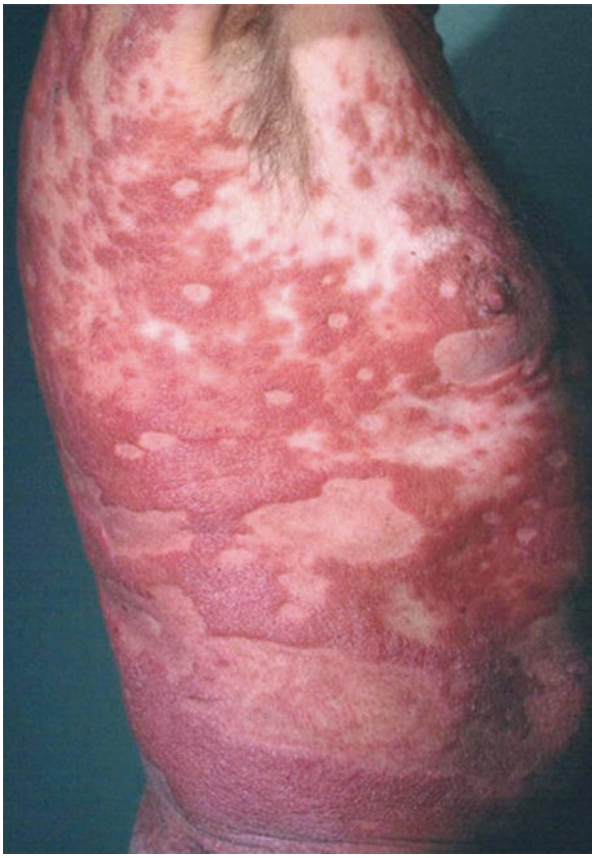

Fig. 10.34 BB leprosy. Multiform clinical aspect in BB patient with type 1 reaction

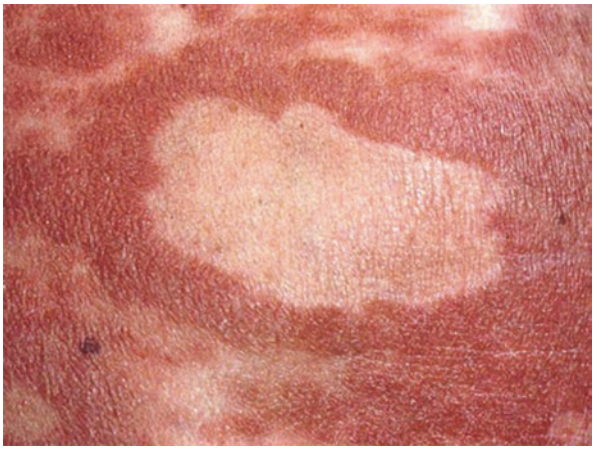

Fig. 10.35 BB leprosy. Saucer lesion with well-defined inner edge. The outer edge is otherwise vague

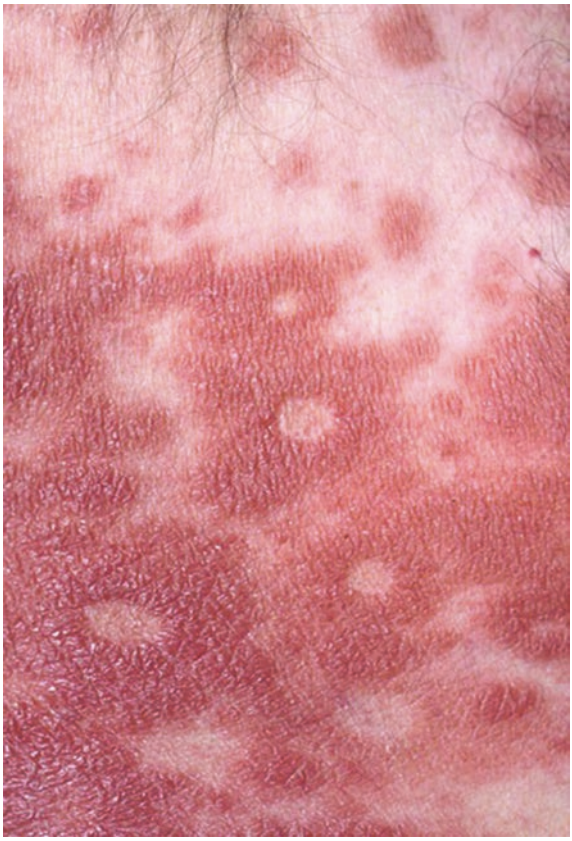

Fig. 10.36 BB leprosy. Swiss cheese lesions

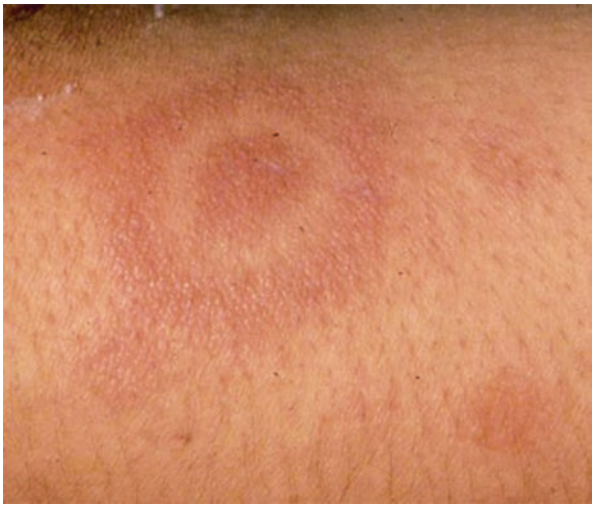

Fig. 10.37 BB leprosy, targetoid lesion

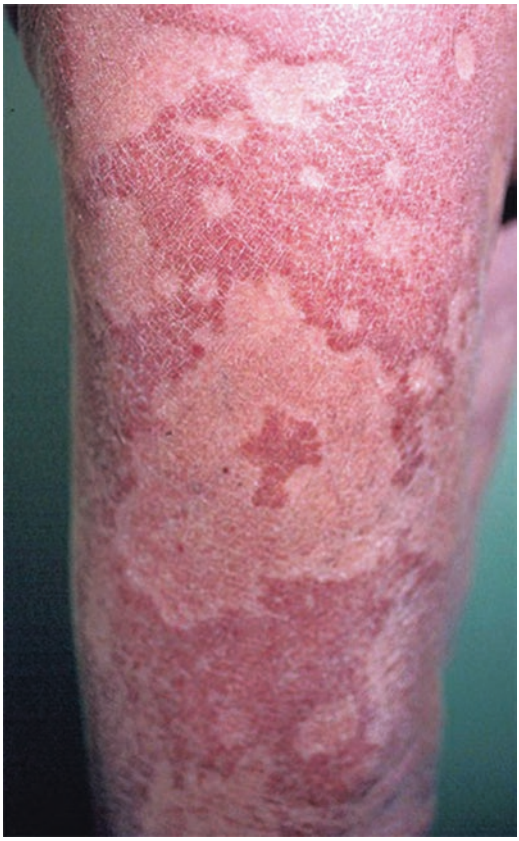

Fig. 10.38 BB leprosy, targetoid lesion in subsiding type I reaction

- Red or coppery color and violaceous in type 1 reaction in light-skinned people
- Extremely variable size (Fig. 10.33)
- Vague edges (Figs. 10.33 and 10.34)

Peripheral nerves are involved in this form with disease evolution. Reactional episodes are frequent in BB. Skin smear presents large numbers of solid AFB. Bacterial index can vary from 2+ to 4+.

10.5.4 Borderline Lepromatous Leprosy (BL)

Lesions of the initial stage of this form are rather grouped together (Fig. 10.39).

M. leprae diffusion on the skin is not limited by CMI, and lesions increase in number, assuming a bilateral symmetric pattern (Figs. 10.40, 10.41, 10.42, and 10.43) which is not as pronounced as can be observed in LLs.

The clinical aspect can be made up of macules (Figs. 10.40 and 10.41), plaques (Fig. 10.42), and nodules (Figs. 10.43 and 10.44).

BL macules have:

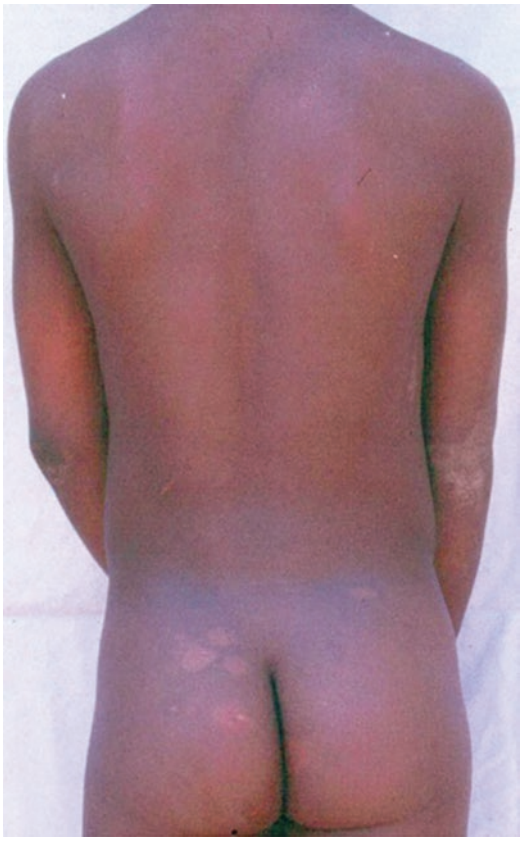

Fig. 10.39 BL leprosy. Early aspect: small macules, some grouped, with ill-defined margins on the buttocks

- Red or coppery color, or slight hypopigmentation (Figs. 10.40 and 10.41)
- Vague edges (Fig. 10.40)
- Small size (Figs. 10.39, 10.40, 10.41, and 10.42)

Macules can enlarge and become infiltrated in the center, changing into plaques with vague edges and dome shape.

The clinical aspect can also exhibit presence of nodules that are erythematous/coppery color or normal skin color (Fig. 10.43), with sharp edges (Fig. 10.44). Sweating remains normal for a long time. Lesion surface is smooth. In advanced stages, there is thinning out of hairs. Lesion arrangement is not as clearly symmetric as in the LL form.

In BL leprosy, peripheral nerves are involved earlier than in the LL form. At the site of predilection, peripheral nerves reveal a tapering shape, thickening on palpation. In BL forms resulting from evolution of BT lesions, there is important nerve involvement, distributed asymmetrically. Lesion arrangement is not as clearly symmetric as in LL. Skin smear carried out on lesions reveals a large amount of bacilli,

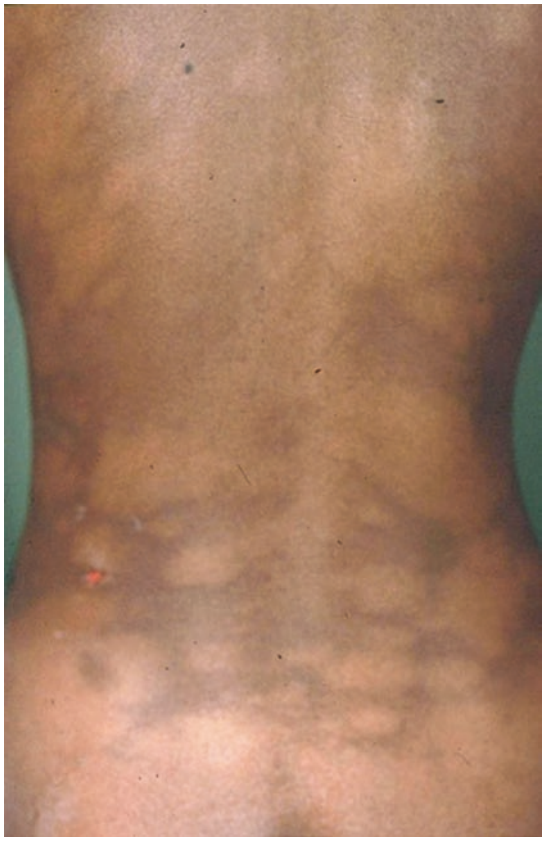

Fig. 10.40 BL leprosy. Bilateral distribution of macules, smooth surface, and vague edges, being less symmetric than macules in LLs because of the smaller number of lesions (BI 5+)

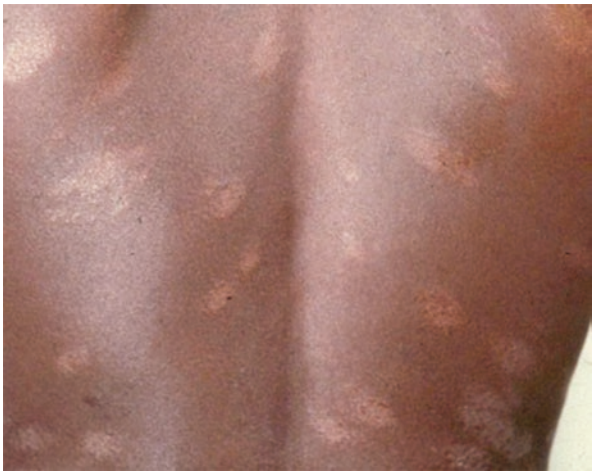

Fig. 10.41 BL leprosy. Macules: symmetric bilateral distribution. The warmer, paravertebral region is spared of lesions. Sensitivity in lesions is maintained

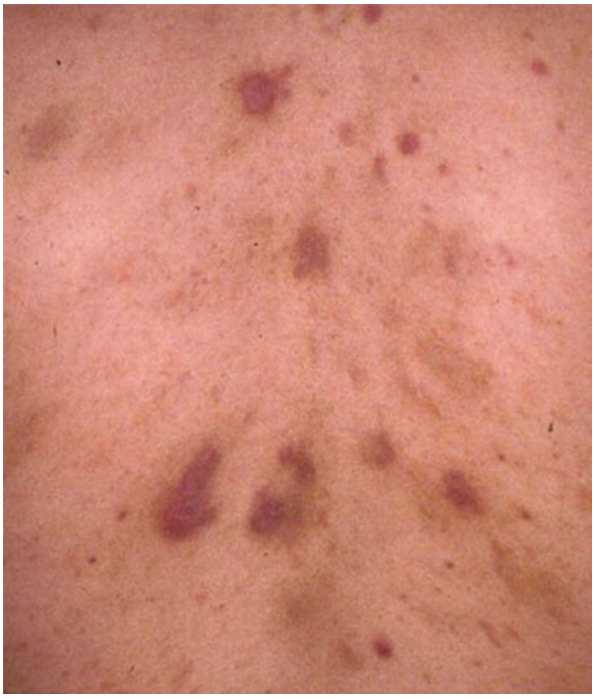

Fig. 10.42 BL leprosy. Plaques with vague edges, with symmetric, bilateral distribution

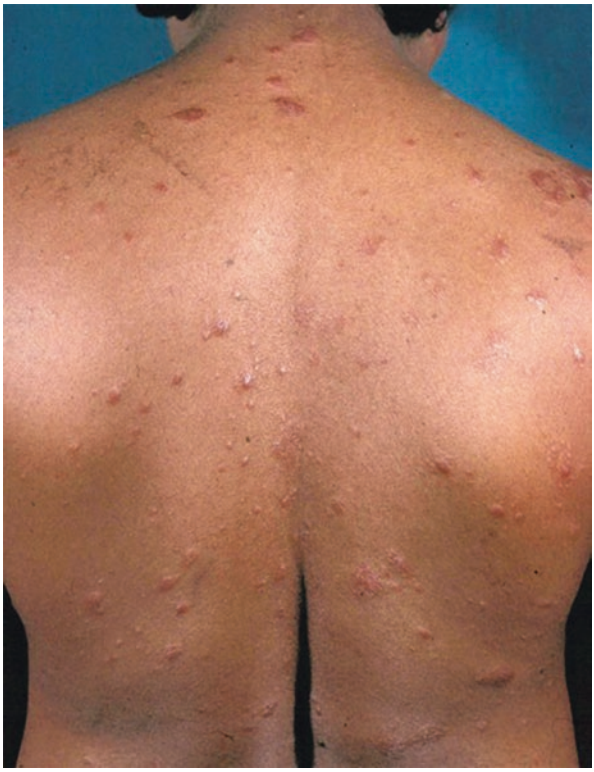

Fig. 10.43 BL leprosy. Nodules with symmetric bilateral distribution, being not as numerous as in LLs (BI 4, 5+)

Fig. 10.44 BL leprosy. Nodules on helix are clearly defined, while in the LLs form, they are vague; from Nunzi and Leiker (1990) Manuale di Leprologia. AIFO-Italia, Bologna

which are sometimes arranged in small globi. The bacterial index varies from 4+ to 5+. In the early phases of disease, nasal smear (swab) is negative but becomes positive in advanced disease.

10.5.4.1 Lepromatous Leprosy (LL)

Anergy enables hematogenous spread of *M. leprae* on the skin as well as in internal organs and determines lesion arrangement and morphology: symmetric bilateral distribution of lesions (Fig. 10.45) sparing warmer areas of the body (axilla, groin, and perineum). Even in areas which are clinically undamaged (known as immune zones), it is possible to find AFB.

The body carries a large bacillary load over long periods, and the first symptoms appear after a long incubation period. The prodromal stage is aspecific with mild symptoms (see Chap. 7). Onset can be acute with type 2 reaction symptoms.

In the lepromatous form, there are two clinical subforms: subpolar (LLs) and polar (LLp). This distinction has practical importance, because patients suffering from LLs are not completely anergic. Under treatment, they can reach a certain degree of CMI and can develop type 1 reaction. Moreover, under treatment, LLs becomes bacteriologically negative in a shorter time compared with the LLp form.

Subpolar lepromatous leprosy (LLs) is characterized by presence of macules, nodules, and plaques which maintain their individuality. LLs can manifest as such from the beginning (Figs. 10.45, 10.46, 10.47, 10.48, and 10.49) or represent the final evolution of borderline forms after a worsening process.

Polar lepromatous leprosy (LLp) appears in extremely anergic patients and begins by affecting the skin of one body region with diffuse infiltration, with indistinct limits.

This clinical aspect is clear on the forehead, where skin folds are more evident, on zygomas, and on the ear. This diffuse infiltration provokes a typical clinical aspect called facies leonine (Fig. 10.50).

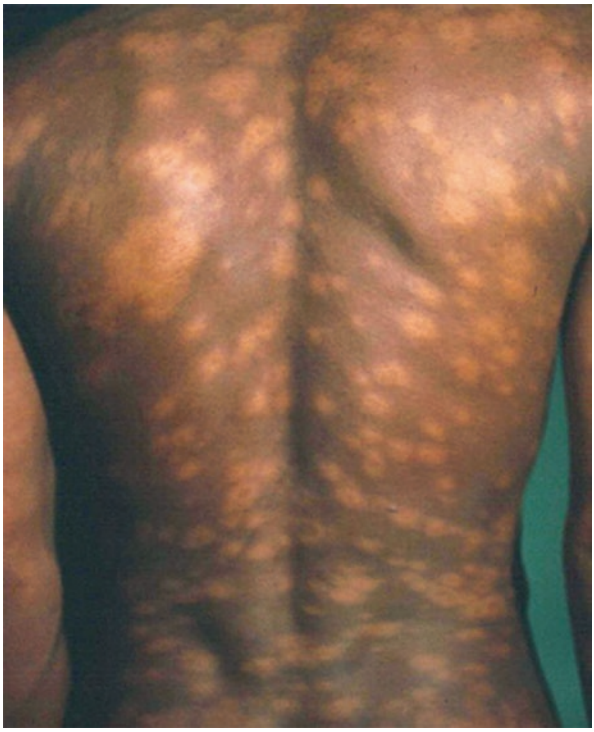

Fig. 10.45 Lepromatous leprosy (LLs). Symmetrical arrangement; a large BT macule on the left side of the back, small annular lesion (BB), and small macules (LLs) with vague edges. These different lesions show the natural history of the patient's disease: beginning with BT, through BB, ending in LLs; from Massone and Nunzi (2009) Note: di leprologia. AIFO-Italia, Bologna

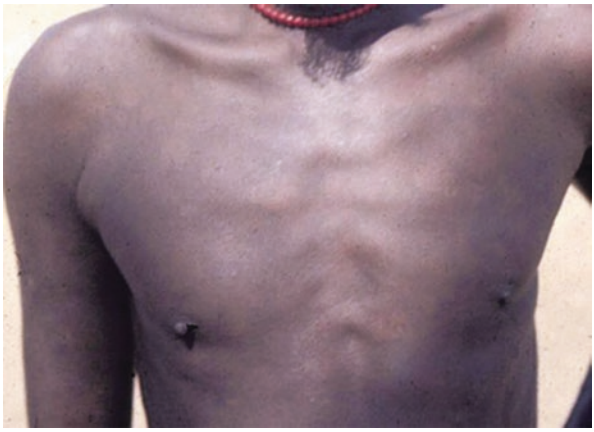

Fig. 10.46 Lepromatous leprosy (LLs). Small, prelepromatous, coppery macules with vague edges and smooth surface; from Massone and Nunzi (2009) Note: di leprologia. AIFO-Italia, Bologna

At the lepromatous pole, there can be macules, nodules, plaques, and diffuse infiltration.

The disease onset can have a mono- or polymorphous aspect. In a small percentage of cases, lepromatous leprosy begins with a prelepromatous phase characterized by small macules with vague edges scattered in a symmetric pattern on the skin. They can be more easily seen with sidelight on the skin (Fig. 10.46).

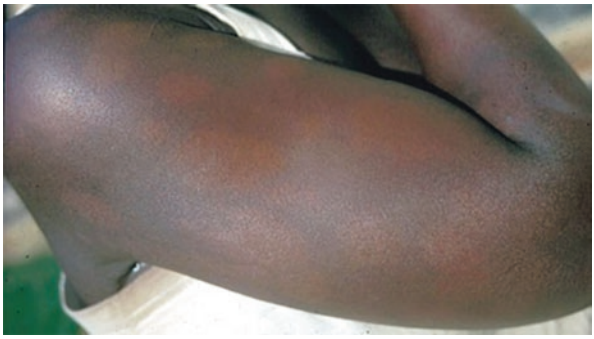

Fig. 10.47 Lepromatous leprosy (LLs). Coppery macules, vague edges, smooth surface, and preserved sensitivity; from Nunzi and Leiker (1990) Manuale di leprologia. AIFO-Italia, Bologna

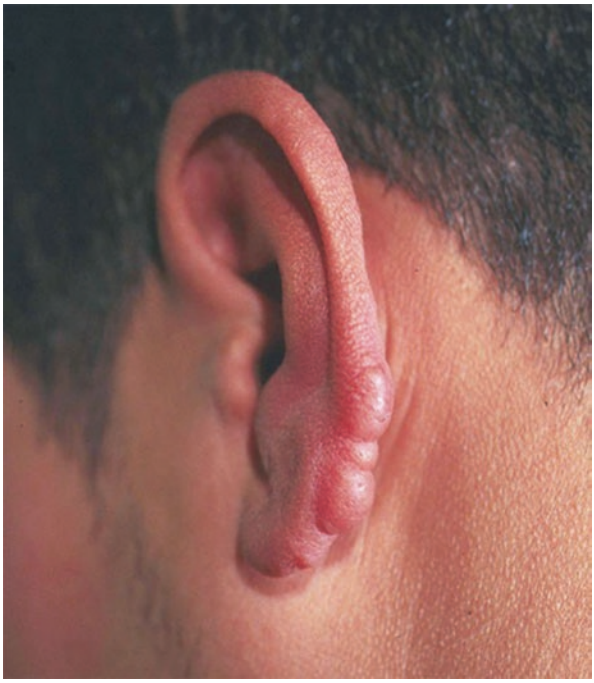

Fig. 10.48 Early lepromatous leprosy (LLs). Erythematous nodules in helix (cold part of the body)

Early LL lesions do not show alterations due to involvement of the cutaneous nerve bundles, and they retain sensitivity and sweating.

Macules (Fig. 10.45, 10.46, and 10.47) of LL are characterized by:

- Symmetrical arrangement
- Red or coppery color or slight hypopigmentation
- Smooth, shiny surface
- Vague edges
- Small size
- Presence of sensitivity and sweating

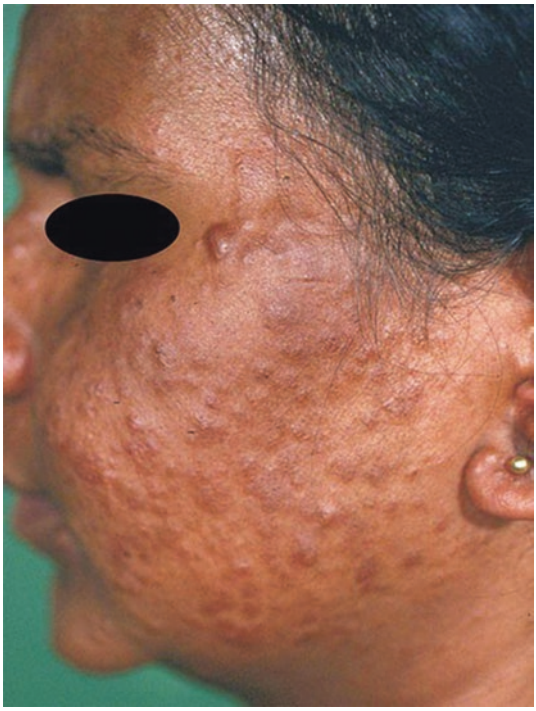

Fig. 10.49 Lepromatous leprosy (LLs). Multiple nodules with different dimension on the face

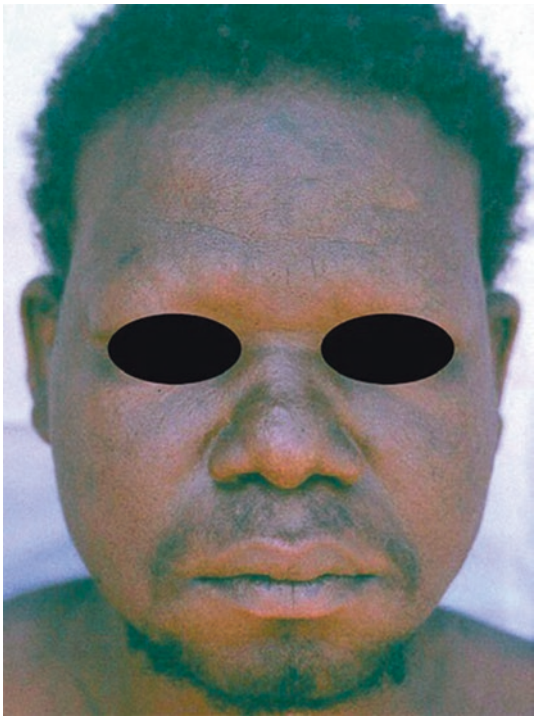

Fig. 10.50 Polar lepromatous leprosy (LLp). Diffuse infiltration on the skin of the face, madarosis, and nasal pyramid collapse (facies leonine)

During progression of the disease, macules can infiltrate and develop into plaques.

Nodules (Figs. 10.51 and 10.52) above the skin surface can represent the onset of the disease, or they can appear after the appearance of macules. They are characterized by:

- Red or coppery or normal skin color
- Vague edges
- Hard consistency

In polar lepromatous leprosy (LLp), the infiltration can spread to forearms, the back of hands, and extensor and lateral surfaces of the legs. In untreated patients, there is progressive body hair loss and appearance of hypo-anhidrosis in involved areas. Madarosis is a typical aspect which begins from the external part of the eyebrow and can involve eyelashes (Figs. 10.50 and 10.51).

Nervous trunk damage is slow and appears later compared with skin lesions. In advanced forms, it appears as a symmetrical, bilateral polyneuritis. Heavy destruction of nervous skin bundles leads to anesthesia in limbs (glove/stocking anesthesia). Anesthetic areas can cover the whole body with the exception of the warmer regions: axilla, groin, or scalp covered with hair.

At sites of predilection, peripheral nerves present uniform swelling. In later phases, nerves take on the consistency of a hard cord. LLs patients who are downgrading from the BT form can have peripheral nerve lesions which maintain the same features as the initial form (asymmetric and serious).

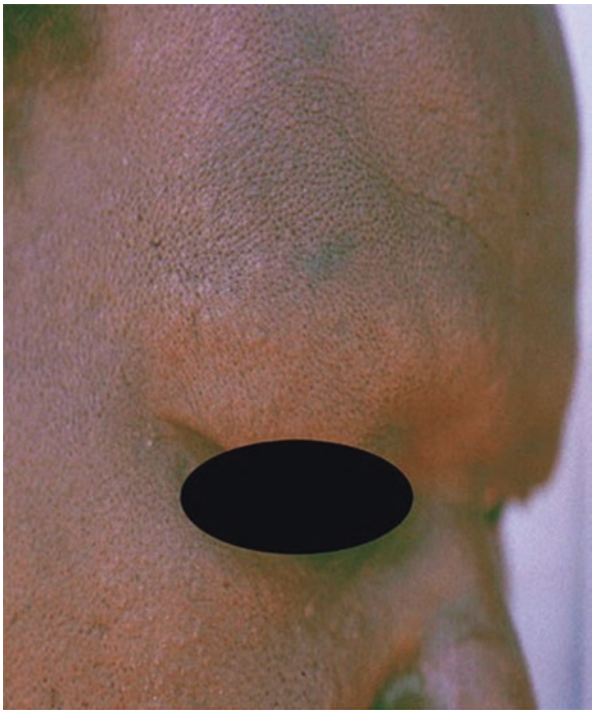

Fig. 10.51 Polar lepromatous leprosy (LLp). Madarosis

In advanced forms of lepromatous leprosy, high incidence of upper respiratory tract involvement (as high as 80%) can be observed, with high bacillary invasion which can lead to destruction of bones of the nasal pyramid (Figs. 10.50 and 10.52c) or the alveolar processes, with loss of superior incisors and perforation of the hard palate. Edema in lower limbs is usually observed in advanced forms.

M. leprae in LL patients is also localized in internal organs. In skin smears done on lesions or on cooler body regions (earlobe, elbow, back of finger, knee), there are enormous quantities of AFB. Bacterial index is 6+ (the maximum of the scale), and there are large-sized hyperchromic globi.

10.6 Unusual Clinical Aspects

10.6.1 Pure Neuritic Leprosy

This type of leprosy is characterized by symptoms caused by *M. leprae* localization in peripheral nerves. Cutaneous lesions are absent (see Chap. 26).

Symptoms and signs are sensorial deficit, muscular weakness, paralysis, and subjective symptoms represented by burning sensation, anesthesia, and shooting pains.

In late untreated pure neuritic leprosy, the distal part of the limbs can present deformities.

Mononeuritic involvement is more common. In the affected nerves, nerve abscess can occur.

In this particular form, biopsy must be done to formulate a diagnosis [5]. To avoid irreversible paralysis, only sensory nerves should be biopsied: the sural nerve and the superficial radial nerve. Before performing the biopsy, the physician must obviously verify that these two nerves have been damaged by *M. leprae* using sensory or neurophysiologic tests.

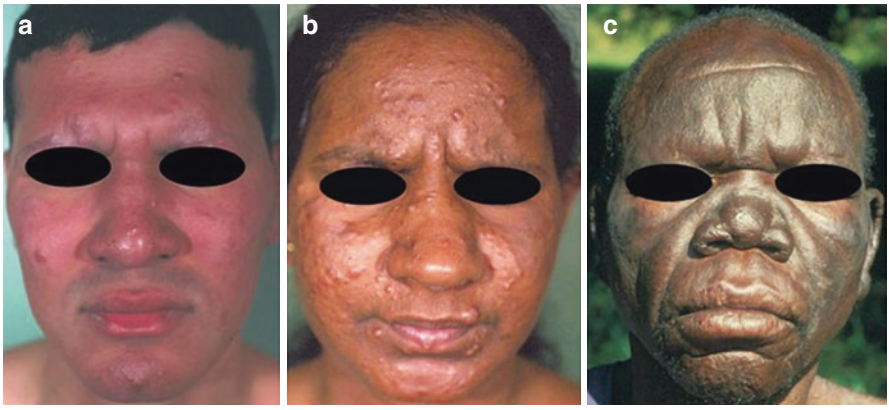

Fig. 10.52 Facies in lepromatous leprosy. (**a**) Early subpolar lepromatous leprosy; there are nodules in cooler parts of the face and a slight infiltrated skin. (**b**) Subpolar lepromatous leprosy; there are several nodules with bilateral arrangement. Rarefied eyebrows on the external part. (**c**) Polar lepromatous leprosy; there is collapse of the nasal pyramid and madarosis. The patient is under MDT treatment (Note: clofazimine pigmentation); from Nunzi E, Leiker DL (1990) Manuale di Leprologia, AIFO-Italia, Bologna

Histopathology of involved nerves enables classification of most patients into the paucibacillary part of the spectrum (TT or BT) or more rarely multibacillary forms.

Pure neuritic leprosy must be distinguished from the usual leprosy forms, having a neuritic onset not associated with skin lesions. After months or years, a certain number of these patients develop skin lesions [6].

10.6.2 Lepromatous Leprosy Characterized by a Single Nodule or Group of Nodules

Lepromatous leprosy cases characterized by a single nodular lesion have been reported [7]; the diagnostic possibility is usually suggested by histology and confirmed by the report of numerous AFB scattered or arranged in globi. Differential diagnosis considers atypical mycobacteriosis. In dubious cases, it is necessary to carry out in vitro cultural tests for AFB or to characterize mycobacteria by polymerase chain reaction (PCR). In literature, cases of leprosy with nodules on one-half of the face or thorax have been reported [8, 9].

10.7 Wade's Histoid Leprosy

This is clinically characterized by firm hemispherical nodules, with well-defined edges and smooth bright surface (Figs. 10.53 and 10.54). It has been called "histoid" due to the histological similarity with dermatofibroma (presence of spindle-shaped

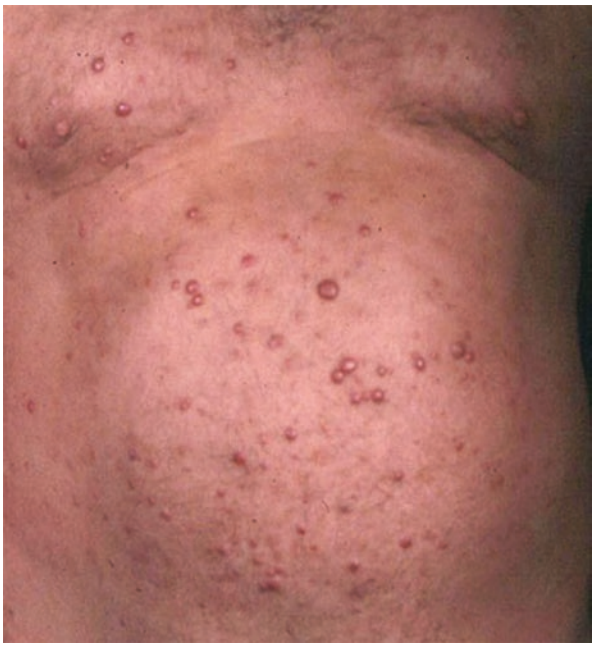

Fig. 10.53 Lepromatous leprosy, Wade's histoid type. Scattered nodules

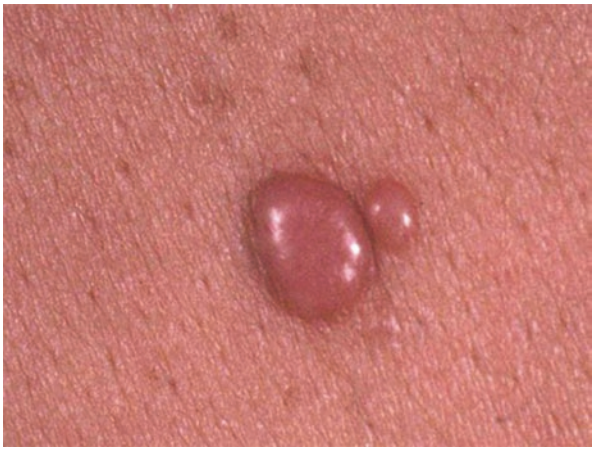

Fig. 10.54 Wade's histoid type. Detail of nodules: well- defined edges. Note: that the LLs nodules have vague edges

cells) [10, 11]. Histoid nodules can also represent the first sign of LLs leprosy. When monotherapy with sulfones was in use, appearance of histoid lesions in treated LLs patients could represent the first clinical sign of sulfone resistance [12].

When numerous, nodules have symmetric distribution, possibly clustering in groups.

Bacterial index determined on slit-skin smear taken from this lesion is high (5+/6+).

10.8 Anticardiolipin Antibodies' Conditioning Aspects of Lepromatous Leprosy

During the nineteenth century, Mexican doctors identified an acute phenomenon, the Lucio phenomenon (LPh), in patients affected by diffuse lepromatous leprosy (DLL or Lucio–Latapí leprosy; see Chap. 22), clinically characterized by the occurrence of painful, red, or purpuric macules, of irregular shape, angulated or "stellar" on the lower extremities, upper extremities, and the trunk. Severe cases shows cutaneous thrombosis and necrosis leading to amputation of fingers (Fig. 10.55). Histopathology of LPh shows a necrotizing panvasculitis.

Reports [13, 14] have shown the presence of anticardiolipin antibodies in patients affected by LPh. Moreover, histopathological changes typical of antiphospholipid antibody syndrome with endothelial cell proliferation, thrombosis, a mild mononuclear cell infiltrate, and ischemic necrosis have been reported in LPh. These evidences raised the hypothesis that the thrombotic manifestations characterizing the LPh may be linked also to antiphospholipid antibody syndrome.

Antiphospholipid antibodies have been found to be increased in leprosy (mainly in MB patients), in different studies, and without differences between leprosy patients not under therapy, taking MDT or those who have completed MDT. However, lepromatous leprosy do not usually present with antiphospholipid antibody syndrome, and gangrene of the extremities in leprosy has been reported to be caused

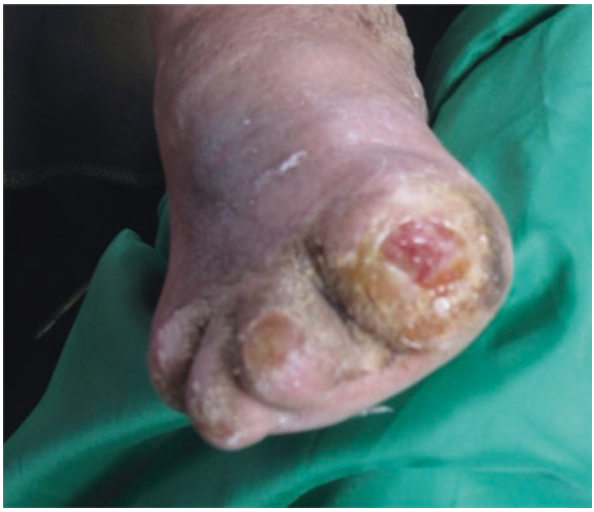

Fig. 10.55 Lucio's phenomenon, toe amputation

also by intimal thickening and medial infiltration, embolization and resultant grafting of Virchow cells, and arterial entrapment due to nerve trunk hypertrophy in the osteoligamentous canal [14, 15].

10.9 Recrudescence

This term indicates clinical and/or bacteriological aggravation of a patient under treatment. An increase by one or more units in the bacterial index shows insufficient therapeutic effect.

Clinically, this can be seen with reactivation of preexisting lesions and appearance of new lesions. In paucibacillary patients, distinction between recrudescence and type 1 reaction can be difficult. In multibacillary patients, differential diagnosis must consider erythema nodosum leprosum (ENL).

Recrudescence may result from irregular intake of drugs or selection of *M. leprae* bacilli resistant to the drugs in use [16].

10.10 Relapse

Relapses are due to the appearance and increase of bacilli after treatment.

Causes of relapse are:

- Wrong classification of a multibacillary patient with consequent prescription of inadequate treatment
- Insufficient chemotherapy due to irregular drug intake
- Persistent bacilli (persisters) that are metabolically inactive or localized at sites where they are not reached by drugs

- Reinfection, in theory possible in multibacillary anergic cases
- Immunosuppressive therapy for other diseases

10.10.1 Relapsing Cases in Multibacillary Leprosy

Clinically relapsing cases are represented by reappearance of lesions which had disappeared under treatment or by appearance of symmetrically distributed new lesions (nodules). For this reason, it is important to record the exact location of lesion in patient's clinical files. Bacilli reappear in lesions with "solid" morphology.

The differential diagnosis between relapse and reversal reaction is based on bacteriological examination. In reversal reaction, the bacterial index is unchanged and the morphological index remains zero.

10.10.2 Relapsing Cases in Paucibacillary Leprosy

There is reactivation of preexisting lesions and/or appearance of new lesions. Relapsing cases and reversal reactions are so similar that is difficult to distinguish between them in differential diagnosis. Onset in reversal reactions is generally more acute than in relapse.

Bacteriological examination cannot give helpful information because it is negative in paucibacillary patients. Also, histopathology can hardly distinguish between relapse and reaction (see Chap. 12).

To reach a differential diagnosis, an empiric approach is needed by giving corticosteroid (diagnosis ex juvantibus): reversal reactions improve quickly with corticosteroid therapy, while relapses do not. It must be underlined that relapsing cases can be associated with reversal reactions. In this case, steroidal therapy gives some results, but it is not able to heal lesions.

10.10.3 Relapsing Cases With Acute Appearance

Relapse appears acutely with type 2 leprosy reaction.

10.10.4 Relapsing Cases With Neuritic Symptoms

In either multi- or paucibacillary forms, the first manifestation can be exclusively neurological. Patients report paresthesia and an increase of anesthetic skin areas which are innervated by damaged nerves; nerves can be swollen, painful, and tender. In these cases, differential diagnosis between relapsing cases and reversal reactions is clinically difficult. Moreover, similar symptomatology can be caused by nerve fibrosis or fibrosis of nerve sheath. Diagnosis of relapse is aided by the presence of solid *M. leprae* in biopsy carried out on the nervous trunk involved.

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Lucio–Latapí Leprosy

11

Mario Magaña

Among the protean clinical and histopathological features of leprosy, diffuse lepromatous leprosy is a multibacillary systemic disease, with multiple organ involvement, and with a negative intradermal reaction to the Mitsuda test (or lepromin skin test, which in the past, it was used as an aid to classification of cases). It presents as a diffuse variant, in which there are no circumscribed elements, i.e., nodules or lepromas, macules, or plaques, but which is characterized by diffuse and massive infiltration of the skin, known as diffuse lepromatous leprosy (DLL) or Lucio–Latapí leprosy. This is a distinct form of multibacillary leprosy reported mainly in Mexico, where it represents 15%–23% of all leprosy diagnoses [1]. Nevertheless, it is also seen in other areas of Central and South America such as Colombia and Brazil. Exceptionally, it has been diagnosed in other regions.

It was Ladislao de la Pascua who first described spotted or lazarine leprosy in 1844; when physicians were not aware of the etiology and nature of this illness, it was known as a chronic and destructive disease. In 1852, Lucio and Alvarado [2], working at the Saint Lazaro Hospital in Mexico City, which assisted only persons with leprosy, studied 41 patients (21 men and 20 women) with this diffuse infiltration of the skin without lepromas (Lucio's leprosy). They observed that 13 of them (6 men and 7 women) developed peculiar painful, red spots on the skin: Lucio's phenomenon.

Almost a century later, Latapí and Chévez-Zamora studied the subject and recognized both aspects observed by Lucio and Alvarado, coining the terms Lucio's leprosy and Lucio's phenomenon, identified as a vasculitis [3].

It is widely accepted that *Mycobacterium leprae* is the causative agent of several forms of leprosy; however, the identification of the new strain of mycobacterium in

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2008 by Han et al. [4] in tissues from patients with DLL suggests that the peculiar geographical and ethnic prevalence, as well as the particular severity of this form of the disease, is due to this distinctive strain: *Mycobacterium lepromatosis*. DLL carries a higher mortality rate than other forms of leprosy [1].

Nowadays, it has been demonstrated that Lucio-Latapí leprosy is caused mostly, if not only, by *M. lepromatosis*. According to Han et al., at least 63.2% of patients with Lucio-Latapí leprosy had *M. lepromatosis*, 20.7% had *M. leprae*, and 16.1% had both bacteria [1].

Patients with lepromatous leprosy have a poor Th1 response, or a dominant Th2 response, with production of IL-4, IL-5, and IL-10, which are able to suppress activation of macrophages against the causal mycobacterium; there is production of antibodies, which may result in an immune-complex disease. Lymphocytes infiltrating the skin of patients show a predominance of CD8 subset (CD4/CD8 = 1/2).

The clinical sine qua non criterion is diffuse nonnodular infiltration of the skin [5]. At the beginning, the skin shows wide infiltration that has been compared with myxedema, the face looks "healthy" (*lepra bonita* or pretty leprosy), and the earlobe turns thick. Later on in the progression of the disease, hands become swollen, puffy, and red, and the legs look edematous. In time, all the skin becomes flaccid and atrophic (Fig. 11.1), giving to some areas such as the legs an ichthyosiform aspect.

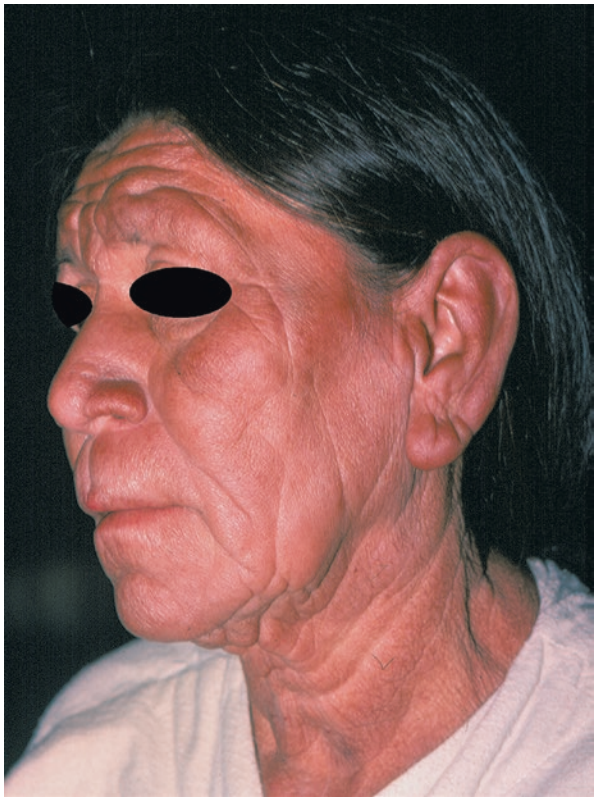

Fig. 11.1 Diffuse lepromatous leprosy in a late stage: flaccid skin, in particular that of the earlobe

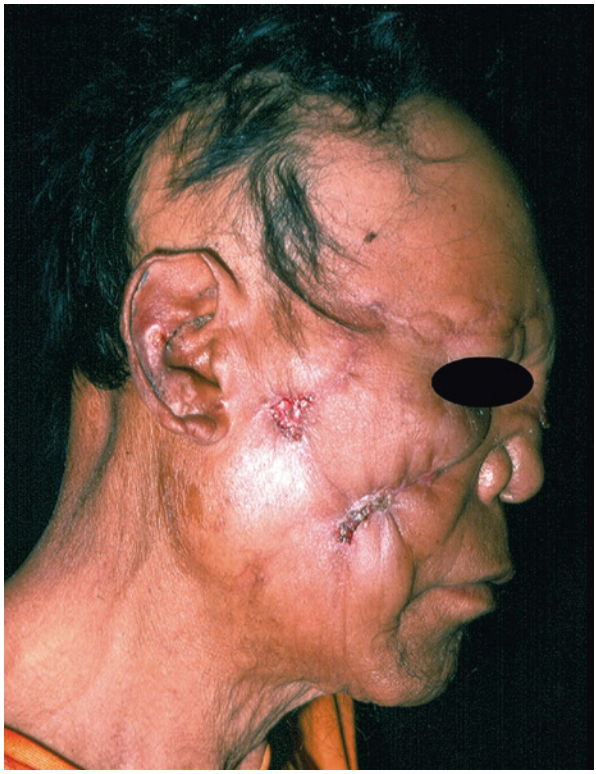

Fig. 11.2 Diffuse lepromatous leprosy with hair loss and damage to the nasal cartilaginous septum

Other features are telangiectasias, nonvisible subcutaneous plaques, widening of nasal root, rhinitis, hoarseness, and septal perforation of cartilage. Patients first notice numbness and then impairment of sensation in hands and feet.

Loss of hair is common, not only from the scalp, which is never total, but also from eyebrows and eyelashes, total or partial (Fig. 11.2). However, this is not a constant finding, and some patients do not lose hair from any area.

Lucio–Latapí leprosy or DLL usually presents in adults of both sexes; it is exceptional in childhood and the elderly. Clinical changes may be so subtle that often the patient notices the disease only because of sudden development of an acute reaction, namely, Lucio's phenomenon.

Although the histopathology of DLL depends on the stage of the disease, there are always acid-fast bacilli (AFB) in variable amounts but usually many, outside and inside of foamy macrophages or Virchow cells; these are the two least criteria to reach the diagnosis under the microscope. Macrophages are mixed with lymphocytes around blood vessels, adnexal structures, and nerves.

In early stages of the disease, the cellular infiltrates localized around vessels are slight and apparently insignificant [6]. In time, there is progressive dissemination of AFB and increasing numbers of Virchow cells, not only throughout the dermis but also into subcutaneous fat. In well-developed stages, macrophages are prominent in

number and infiltrate in a diffuse pattern; blood vessels begin to show involvement as thickening of their walls.

Besides the clinical and histopathological parameters, bacillary assessment is also very useful for diagnosis, and mostly for classification of a patient with leprosy.

In advanced diffuse lepromatous leprosy, the bacteriological index (see Chap. 20) is usually 5+ or 6+ and decreases after 6 months or more of treatment.

According to the World Health Organization, treatment should be with multibacillary multidrug therapy: rifampicin 600 mg/month, clofazimine 50 mg/day or 300 mg/month, and dapsone 100 mg/day, all three for at least 1 year. However, in Mexico, these patients are treated for 2 years and sometimes even longer, depending on their clinical, bacteriological, and histopathological response.

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Histopathology of the Skin in Leprosy

12

Cesare Massone and Antônio Pedro Schettini

12.1 Introduction

Leprosy skin histopathology is characterized by granulomatous infiltrate with only few exceptions (indeterminate leprosy and some particular forms) [1–8]. The clinical spectrum of the disease described in Chap. 6 (Classification) has its direct correlate on histopathology, reflecting the different grades of cell-mediated immune response (CMI) as described in Chaps. 4 (Host Response to *M. leprae*) and 5 (Pathogenesis).

Leprosy diagnosis cannot be made on histopathology alone (unless the bacillus is identified in the biopsy); clinical data such as the number, distribution, and type of lesions, with or without anesthesia, are mandatory information to diagnose, but mainly to classify, the disease. Therefore, clinico-neurologic–pathologic correlation is mandatory, particularly in paucibacillary leprosy and reactional states [9].

Skin biopsy must be deep (6-mm (OR 5MM) punch biopsy including part of the subcutaneous fat), without artifacts (squeeze), must be performed at the correct site of the lesion (Table 12.1), and must be included in buffered formalin. Incisional biopsy is ideal for deep infiltrated lesions involving the subcutaneous fat.

Hematoxylin and eosin (H&E) and one of the variants of the Ziehl-Neelsen stain like Fite–Faraco (FF) stains must be performed on each biopsy by an experienced laboratory. Serial sections are sometimes needed. Immunohistochemistry for assessment of the lymphocytic infiltrate is never necessary for diagnosis. Polymerase chain reaction (PCR) for *Mycobacterium leprae* is useful only in a limited number of cases.

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Table 12.1 Correct site of biopsy on leprosy lesions

Suspected leprosy type	Site of biopsy
Indeterminate leprosy (I)	Center of the lesions, or better center of the anesthetic area
Tuberculoid leprosy (TT) and borderline tuberculoid leprosy (BT)	Infiltrated margin of the lesion
Borderline leprosy (BB) Borderline lepromatous leprosy (BL) and lepromatous leprosy (LL)	Most edematous and infiltrated area of the lesion Center of the macule or nodule
Type 1 reaction (T1R)	Most edematous and infiltrated area of the lesion
Erythema nodosum leprosum (ENL)	Center of the nodule; deep biopsy (up to subcutaneous fat)

12.2 Modified Fite Stain for Acid-Fast Bacilli (AFB)

FF is the method of choice for staining AFB; control sections should be used, and "whenever there is doubt, there should be no hesitation in staining further sections" [10].

There are several recipes for modified stain for AFB. Ridley [10] reports the following:

1. Remove wax in two changes of xylene/peanut oil (3:1) mixture, 7 min each change.
2. Wipe off excess oil from back of slide.
3. Blot section very gently with fine filter paper, three times.
4. Wash in running water, 5 min.
5. Rinse in distilled water.
6. Stain in carbol fuchsin, 30 min.
7. Wash in running tap water, 2 min.
8. Decolorize in 1% acid alcohol to pale pink.
9. Wash in running tap water, 2 min.
10. Counterstain in 0.15% methylene blue, five or six dips.
11. Wash in running water until section is pale blue, about 3 min.
12. Dehydrate quickly in absolute alcohol, three changes.
13. Clear in xylene with two changes, and mount.

The National Centre for Hansen Disease of Genoa uses the following modified formula from Job and Chacko [3]:

1. Cut slides with tissue sections of 5 lm.
2. Deparaffinize slides with a mixture of peanut oil and xylene (or xylene substitute)*, two times for 12 min each.
3. Discharge the excess of the mixture and dry gently (for compression) with paper, three times.

4. Cover the sample with carbol fuchsin (same solution as Ziehl–Neelsen) for 20 min.
5. Rinse gently with water, until the water flows off clear.
6. Decolorize slides with 2.5 mL of mixture** of sulfuric acid and ethanol.
7. Rinse with tap water.
8. Slides must be light pink; otherwise, repeat the decolorization with about 1 mL of the same solution used at point 6.
9. Counterstain with 1% methylene blue, for 5 min.
10. Rinse the stain with tap water until the solution is not throwing blue dye anymore.
11. Allow slides to dry in air, away from sunlight.
12. Mount with balsam.

*Peanut oil/xylene = 1:2

**Working solution: 5 mL 95% sulfuric acid, 25 mL ethanol absolute, 70 mL deionized water

Slides must be observed with a 1009 objective lens in oil. AFB are assessed on histopathological sections exactly in the same way as in the smear according to the following logarithmic indices [bacterial index of granuloma (BIG)]:

- 0 Absence of Bacilli
- 1+·····1–10 bacilli in 100 high-power fields (hpf)
- 2+·····1–10 bacilli in 10 hpf
- 3+·····1–10 bacilli for hpf
- 4+·····10–100 for hpf
- 5+·····100–1000 for hpf
- 6+·····Many clumps, "globi" (1000 bacilli)

12.3 Histopathological Patterns of Leprosy

The inflammatory cells present in leprosy lesions are epithelioid cells, macrophages, lymphocytes, plasma cells, and in specific cases also neutrophils and mast cells. According to the CMI response to *M. leprae*, different types of granulomatous reaction can be observed. In each biopsy, nerves have to be carefully checked, as involvement of the nerve and presence of AFB inside the nerve are diagnostic features of leprosy.

12.3.1 Epithelioid Granuloma

This type of infiltrate can be seen in TT, BT, and BB. TT is characterized by noncaseating granulomas formed by mature epithelioid cells and "encapsulated" by many lymphocytes (Figs. 12.1 and 12.2). Multinucleated giant cells are present, particularly numerous large Langhans cells are pathognomonic. Plasma cells are rare.

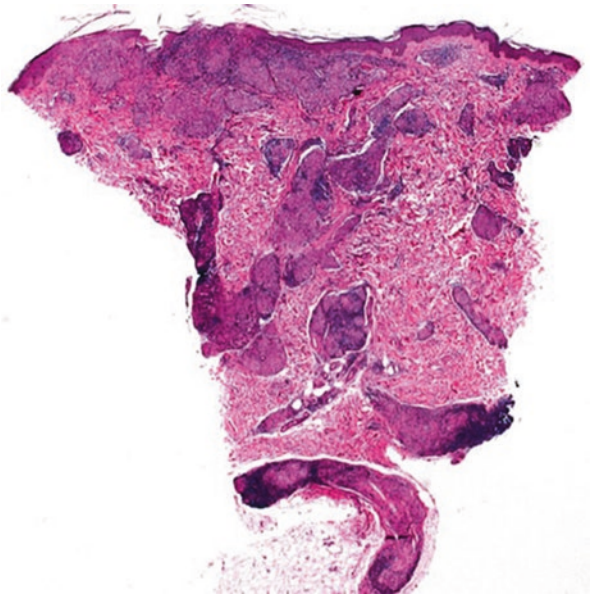

Fig. 12.1 TT. Multifocal granulomatous infiltrate, "eroding" the epidermis

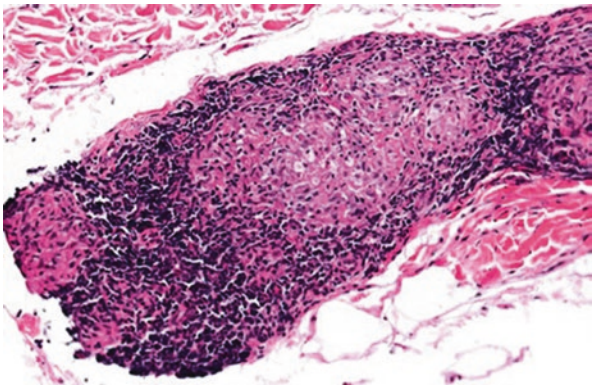

Fig. 12.2 TT is characterized by noncaseating granulomas formed by mature epithelioid cells and "encapsulated" by many lymphocytes

Fibrin in the center of the granuloma is rarely observed. The epidermis is often atrophic and "eroded" by the granulomas present in the superficial dermis (Fig. 12.3). There is no clear subepidermal zone.

The granulomatous infiltrate is more often multifocal with a typical periadnexal and perineural distribution, in both the superficial and deep dermis. Skin adnexa (with involvement of the hair erector muscle and sweat glands) may also be infiltrated and destroyed by the granuloma (Fig. 12.4). Extension of the infiltrate to the subcutis is possible.

Nerves are enlarged and swollen. The perineurium is intact and may be surrounded by lymphocytes. In other cases, nerves are infiltrated by granulomas and may be destroyed beyond recognition (Figs. 12.5 and 12.6). Rarely, a patch of fibrinoid necrosis may be observed. Even when is not possible to recognize the nerve,

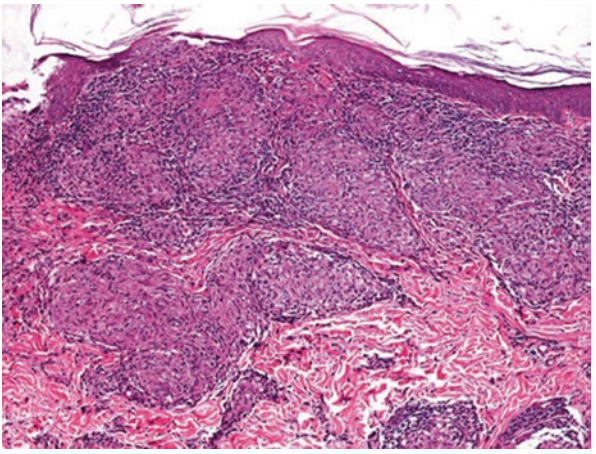

Fig. 12.3 TT. The epithelioid granulomas "erode" the epidermis

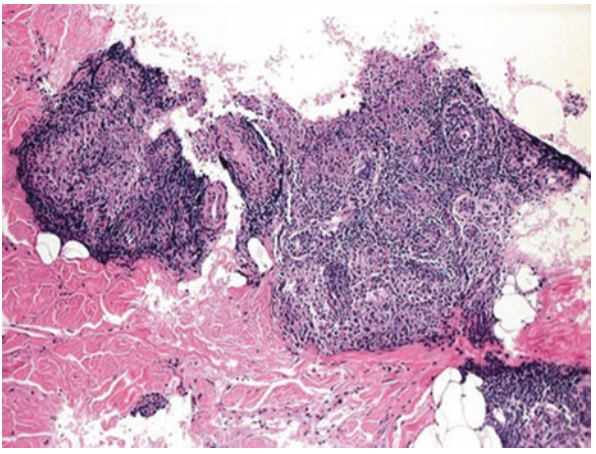

Fig. 12.4 TT. Epithelioid granulomas infiltrate and destroy the skin adnexa

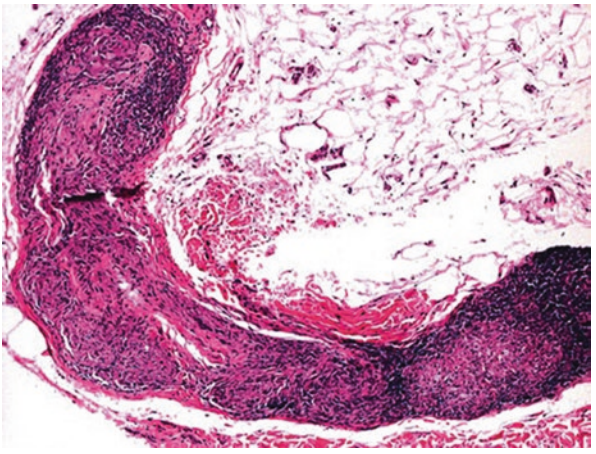

Fig. 12.5 TT. Nerves are enlarged, swollen, and infiltrated by epithelioid granulomas

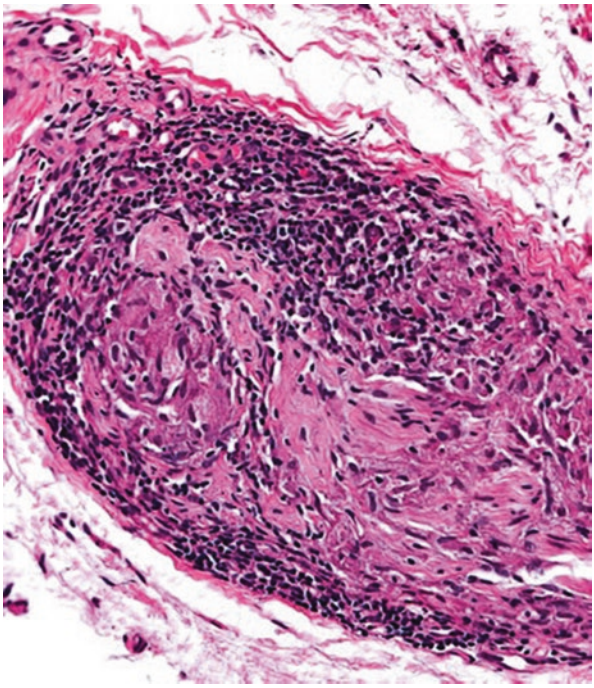

Fig. 12.6 TT. Nerves are enlarged, swollen, and infiltrated by epithelioid granulomas

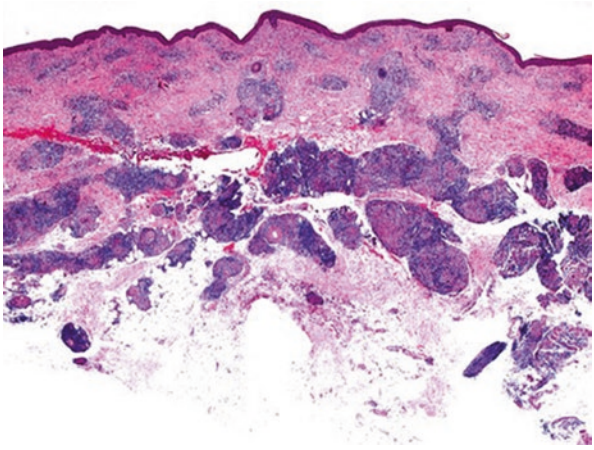

Fig. 12.7 BT. Multifocal granulomatous infiltrate, similar to TT. The epidermis is usually spared

the presence of an elongated infiltrate ("sausage-type") in the deep dermis–subcutis is highly suggestive of leprosy. The granuloma tends to track along nerve forming a serpiginous or linear pattern. Careful search may reveal few remnants of nerve tissue in the granuloma. Some authors report that S100 stain is superior to H&E in identifying nerve fragmentation in TT, but S100 staining is usually not needed.

AFB are usually absent (AFB 0–1+), and PCR is negative in most of the cases. BT presents a similar infiltrate (Fig. 12.7); differences reside in the less mature epithelioid cells and infiltration of lymphocytes (reduced in comparison to TT) within granulomas.

Granulomas are less organized, and tubercle formation is unusual (Fig. 12.8). Undifferentiated, medium-sized giant cells are typical of BT. The epidermis may or may not be eroded by granulomas. Nerves typically are moderately swollen and show perineural and intraneural granulomas. The perineurium is infiltrated by lymphocytes.

AFB is 0–2+; PCR is positive in only almost half of cases.

BB is also characterized by granulomas with immature epithelioid cells but without formation of well-defined epithelioid granulomas (Fig. 12.9). Giant cells are not present, and lymphocytes are diffusely distributed. Macrophages represent also a fairly significant proportion of cells. There is often edema. The epidermis is atrophic. The subepidermal zone is not involved. Nerves are not swollen but are infiltrated by epithelioid cells and lymphocytes and partly destroyed. Lamination of the perineurium (reactive proliferation of perineural cells) may be observed. AFB is 3–4+.

The main differential diagnoses of TT and BT are all granulomatous dermatitis like sarcoidosis, tuberculosis, leishmaniasis, and secondary syphilis.

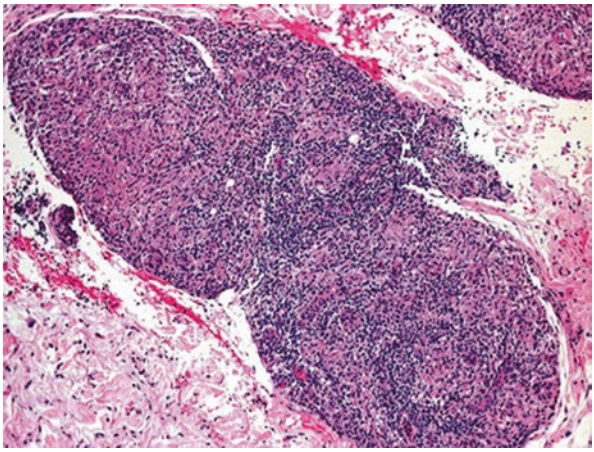

Fig. 12.8 BT. There is less maturation of epithelioid cells and pronounced infiltration of lymphocytes within granulomas

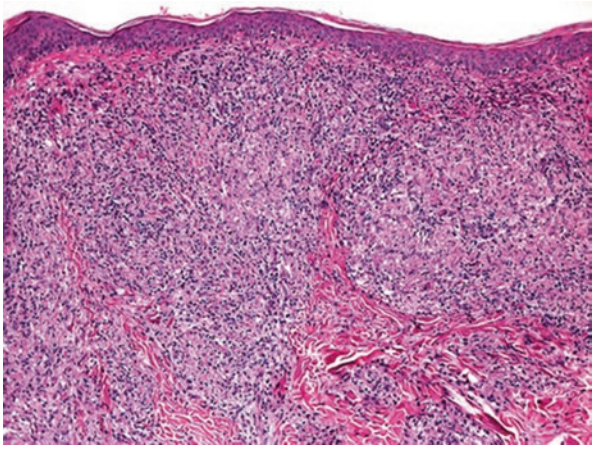

Fig. 12.9 BB. Granulomas with immature epithelioid cells but without formation of well- defined epithelioid granulomas. Giant cells are not present, and lymphocytes are diffusely distributed. Macrophages represent also a fairly significant proportion of cells. There is often edema. The epidermis is atrophic

Type 1 reaction (T1R) occurs mainly in BT, BB, and sometimes also BL patients (Figs. 12.10, 12.11 and 12.12). Histological features are (a) edema in the superficial dermis, (b) edema in the granuloma with disorganization of the granuloma, (c) appearance of foreign-body giant cells (sometimes with vacuoles due to intracellular edema) and large Langhans giant cells, (d) epidermal erosion with spongiosis, and (e) fibroplasia. In severe reaction, breakdown of the granuloma and even necrosis or ulceration can occur, and few collections of neutrophils may be seen. As T1R subsides, there is reduction in the edema and formation of well-organized tubercles. According to Lockwood et al., the five key features are dermal edema, intragranuloma edema, giant cell size, giant cell number, and HLA-DR expression [11–13].

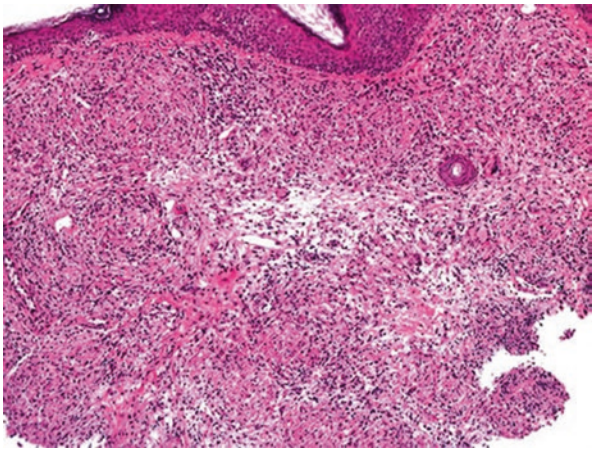

Fig. 12.10 BB with T1R. Apart from features of BB, there is more pronounced edema in the dermis with the appearance of many large Langhans giant cells and foreign-body giant cells

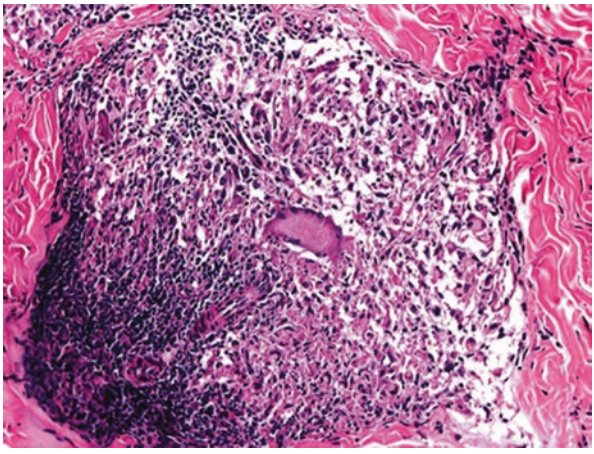

Fig. 12.11 BT with T1R. Edema in the granuloma with disorganization of the granuloma and presence of large Langhans giant cells

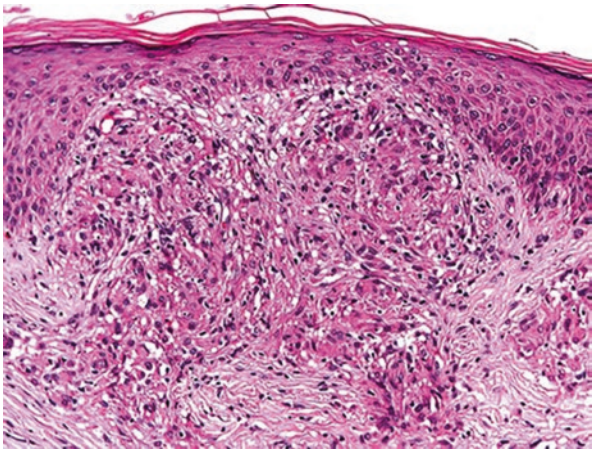

Fig. 12.12 BT with T1R. Under MDT and steroid therapy, histopathological changes might be different, as in this case, where only disorganization of the granuloma and intracellular edema together with vacuolization are present. There is also fibrosis in the dermis

12.3.2 Macrophage Granuloma With Virchow Cells

LL is characterized by collections and sheets of macrophages within the dermis and not very many lymphocytes, diffusely scattered or in a clump. There are no epithelioid cells. Plasma cells are usually present. The epidermis is atrophic, and a clear grenz zone in the superficial dermis between the infiltrate and the epidermis is typically present. Macrophages are more often diffusely distributed in the dermis, but also a "patchy nodular" arrangement can be seen (Figs. 12.13 and 12.14). Skin adnexa are surrounded by macrophages and are atrophic. Subcutaneous and deep dermal infiltration can be present.

Macrophages present gray cytoplasm with foamy changes (lepra cells, Virchow cells; Fig. 12.15), varying from mild foamy appearance in early lesions to large vacuoles in older or regressing lesions (occurring also in multinucleated giant cells; Fig. 12.16).

Nerves show perineural collection of macrophages and may have onion-skin perineurium but without significant infiltration, or they may be fairly normal (Fig. 12.17). Nerves may be somewhat hyaline or fibrosed, but not swollen.

Numerous AFB (5–6+) are present within macrophages, sweat and sebaceous glands, hair follicles, arrectores pilorum muscles, Schwann cells, perineural cells, and vascular endothelium (Figs. 12.18, 12.19, and 12.20). AFB may be arranged in parallel arrays, forming clusters, or densely packed in large masses known as "globi." AFB have solid appearance in untreated patients, while they are granular or fragmented in patients under or shortly after multidrug therapy (MDT; Fig. 12.20).

BL is also characterized by macrophage granulomas, but with more numerous lymphocytes filling a segment of the granuloma to its periphery. In some cases, lymphocytes predominate over macrophages (Figs. 12.21 and 12.22). A solitary clump of epithelioid cells among the macrophages may be seen. The infiltrate can

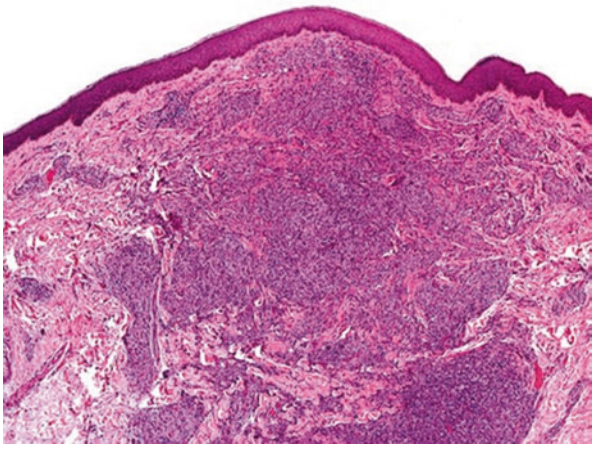

Fig. 12.13 LL. Patchy nodular infiltrate of macrophages in the dermis without granuloma formation. A clear grenz zone in the superficial dermis between the infiltrate and the epidermis is typically present

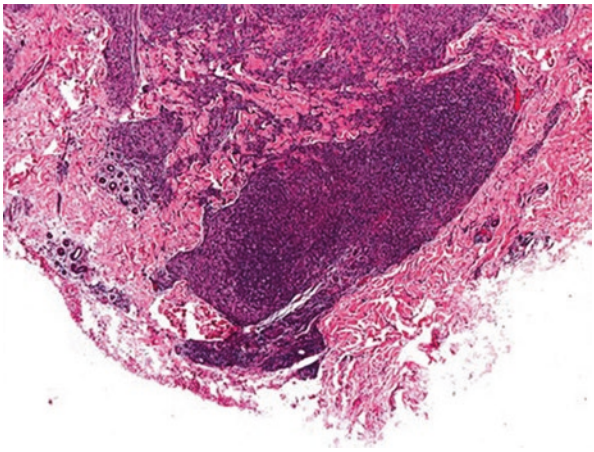

Fig. 12.14 LL. Skin adnexa are surrounded by macrophages and are atrophic

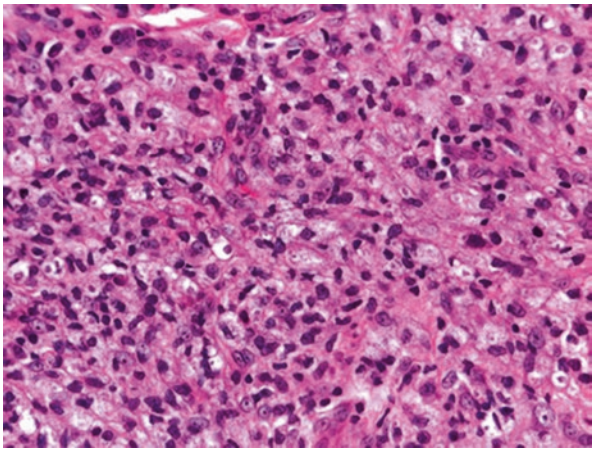

Fig. 12.15 LL. Macrophages present gray cytoplasm with mild foamy changes [lepra cells, Virchow cells in early lesions (same case as Figs. 12.13 and 12.14)]. Plasma cells are present

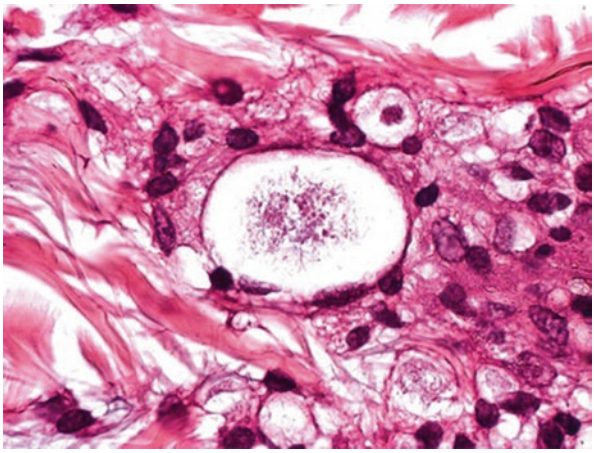

Fig. 12.16 LL. In older or regressing lesions, large vacuoles appear

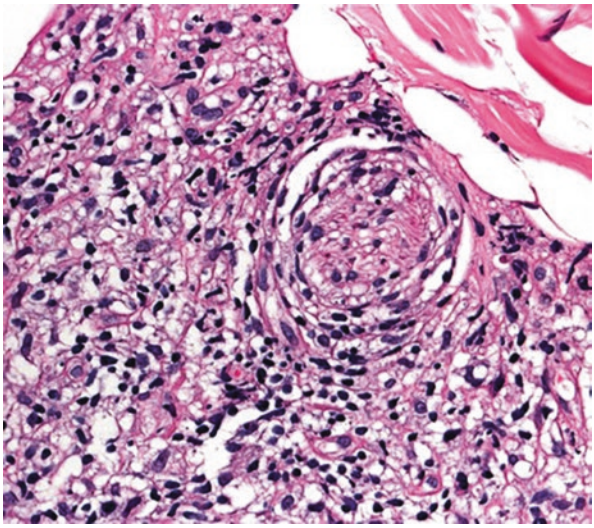

Fig. 12.17 LL. Nerves show perineural collection of macrophages and may have onion-skin perineurium but without significant infiltration

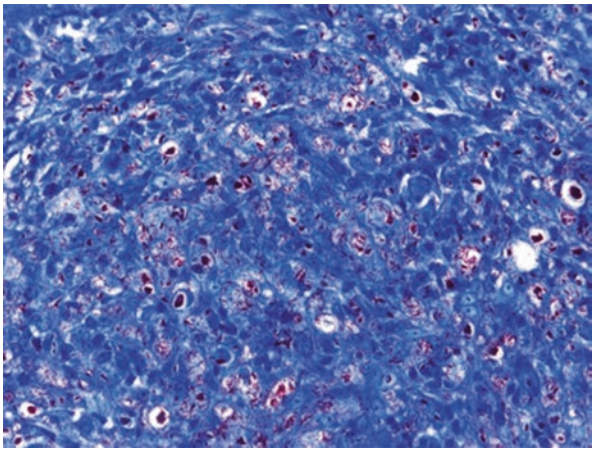

Fig. 12.18 LL. Numerous AFB (6+) are present within macrophages, arranged in parallel arrays, forming clusters, or densely packed in "globi." AFB have solid appearance in untreated patients (same case as Figs. 12.13, 12.14, and 12.15)

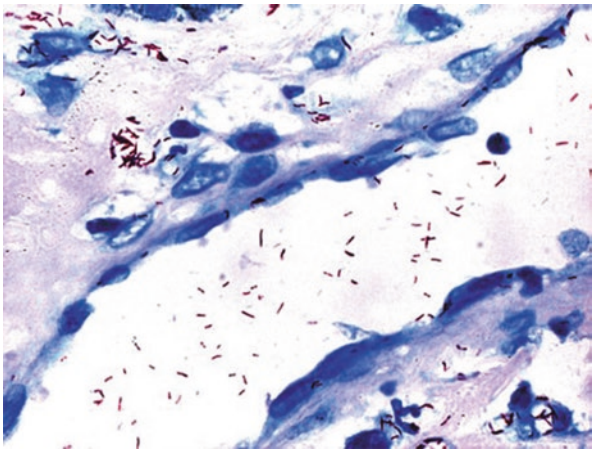

Fig. 12.19 LL. Solid AFB are present also in vessels

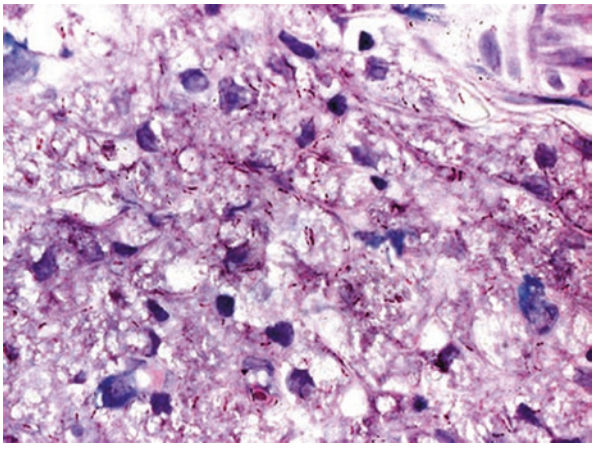

Fig. 12.20 LL. AFB are granular or fragmented in patients under or shortly after MDT

be diffuse, patchy nodular, perivascular, or periadnexal but always separated by a typical narrow zone of collagen from the epidermis. Macrophages have foamy changes, especially in regressing lesions, but no vacuoles. Nerves show onion-skin perineurium with lymphocytes forming a cuff around a nerve bundle. AFB (4–5+) are present inside macrophages, Schwann cells, endothelial cells, and arrectores pilorum muscles.

PCR is obviously positive in both LL and BL and is not necessary for diagnosis. Histopathological differential diagnoses of both LL and BL include mainly other diseases presenting foamy macrophages such as xanthomas and xantho-granulomas, post-kala-azar dermal leishmaniasis, and paraffinoma.

Infections caused by nontuberculous (atypical) mycobacteria are an important emerging issue because they also present granulomatous infiltrates with histiocytes and neutrophil abscesses similar to leprosy. Cultures and molecular investigations are mandatory in case of doubt.

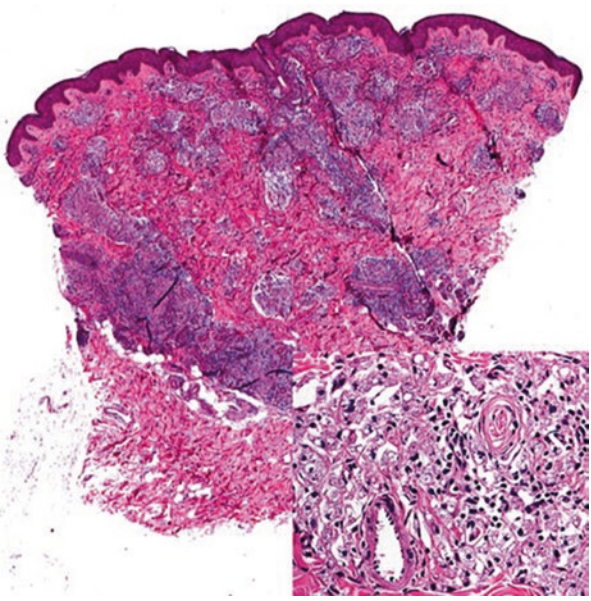

Fig. 12.21 BL. Macrophage granuloma with lymphocytes inside. In detail, Virchow cells and a nerve with onion appearance

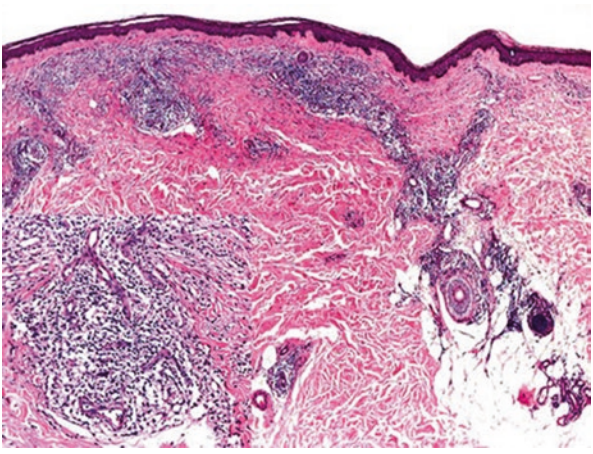

Fig. 12.22 BL. In some cases, lymphocytes predominate over macrophages. In these cases, diagnosis might be a challenge

If T1R occurs in BL, epithelioid granulomas with giant cells and edema are observed, together with Virchow cells and macrophage granuloma.

ENL can occur in both LL and (less commonly) BL. The key to diagnosis in the acute stage is edema in the dermis and neutrophilic infiltrate with background changes of preexisting lepromatous lesions. Eosinophils, plasma cells, and mast cells are present. Typically, the infiltrate is centered on small granulomas in the subcutis with clusters of neutrophils around old foamy macrophages. This has been described by Ridley as "pink node type" or classic ENL (mild form; Figs. 12.23, 12.24, and 12.25). In histopathological textbooks, ENL has been classified as a

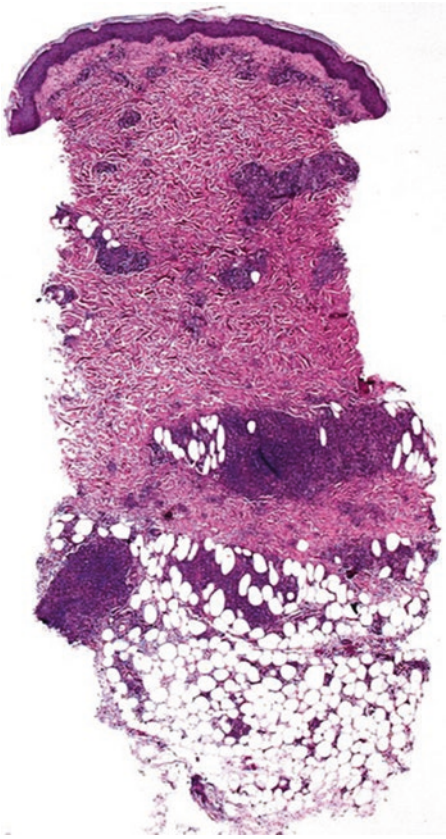

Fig. 12.23 "Pink node type" or classic ENL. The infiltrate is centered on small granulomas in the subcutis with clusters of neutrophils around old foamy macrophages

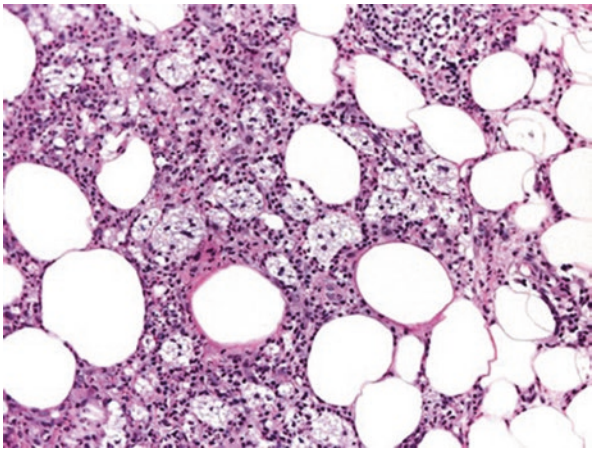

Fig. 12.24 "Pink node type" or classic ENL. Lobular panniculitis with neutrophils and foamy macrophages

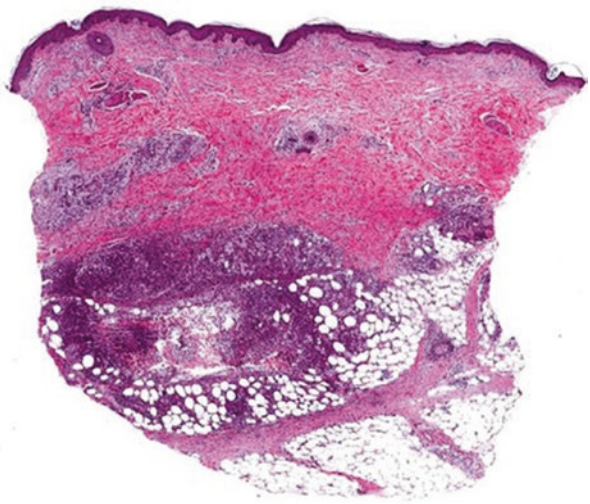

Fig. 12.25 "Pink node type" or classic ENL. Features of LL have to be present in the dermis

mostly lobular panniculitis with vasculitis, but it is worth noting that vasculitis is not a conspicuous feature in classic ENL. In fact, classical features of small or medium vasculitis, necrotizing changes and thrombus formation, have been demonstrated in classic ENL lesions in almost 25% of cases. ENL is a dynamic process, and of course the timing of the biopsy influences the histopathological features, with vasculitic changes being seen more often in very early lesions.

Both superficial and deep dermis are involved in 65–85% of cases, but in some cases, the process involves exclusively the dermis with mixed infiltrate of neutrophils (sometimes eosinophils) and lymphocytes, dispersed among foamy macrophages. In some cases, the reaction can be severe and produce degeneration of collagen and necrosis (necrotizing ENL, severe ENL; Fig. 12.26) of both the dermis and epidermis (clinically ulcerated lesions). In such cases, ENL may resolve with dermal fibrosis.

Solid AFB are seen in lesions from untreated patients, while granular or fragmented AFB are usually seen in patients under therapy. In cases occurring after MDT, FF can be negative.

The term "erythema nodosum leprosum" is a misnomer that can mislead both clinicians and histopathologists. The definition of ENL is inappropriate mainly as regards its clinical similarity with true EN: the term "type 2 leproreaction" should more correctly be used. The main histological differential diagnoses include true EN, erythema induratum of Bazin, Sweet's syndrome, pyoderma gangrenosum, and deep mycotic infections. Clinicopathologic correlation is necessary to distinguish necrotizing ENL from Lucio's and Alvarado phenomenon (see Chap. 22) [14–16].

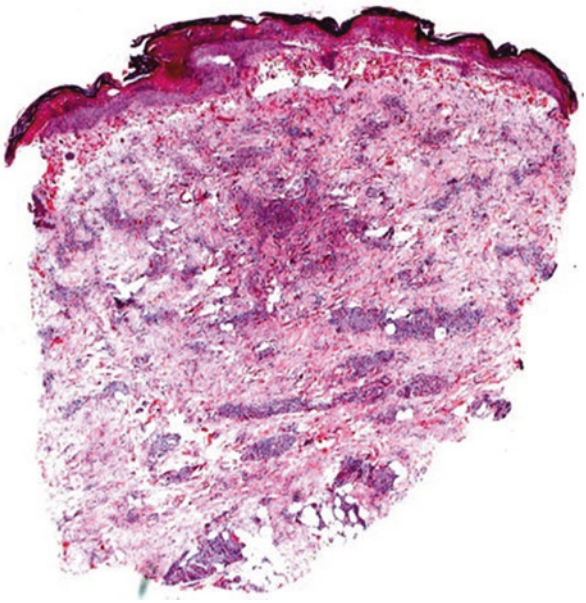

Fig. 12.26 Necrotizing or severe ENL. In this case, a leukocytoclastic vasculitis with necrosis of both the dermis and epidermis is present. Neutrophils and hemorrhages are constantly seen

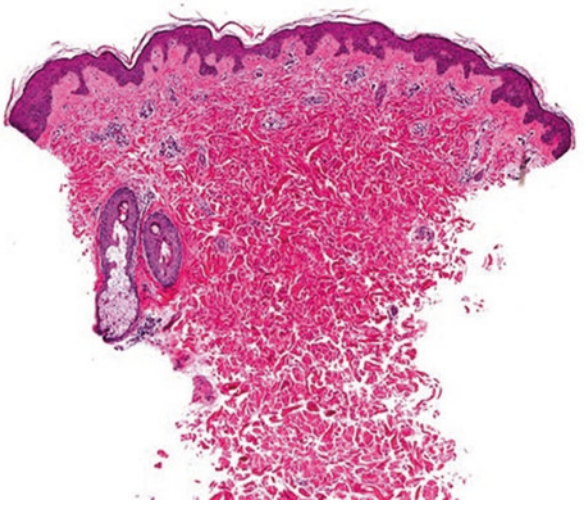

Fig. 12.27 I. An only superficial, perivascular lymphohistiocytic infiltrate is present in one-third of cases

12.3.3 Perivascular Infiltrate

I presents with a perivascular lymphohistiocytic infiltrate that can be only superficial or more often superficial and deep with periadnexal distribution (Figs. 12.27, 12.28, and 12.29). In some cases, the infiltrate is more prominent around the adnexa (sweat and sebaceous glands, hair follicles) than around the vessels. A perineural infiltrate can be seen in one-third of cases, and lymphocytes "cuffing" the nerve

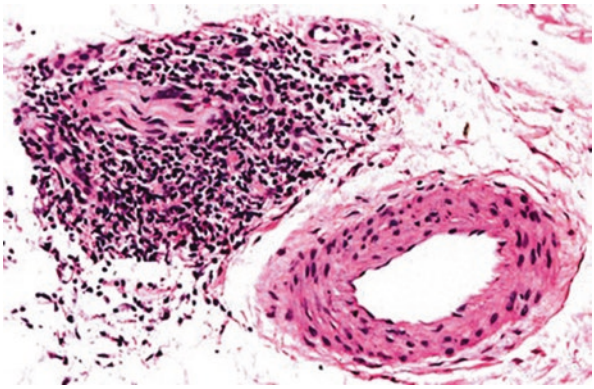

Fig. 12.28 I. In another one-third of cases, lymphohistiocytic perineural infiltrate is seen. Nerves are however not enlarged

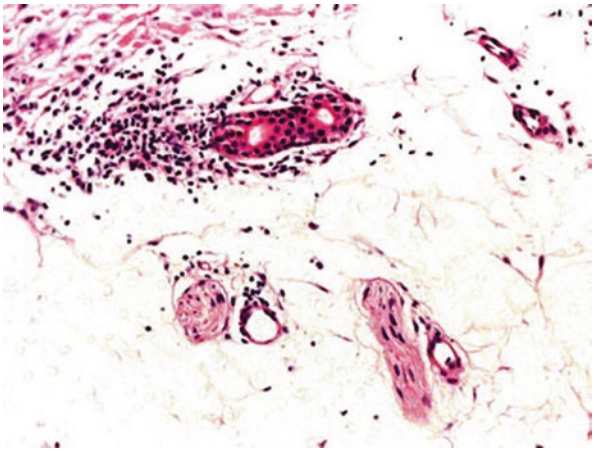

Fig. 12.29 I. In another one-third of cases, the infiltrate is only perivascular and periadnexal

fibrils is a good hint for the diagnosis. Mast cells are increased. Nerves are however not enlarged. Granulomas are not seen. The epidermis may present focal vacuolar degeneration of basal keratinocytes and exocytosis of lymphocytes. I lesions are clinically hypopigmented. Even if reduction of melanin in the basal layer and decreased number of melanocytes have been recorded, the exact mechanism of hypopigmentation has not yet been clarified.

AFB are difficult to find, and FF serial sections (up to 30 sections have to be screened) following a dermal nerve are needed to find a single or small group of AFB. The subepidermal zone just beneath the basal layer and the arrectores pilorum muscles are sites where AFB might be also seen. PCR is positive only in a small proportion of cases, being in these cases of some help for diagnosis.

Diagnosis of I without detailed clinical information is not possible, as the histopathological differential diagnosis is with the large group of dermatitis with perivascular and periadnexal lymphohistiocytic infiltrate. The infiltrate can be in some cases so minimal that interpretation can be subjective. In such cases, the final diagnosis rests with the clinician.

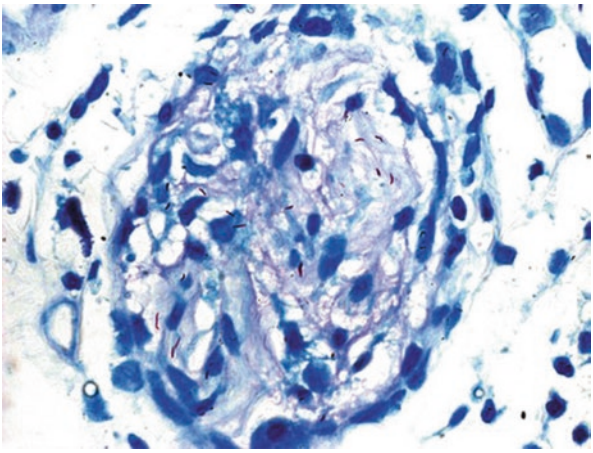

Fig. 12.30 I leprosy in evolution toward multibacillary leprosy. The finding of solid AFB indicates evolution toward the lepromatous pole

I leprosy can heal or progress to one of the abovementioned forms of disease. If I is progressing toward the tuberculoid pole, small epithelioid granulomas might be seen, and these cases have to be classified as early TT. On the other hand, if I is progressing toward the lepromatous pole (Fig. 12.30), focal macrophages and clumps of AFB in the nerves are seen. Cases progressing toward BL might be characterized by predominant perivascular and periadnexal lymphocytic infiltrate rather than macrophages, representing a diagnostic pitfall. Indeed, these cases have to be classified as early BL.

12.4 Diagnosis of Leprosy

Leprosy diagnosis can be easy (multibacillary cases) or very difficult (paucibacillary cases), especially for inexperienced pathologists in nonendemic areas. In fact, due to increasing immigration flow from endemic areas, leprosy cases might be more frequently observed also in nonendemic areas such as Europe. Although leprosy remains a rare disease, histological confirmation is mandatory in all cases (i.e., in Italy, it is requested by official guidelines of the Ministry of Health), thus representing a potential pitfall for dermatopathologists without specific experience [17].

The gold standard for diagnosis of leprosy is strictly related to three major points: well-done and deep, 6-mm punch biopsy performed at the correct site of the lesion, well-cut and stained H&E and FF slides, and detailed clinical information. When this sequence is respected, the majority of cases can be definitively diagnosed and classified. Problems arise, e.g., when biopsy is performed on a nondiagnostic area, slides present artifacts, FF is not reliable, or clinical data are lacking, making histological reports aspecific or inconclusive, with delay in treatment and possible onset of neural complications.

12.5 Issues About Histological Classification

Unfortunately, the Ridley–Jopling clinical classification does not always correlate with the histological features, and in some cases, there is a discrepancy between clinical and histopathological classification. Different authors have found overall agreement between clinical and histological type of 58–70%. The major disparity is seen in borderline cases. Parity in the polar group is maximum, because they are stable and show fixed histopathology, while borderline and indeterminate groups may have different histopathology at different sites. Moreover, multiple biopsies performed on the same BL lesions might also not correlate with clinical features. The explanation can be found in differences in sample size, choice of biopsy site, depth of biopsy, quality of H&E and FF sections, and the number of sections examined, again underlying the importance of the abovementioned gold standard for diagnosis of leprosy [1–4].

Anyhow, the discrepancy between the clinical and histological picture in borderline patients is not surprising as they are characterized by nonstable systemic immunity (see Chap. 5, "Pathogenesis") that influences the clinical features. Moreover, differences in the local skin CMI may also occur.

T1R histological changes are recognizable in 60% of cases only, causing underdiagnosis by histopathologists in comparison with clinicians [1–4].

Finally, while the polar forms TT and LL are quite easily diagnosed and classified, borderline cases may sometimes present overlapping features, resulting in practical difficulty in exactly classifying a patient. For this reason, combinations of symbols such as TT-BT, BT-BB, BB-BL, and BL-LL are used for cases that show features intermediate between two groups [1–4].

Currently, the WHO recommends "the diagnosis of leprosy may be based on clinical examination, with or without slit-smears, or pathological examination of biopsies," and diagnosis and classification are based on the number of lesions (see Chap. 6). But on referral center, histopathology is essential to confirm the diagnosis. Moreover, an accurate classification is needed to standardize research on immunology and pathogenesis, molecular biology, and treatment of leprosy. Moreover, a correct classification is mandatory to differentiate between leprosy reactions and relapse. Last, a more accurate diagnosis avoids over- and undertreatment of leprosy.

12.6 Unusual Forms

Histoid leprosy (HL) is a clinical and pathologic variant of multibacillary leprosy, usually occurring in patients on long-term diaminodiphenyl sulfone, with initial improvement followed by relapse. HL can occur in LL, BL, and IL patients [18, 19].

The term was coined by Wade in 1963 to describe firm, reddish, or skin-colored, dome-shaped, or oval papules and/or nodules, regular in contour with translucent shiny and stretched overlying skin that on histology show a

circumscribed macrophage granuloma with predominance of spindle-shaped cells and/or polygonal cells, and the unusually large number of AFB. Some cases may resemble a fibrohistiocytic tumor. Small collections of foamy macrophages may be present.

Lucio–Latapí leprosy and the Lucio–Alvarado phenomenon have been discussed in Chaps. 11 and 22.

Leprosy in HIV patients has been described in Chap. 35.

Histology of leprosy lesions on special sites (oral cavity, nasal cavity, scalp, and genital areas) is similar to those of the skin.

12.7 Regressive Lesions

Leprosy patients under or after MDT can be also biopsied to follow-up the clearance of the lesions. Clearance of infiltrate and AFB takes years depending on the granuloma fraction and BIG.

TT and BT show progressive disappearance of the granuloma with gradual loss of epithelioid cells and without fibrosis. TT and BT regressing lesions assume progressively the picture of a nonspecific perivascular chronic inflammation, mainly constituted by lymphocytes. Resolution of the process after successful therapy lasts 1–3 years, but it is worth noting that perineural granulomas in TT have been reported to persist also 18 months after cessation of effective therapy [11].

In BL and LL, regressive lesions are characterized by "fatty degeneration" (a sort of "postbacillar thesaurismosis") with the appearance of more and larger vacuoles (even giant vacuoles, especially in LL). The lymphocytes become more diffusely spread. Foamy cells disappear slowly, leaving a perivascular lymphocytic infiltrate with few foamy macrophages. AFB are granular or fragmented, and their number also reduces progressively. Skin adnexa are atrophic and disappear without fibrosis. Nerve bundles are replaced by fibrosis. After successful MDT, lesions can clear histologically in 2–5 years or more, but in some patients, a few foam cells seem to persist for life, for unexplained reasons [11].

12.8 Relapse

Relapse may take place at the site of a preexisting lesion, at a fully resolved site, or at a previously uninvolved site.

In TT and BT relapse, there is reappearance or new appearance of granulomas, composed mainly of epithelioid cells, lymphocytes, and Langhans giant cells, with the same distribution as in active lesions. Differentiating relapse from T1R is extremely difficult.

In the early phase of BL and LL relapse, small and large foci of newly arrived lymphocytes and spindle-shaped macrophages with pink granular cytoplasm are identified along with a few small clumps of foamy macrophages. Once the lesion is well established, the foamy change becomes obscured by collections of

spindle-shaped and immature macrophages. The main feature is that solid staining AFB reappear in biopsy specimens. Coexistence of granular or fragmented AFB is possible.

It is not possible to distinguish histologically between relapse and reinfection.

12.9 Diagnostic Clues

In summary, histological features that should always induce consideration or exclusion of leprosy on biopsy of a patient coming from endemic areas are:

- Multifocal granulomatous infiltrate with epithelioid granulomas
- "Erosion" of the epidermis by epithelioid granulomas
- Perineural and periadnexal granulomatous infiltrate
- Swollen and enlarged nerves
- Necrosis of the nerve
- Perineural lymphocytic infiltrate
- Lymphohistiocytic infiltrate more prominent around adnexa than around vessels
- Macrophage granuloma with foamy cells
- Foamy cells with large vacuoles
- Neutrophils in a macrophage granuloma with foamy cells
- The six Ls of plasma cells: lues, lupus vulgaris, Lyme/borreliosis, leprosy, leishmaniasis, and lymphoma [5]
- Pale infiltrate: lues, lupus vulgaris, leprosy, and leishmaniasis [5]

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Differential Diagnosis of Leprosy: Skin

13

Enrico Nunzi and Salvatore Noto

Interaction between *Mycobacterium leprae* (*M. leprae*) and the host's immune system results in a wide range of lesions on the skin, giving rise to either mono- or polymorphous clinical aspects.

In active leprosy, these clinical aspects are characterized by [1]:

- Macules, which appear red in light-skinned patients and coppery in dark-skinned patients or are hypopigmented
- Papules, in groups or along the edges of hyperergic lesions
- Nodules, either scattered or in groups
- Plaques, caused by coalescence of papules or nodules or by infiltration of macules

In the hyperergic forms (TT, BT), skin lesions have asymmetric distribution, as well as early involvement of peripheral nerve autonomic branch leading to anhidrosis. This gives to lesions a dry, rough, and opaque appearance. Xerosis of lesions may cause pityriasiform desquamation.

Leprosy reactions are characterized by acute appearance of nodules and plaques, or by increase in erythema, infiltration, and edema in preexisting lesions.

On reactional lesions, vesicles, bullae, and ulcers may also appear.

Scaling occurs in healing lesions of type 1 leprosy reaction. Around subsiding erythema nodosum lesions, there could be collarette scaling.

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Residual lesions consist of hyperchromic macules, scars (resulting from ulcerative lesions), and atrophy occurring upon dissolution of the dermic infiltrate secondary to *M. leprae* elimination.

Trophic skin lesions following damage to peripheral nerves include ulcers, sclerosis, and scars.

This broad range of manifestations prompts us to take into consideration numerous skin diseases to be distinguished from leprosy in differential diagnosis.

13.1 Macule

A macule is a circumscribed, flat lesion with different surrounding skin color, which in leprosy may be due to:

- Erythema: the macule appears red in light-skinned patients, whereas in dark-skinned patients, the erythema gives the lesion a coppery color.
- Hypopigmentation: more easily detected in dark-skinned patients. The diminished pigmentation in leprosy is homogeneous and never complete; hypopigmentation is more pronounced in paucibacillary forms.
- Hyperpigmentation: the hyperchromic macule is to be regarded as a residual lesion. In the course of treatment with clofazimine, slate-gray hyperpigmentation appears in light-skinned individuals in affected areas exposed to sunlight. Macules in leprosy are:
- Permanent.
- Roundish or oval in shape.
- Nonpruriginous.
- Without vesicles.
- Chronic in evolution.
- Resistant to topical therapies.

13.1.1 Erythematous Macule

The erythematous macule is the only basic lesion which may be present in every clinical form of leprosy.

The single macule of indeterminate leprosy (I) is the most difficult lesion to diagnose; sensitivity and sweating are normal or slightly decreased. Only histopathology may help in diagnosis.

Large macules with asymmetric arrangement, in the paucibacillary hyperergic forms (TT, BT), where the surface appears rough and dry, show evidence of early reduction in sensitivity, sweating, and hair growth. These lesions can develop a ring-shaped appearance.

Patients presenting a large number of lesions with asymmetric arrangement also present *M. leprae* on skin smear.

Large macules with symmetric arrangement, with bizarre shapes and annular arrangement, are typically present in the central part of the spectrum (BB). Here, skin smear will be positive.

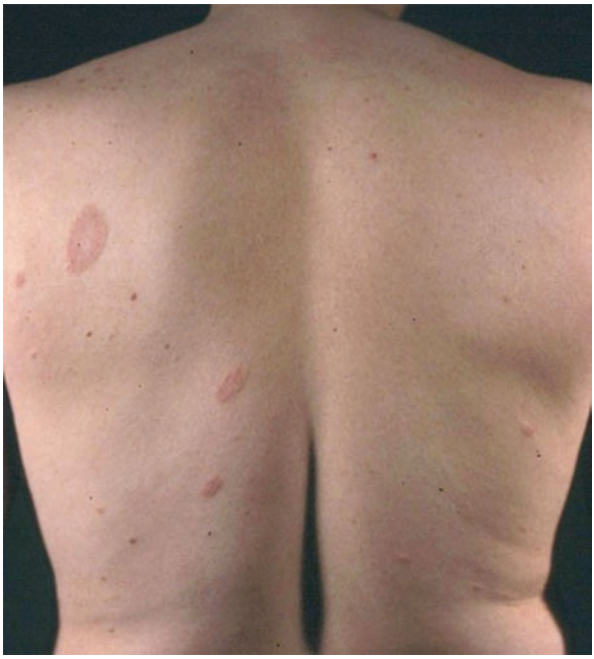

Fig. 13.1 Gibert's pityriasis rosea. Annular herald patch and few small secondary macules

Numerous small macules without anesthesia with symmetric arrangement are typical of multibacillary forms (BL, LLs). The diagnosis is confirmed by the presence of AFB.

Single or groups of a few macules of indeterminate or tuberculoid leprosy must be distinguished from the macules occurring in fixed drug eruption, early-stage morphea, Lyme disease, and cutaneous mycosis and from the herald patch of Gibert's pityriasis rosea (Fig. 13.1).

The clinical evolution enables the formulation of the diagnosis: hyperpigmentation with self-resolution (fixed drug eruption), sclerosis (morphea), and erythema migrans (Lyme disease). Tinea corporis presents peripheral fine scaling, microvesicles, and itching.

The numerous small macules with symmetrical arrangement of multibacillary leprosy must be distinguished from syphilitic roseola by positivity of lues serological tests. Gibert's pityriasis rosea has erythematous macules with peripheral scaling and heals by autoresolution. Tinea corporis disseminata should also be considered in differential diagnosis.

Ring-shaped and circinate lesions with asymmetric arrangement are typical in the hyperergic form of the leprosy spectrum, presenting anesthesia and having raised edges; they must be differentiated from numerous other diseases. Absence of desquamation and vesiculation, together with lack of itching, enables exclusion of many dermatologic diseases. In differential diagnosis, the following must be included: tinea corporis (Fig. 13.2), Gibert's pityriasis rosea, lichen ruber planus, erythema annulare centrifugum, erythema multiforme, and subacute lupus

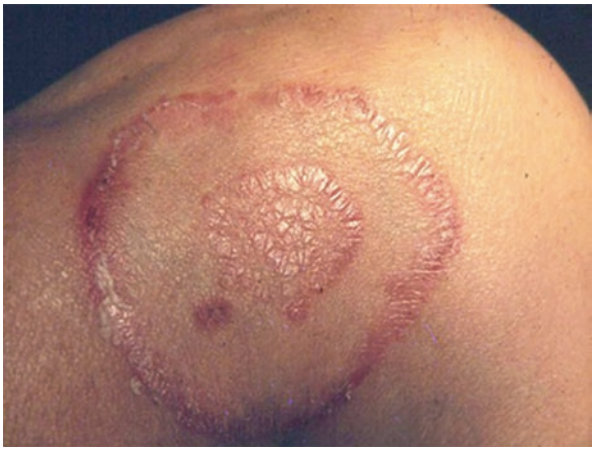

Fig. 13.2 Tinea corporis, large target lesion. Note vesicles on the external border, not present in leprosy. Target lesions can be observed in the BB form of leprosy, which presents skin lesions with symmetric arrangement and acid-fast bacilli

erythematosus. In the early stage of trypanosomiasis (*Trypanosoma gambiense*), erythematous annular patches occur on limbs and upper thorax.

13.1.2 Hypochromic Macule

Due to color loss which can be present in leprosy, macules are hypochromic, so in differential diagnosis, each skin disease with achromic macules has to be excluded: achromic nevus, nevus anemicus, and vitiligo. These diseases may coexist with leprosy in the same patient [2].

The hypopigmented variety of pityriasis versicolor, often localized on the upper trunk, shoulders, and arms, is more easily observed in Western countries during the warmer months of the year. In the active phase, hypochromic macules are characterized by pityriasiform scaling. When observed under Wood's light, macules show pale yellowish fluorescence. The shape and size of macules are variable as a result of coalescence of initial small lesions.

Pityriasis alba is rather common in children, as cutaneous manifestation of atopy (Fig. 13.3). Clinically, it appears with roundish, oval patches with faded margins. It is erythematous in the early stages. Later the erythema subsides, leaving a hypopigmented lesion with dry surface and occasionally fine scaling. The face is the most heavily affected area, but lesions may also appear on the upper part of the thorax, arms, buttocks, and thighs (Fig. 13.4). The macule (either single or multiple) shows diameter of less than 2 cm when located on the face and larger when occurring on the trunk. The eruption persists for months, with the appearance of new lesions and self-resolution of older ones. In Western countries, the lesions become more evident in summer because of the pigmentation of surrounding skin.

Oval or roundish hypopigmented macules may constitute a residue of inflammatory lesions (postinflammatory hypopigmentation) of either bacterial (impetigo), fungal (tinea corporis), or viral infections (herpes zoster), or allergic contact dermatitis.

Early post-kala-azar dermatitis may consist in hypopigmented macules occurring simultaneously with bilateral distribution in several areas of the body (thorax, back, arms, and neck).

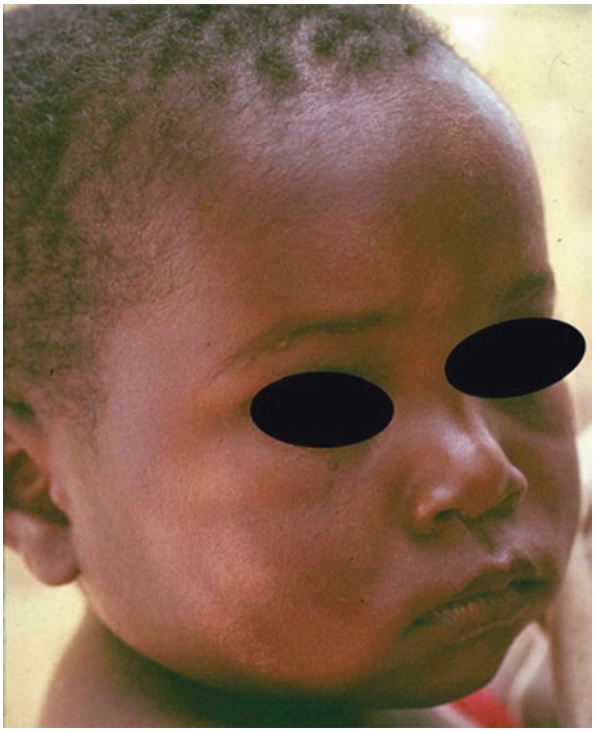

Fig. 13.3 Pityriasis alba

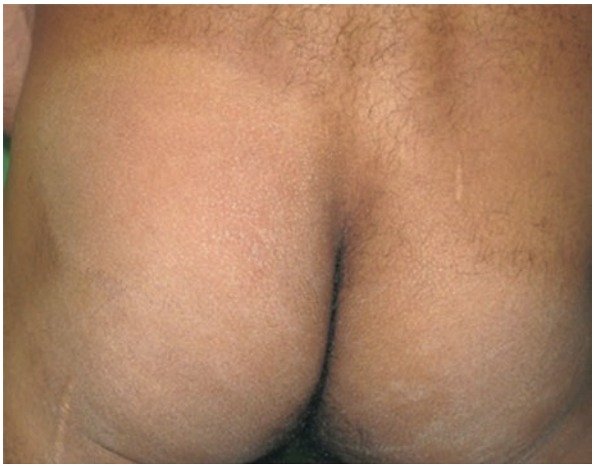

Fig. 13.4 Pityriasis alba. Sensitivity in hypochromic lesions is present

13.1.3 Hyperchromic Macule

This can be a residual sign of cutaneous involvement in leprosy. Case history, along with the possible presence of active leprosy lesions at the same time, can clarify the diagnosis.

Some cases of tuberculoid leprosy have been reported as "primary hyperchromic" macules by authors on account of the successful therapeutic response [3].

Differential diagnosis may include residual lesions of postinflammatory hyperpigmentation, Kaposi's sarcoma, morphea, erythema dyscromicum perstans, and fixed drug eruption. One may take into consideration tinea nigra, clinically characterized by brownish macules localized on palms and soles, and the hyperchromic form of pityriasis versicolor. In epidermomycosis, detection of fungus with KOH enables clear diagnosis.

13.2 Papule

A papule is a circumscribed, superficial, solid, elevated lesion. In hyperergic forms (TT, BT), they assume asymmetric distribution.

Erythematous/coppery papules are frequently associated with other basic lesions, favoring polymorphous clinical aspects.

Red or coppery papules are grouped together along the edges of the ring-shaped lesions, forming clinical aspects to be distinguished from Leiker's granuloma multiforme (Fig. 13.5) [4], localized or widespread granuloma annulare (Fig. 13.6), lichen ruber planus, late secondary syphilis, post-kala-azar dermatitis, and secondary late yaws (Fig. 13.7).

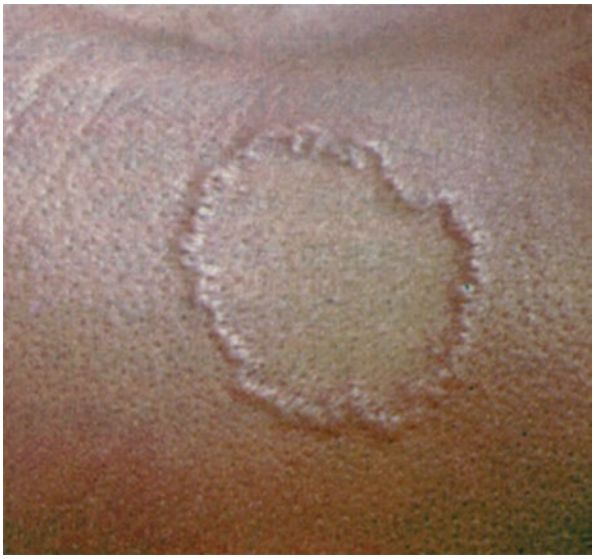

Fig. 13.5 Leiker's granuloma multiforme. This lesion was easily confused with TT leprosy before being identified by Leiker. Clinically, there is no impairment of sensitivity

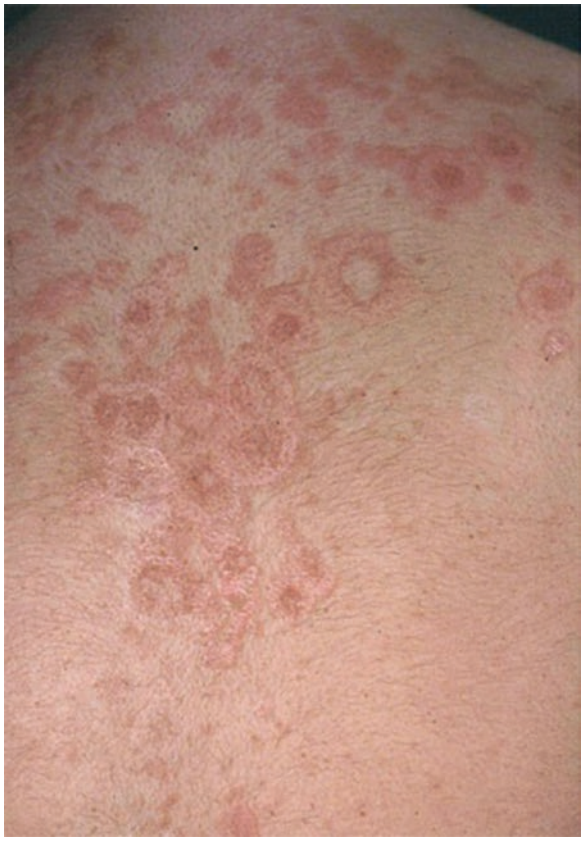

Fig. 13.6 Widespread granuloma annulare. Annular aspect, symmetric distribution; the differential diagnosis is BB leprosy, but—of course—in lesions of granuloma annulare, there are no AFB

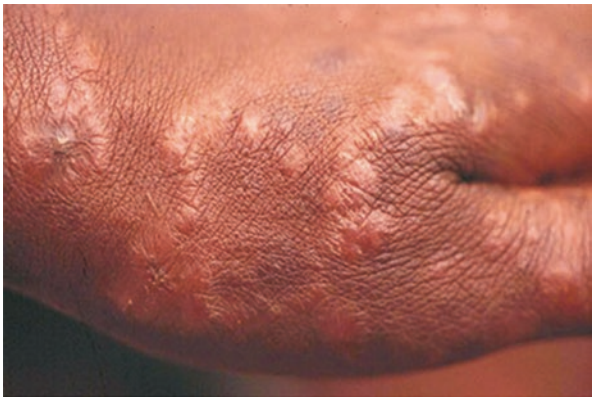

Fig. 13.7 Secondary late yaws. Asymmetric bilateral lesions; the differential diagnosis is with BT leprosy. Clinically, there is no impairment of sensitivity

13.3 Nodule

A nodule is a circumscribed, solid lesion that in leprosy is typical of multibacillary forms. The nodule is due to an inflammatory infiltrate in the whole dermis except the papillary layer.

Nodules characterize the anergic forms (BL, LLs) and have symmetric distribution (except in some anecdotal cases), and many AFB are present. These nodules have slow onset and can be red/coppery or of normal skin color. Edges are vague, are firm in consistency, and when they protrude are defined as tuberous lesions.

The differential diagnosis may include Recklinghausen disease (Fig. 13.8), sarcoidosis (Fig. 13.9), Oriental sore (cutaneous leishmaniasis), anergic cutaneous leishmaniasis, post-kala-azar dermatitis [5], onchocerciasis (Figs. 13.10 and 13.11), mycetoma, keloids, Kaposi's sarcoma, and skin lymphoma [6].

Wade's "histoid" leprosy is characterized by the presence of scattered nodules which are hard in consistency and distinctly delimited from the surrounding skin.

These lesions must be differentiated from several diseases, including dermatofibroma, anergic cutaneous leishmaniasis, molluscum contagiosum, and keloids.

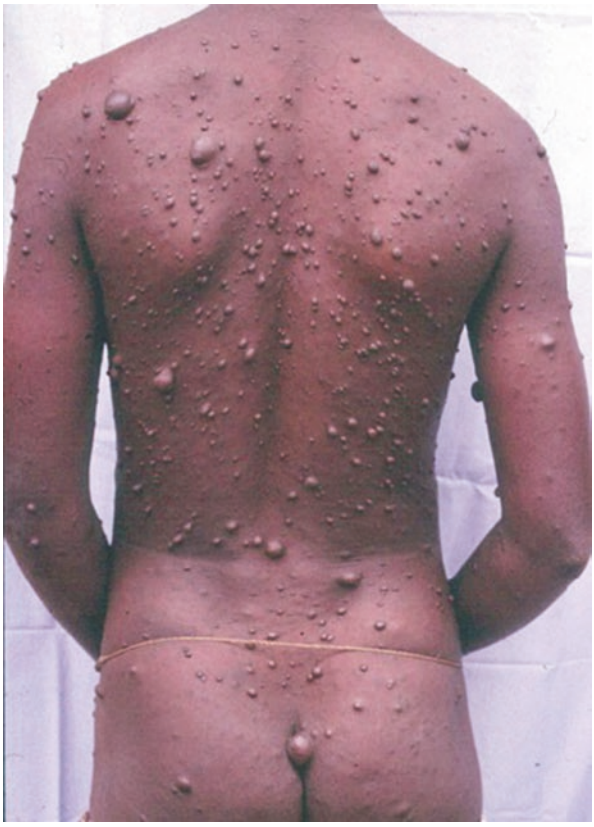

Fig. 13.8 Recklinghausen disease. Cutaneous neurofibromas: soft, sessile, dome-shaped, pedunculated

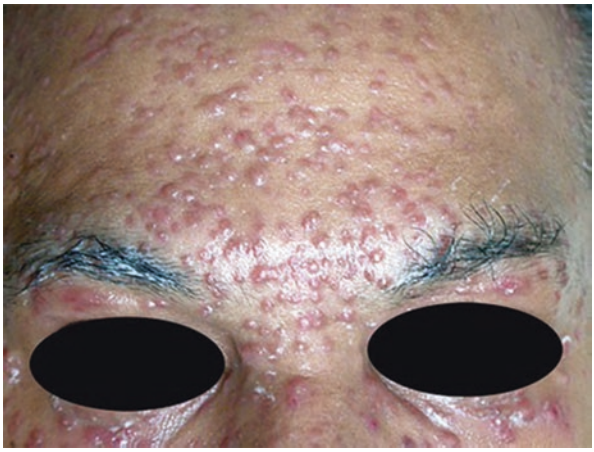

Fig. 13.9 Sarcoidosis. Symmetric distribution of nodules; there are no AFB in lesions

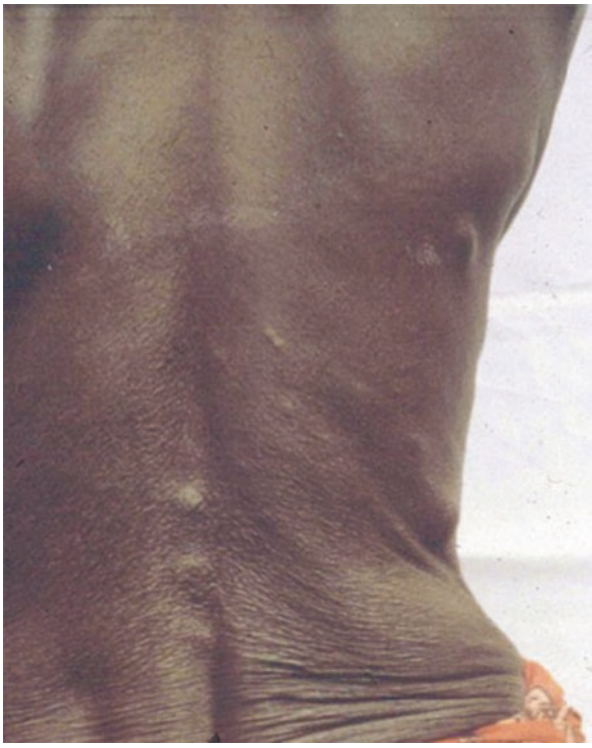

Fig. 13.10 Onchocerciasis. Subcutaneous nodules located on bone prominences; severe pruritus is present; from Nunzi E, Leiker DL (1990) Manuale di Leprologia. AIFO-Italia, Bologna

The nodules of acute erythema nodosum leprosum (ENL), which are localized in the dermis and hypodermis, must be differentiated from nodular vasculitis, having different etiology. The diagnosis could be difficult when ENL represents acute onset of leprosy. Medical history and AFB presence in "cooler" areas of the body (earlobes, extensor aspect of elbows and knees, and upper side of fingers) will help in diagnosis.

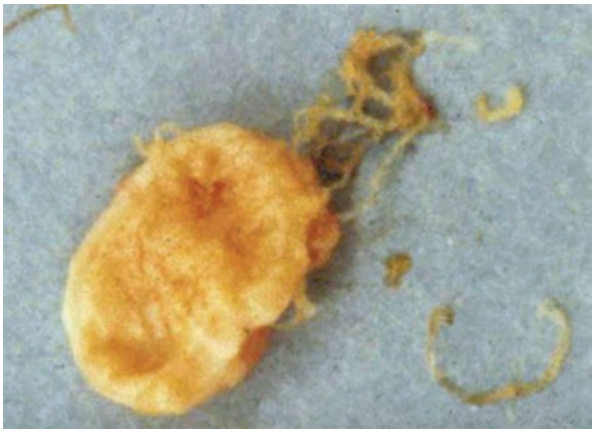

Fig. 13.11 Onchocerciasis. Section of nodule containing adult filariae

13.4 Plaque

A plaque is an elevated, roundish area caused by either spread and coalescence of papules and nodules or central granulomatous infiltration of a macule.

In the hyperergic part of the spectrum, plaques can assume annular appearance with central healing.

Differential diagnosis may include cutaneous leishmaniasis, lupus vulgaris, atypical mycobacterioses, late secondary syphilis, sarcoidosis [7, 8], and mycosis fungoides (Fig. 13.12).

In these lesions, it can be difficult to determine the loss of sensitivity, while presence of AFB and histopathological examination permit diagnosis.

13.5 Diffuse Infiltration

Diffuse cutaneous infiltration is a characteristic of polar lepromatous leprosy (LLp) and must be differentiated from skin lymphoma, actinic reticulosis, and diffuse cutaneous leishmaniasis.

In diffuse infiltration of the skin due to leprosy, slit-skin smear is positive.

13.6 Regional Manifestations

13.6.1 Eyebrows

In polar lepromatous leprosy (LLp), eyebrow involvement is characterized by diffuse infiltration with madarosis, and these features must be differentiated from other infiltrating pathologies: skin lymphoma and follicular mucinosis.

In hypothyroidism and secondary syphilis, thinning out of the external part of eyebrows can be noted, while generalized hair loss can be seen in alopecia areata totalis.

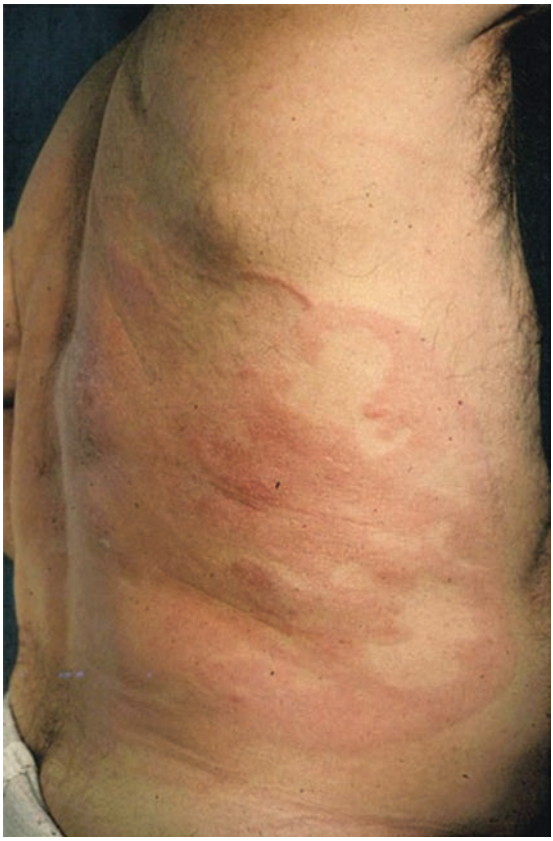

Fig. 13.12 Mycosis fungoides

13.6.2 Ear

The ear may be affected by isolated nodules or diffuse infiltration in lepromatous form. Differential diagnosis must be made with skin lymphoma and diffuse cutaneous leishmaniasis.

Infiltration of the earlobe may also be observed in lupus vulgaris and in lupus erythematosus.

13.6.3 Nose

In advanced stages of LLp, massive bacterial infiltration of the upper respiratory mucosa and of the nose can lead to collapse of the nasal pyramid. Differential diagnosis must be made in relation to mucocutaneous leishmaniasis and relapsing polychondritis (Fig. 13.13). Tertiary yaws may be characterized by an ulcerous and mutilating rhinopharyngitis (gangosa), but in this disease, serological tests for lues are positive

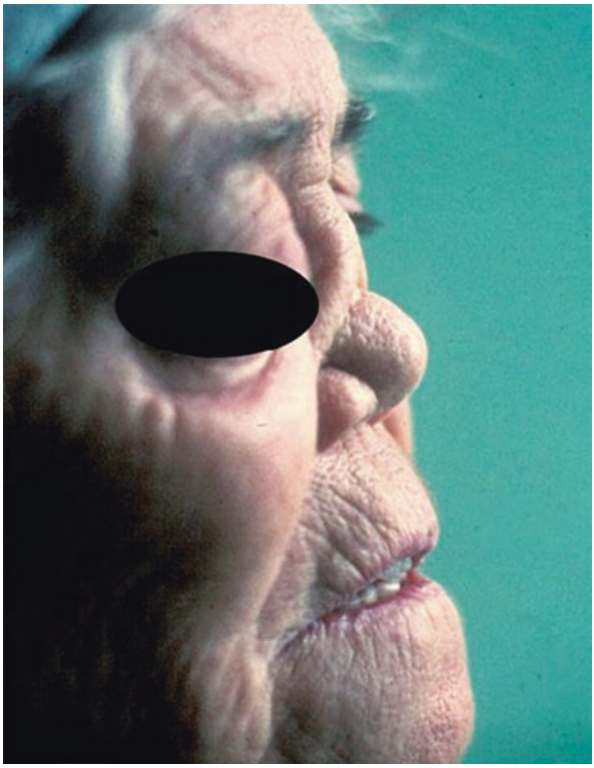

Fig. 13.13 Relapsing polychondritis may cause destruction of the nasal cartilage, resulting in a pseudo-saddle nose; from Crovato F in Leiker DL, Nunzi E (1986) Leprosy in the light skin. An illustrated Manual. AIFO-Italia, Bologna

13.6.4 Hands

Hands may be the site of specific localization of *M. leprae*. The skin of the back of the hands may be affected by diffuse infiltration in LL, resulting in atrophy after effective treatment.

Localization of *M. leprae* in the phalanx, occurring in LL, may lead to dactylitis and pathologic fractures of bones.

The most frequently observed consequences on the hands of leprosy patients are due to involvement of autonomic, sensitive, and motor branches of peripheral nerves. Dryness of the skin, anesthesia, and muscular paralysis favor the occurrence of traumatic cutaneous lesions that may lead to infections involving subcutaneous tissues and bones with ulcerations, fistulae, and scars.

Such polymorphous clinical aspect is included in differential diagnosis with syndromes affecting the peripheral nerves of the hands such as carpal tunnel syndrome, cervical rib syndrome, Dupuytren's disease, and progressive systemic sclerosis.

13.6.5 Lower Limbs

The lower limbs may also be the site of lesions of leprosy. Like the hands, most of the lesions on the lower limbs are due to involvement of peripheral nerves.

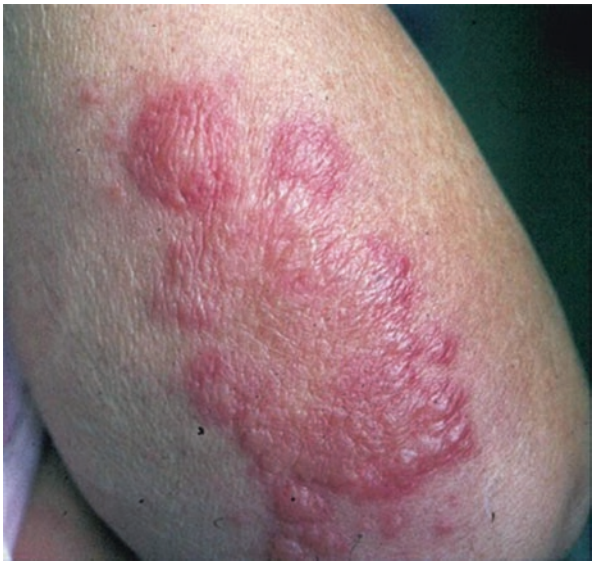

Fig. 13.14 Sweet's syndrome

In differential diagnosis, diseases which are characterized by trophic lesions, such as ulcers and bone reabsorption, must be considered.

Several diseases presenting ulcers and other disabilities on the lower limbs should be taken into account, including atypical nontubercular mycobacterioses, cutaneous tuberculosis, syphilis, cutaneous leishmaniasis, neoplasia, pyoderma gangrenosum, necrobiosis lipoidica, arterial hypertension, chronic venous insufficiency, sickle cell anemia, hereditary neuropathies, and diabetes mellitus.

13.7 Skin Manifestations in Leprosy Reactions

Type 1 leprosy reaction does not usually raise the question of differential diagnosis, since it often occurs in patients under treatment and is clinically characterized by modifications of preexisting lesions which involve an acute inflammatory phenomenon. In some cases, the patient may seek medical attention, for the first time, during type 1 leprosy reaction. This reaction must be differentiated from pathologies which begin acutely with inflammatory plaques, such as erysipelas and Sweet's syndrome (Fig. 13.14). In these cases, examination for AFB is negative. Diagnosis must be based on investigation of neuropathy and on histopathology.

Erythema nodosum leprosum (ENL), the main cutaneous expression of type 2 leprosy reaction, is characterized by acute eruptions of nodules. In BL, ENL may appear with plaques which are depressed in the center and becoming hyperpigmented. Differential diagnosis must be made with fixed drug eruption and nodular vasculitis, which have different etiology. Wheals caused by immunocomplexes can appear in type 2 leprosy reaction.

In acute onset of leprosy, anamnesis and AFB investigation will help in diagnosis.

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Part IV

Leprosy and Nerves

Peripheral Nerves in Leprosy

14

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and José Antonio Garbino

14.1 Normal Nerves

Peripheral nerves arise from the spinal cord and connect the central nervous system (CNS) with the rest of the body. They contain axons and dendrites from several different types of neurons, serving various effector organs and sensory endings. Sensory nerves are outgrowths from cells of the spinal ganglia, which are derived from the neural crest. The neuron cell bodies innervating the muscles are situated in the anterior horns of the grey matter in the spinal cord, for the face in the brainstem. The cell bodies contain the nuclear and the perinuclear cytoplasm in which most of the cell organelles are produced and consequently proteins are synthesized, not only for the cell bodies but for the axons and dendrites as well [1]. Afferent (sensory) nerve fibres carry information to the CNS, while efferent (motor) fibres carry impulses from the CNS to the target organs and tissues. Mixed nerves have both types of nerve fibres and represent the majority of adult peripheral nerves. In this chapter, the term axon will be used for both axon and dendrite, only when appropriate they will be differentiated.

The nerve trunks and their peripheral branches are composed of more or less parallel bundles of nerve fibres, which are in fact braided and intertwined. These are axons ensheathed by Schwann cells forming the myelin sheath: unmyelinated and

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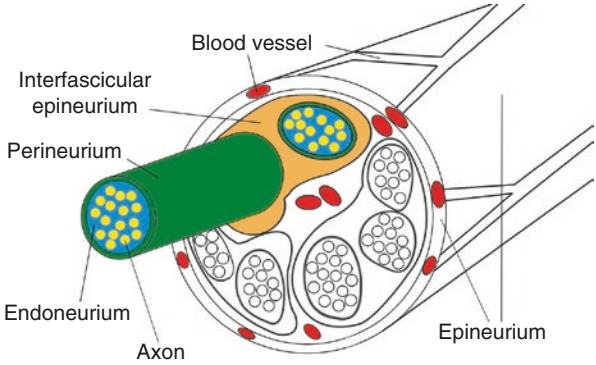

Fig. 14.1 Anatomy of the nerve (Reproduced with permission, © Bernard Naafs 2021. All Rights Reserved)

myelinated nerve fibres. The myelinated nerve fibre consists of one axon and a single row of Schwann cells. Schwann cells wrap tightly around the axon, the node of Ranvier between each of the Schwann cells. Non-myelinating Schwann cells envelop multiple small diameter axons, each axon located in a cavity of the Schwann cell and some of them may form the 'Remak' bundles. These are usually C fibre (see below) axons grouped together. The Schwann cell keeps them from touching each other by squeezing its cytoplasm between the axons. The Schwann cells provide support and nutrition to the axons, ensuring their survival [2]. In between and around these axons and their Schwann cells is the endoneurium, a loose delicate connective tissue, containing blood vessels, however no lymph vessels (Fig. 14.1). Within the trunk, the fibres are grouped together in fasciculi, which may contain a few to hundreds of fibres. The size, number and pattern of the nerve fibres vary in different nerves, and at different points of the nerve, the fasciculi are interwoven [1, 3].

A dense, well-vascularized, but irregular connective tissue sheath, the epineurium, surrounds the whole trunk. A smaller, similar, but less fibrous, perineurium encloses each fascicule. A peripheral nerve trunk contains numerous fibres, some originating from anterior horn cells, carrying motor impulses, and others that are peripheral dendrites of dorsal root ganglia, carrying pain, itch, pressure, temperature and stretch impulses. Some are part of the autonomic nervous system [1, 3].

Based on the total fibre diameter and the speed of impulse conduction, nerve stem fibres are classified as A, B or C fibres [1, 4]:

- Class A are myelinated somatic afferent and efferent fibres, being divided into Aα and Aβ (large-diameter motor and sensory fibres) and Aγ motor and Aδ sensory (medium-diameter myelinated fibres). Aδ activated among others by noxious sensory stimuli.
- Class B are myelinated preganglion fibres of the autonomic (sympathetic and parasympathetic) nervous system.
- Class C are nonmyelinated autonomic and sensory fibres, activated by internal autonomous stimuli and noxious sensory stimuli [5, 6].

14.2 Myelinated Fibres

The diameters of myelinated fibres vary in human peripheral nerves between 2 and 22 μm (microns). There is a direct relationship between the myelin thickness and the axon diameter. Myelin is a specialized extension of the Schwann cell surface membrane. It is formed when tongues of the Schwann cell extend and start to wrap around the axon. The cytoplasm is expelled from the wrap when the layers of the outer membrane of the Schwann cell fuse and form myelin layers. The myelin sheath is a multilamellar structure rich in glycosphingolipids, long-chain saturated fatty acids and cholesterol. Cholesterol is the predominant lipid among the molecular constituents of peripheral myelin (20–30%), regulating its fluidity, permeability and formation speed [7, 8].

The myelin sheath along the axon is divided into internodes, each consisting of a single Schwann cell with its myelin extension wound around the axon. In between the internodes are bare parts of the axon called nodes of Ranvier (Fig. 14.2). The distance between the nodes varies between 0.2 and 1.8 mm. The Schwann cell is important for the integrity of the axons. Along myelinated axons, the stimulus is passed by saltatory conduction from node to node. When the internodes are longer, the conduction is faster [1, 3].

14.3 Nonmyelinated Fibres

Most of the axons in a normal mixed nerve are small unmyelinated fibres, less than 3 μm in diameter [1]. Unlike myelinated fibres, a single Schwann cell is connected with more than one axon (Fig. 14.2). The Schwann cells are arranged in series along

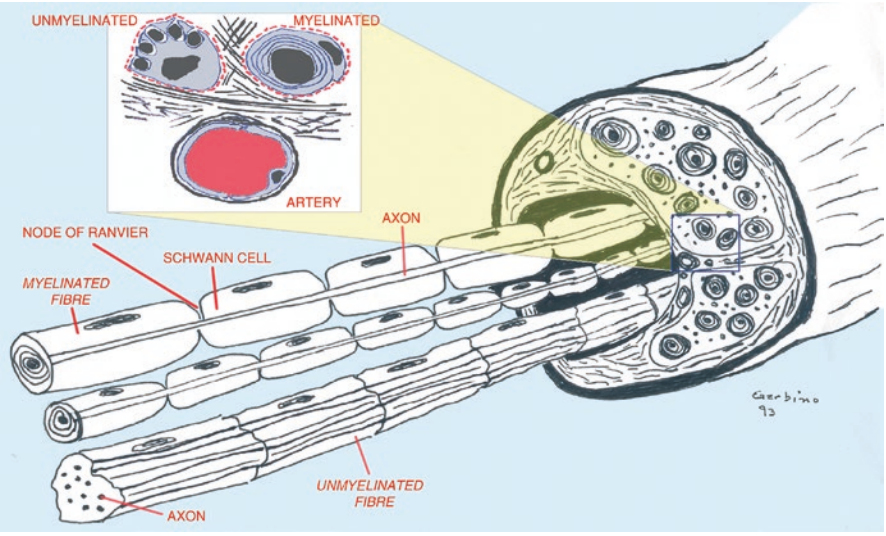

Fig. 14.2 Myelinated and unmyelinated fibres (Courtesy of J.A. Garbino)

groups of unmyelinated axons. The Schwann cells interdigitate with each other. Conduction occurs along the surface of the axons and is much slower than the saltatory conduction of the myelinated axon [4].

14.4 Schwann Cells

Schwann cells constitute 90% of the endoneural cells. In normal nerves, they are all associated with axons. Myelinated and unmyelinated axons have different relationships with the Schwann cells. Schwann cells, when damaged, proliferate in response to growth factors. The terminal differentiation of Schwann cells is regulated by their relationship with the axons of different calibres. Axons of larger diameters express high levels of the growth factor neuregulin-1 (NRG1) and promote myelinating differentiation, whereas small-diameter axons have lower levels of NRG1, leading to differentiation in non-myelinating Schwann cells [9]. Precursor Schwann cells provide trophic support to the axons, thus establishing a state of interdependence between them [10].

The differentiation of immature Schwann cells is essentially stimulated by the growth factors NRG1 and fibroblast growth factor 2 (FGF2) [11]. The activation of pro-myelinating pathways also has NRG1 as one of the best-characterized extrinsic signals [12], although the myelinating phenotype in Schwann cells involves the inactivation of a number of immature cell markers such as GFAP, L1-CAM, NCAM-1 and p75NTR [13].

The proper maturation of Schwann cells is ensured by a group of key transcription factors including Sox-10 (SRY-box 10), Oct-6 (POU class 3 homeobox 1) and Krox-20 (early growth response 2/EGR2) [14, 15]. Krox-20 is a fundamental inducer of the expression of myelin proteins, MBP (myelin basic protein), MPZ (myelin protein zero/P0), MAG (myelin-associated glycoprotein) and PRX (periaxin) [16]. However, during remyelination the internodes are shorter resulting in slower conduction. Besides being important in the conduction of (electric) stimuli, Schwann cells are phagocytic (defence) cells.

14.5 Nerve Sheaths

There are three connective tissue structures in and around a peripheral nerve (Fig. 14.1). The outermost layer, the epineurium, is a relatively loose connective tissue that binds the fascicles into a single nerve trunk. Within it (and around a third connective tissue, the endoneurium) is the second connective tissue layer, the most organized, namely, the perineurium. This consists of layers of flattened cells ranging from 1 to 12 layers depending on the size of the fascicle. It is a sheath that protects the whole of the peripheral nerve system. Each layer is separated from each other by longitudinally arranged collagen fibres with occasionally a fibroblast. Within the layer, adjacent perineurial cells, which are epithelioid myofibroblasts,

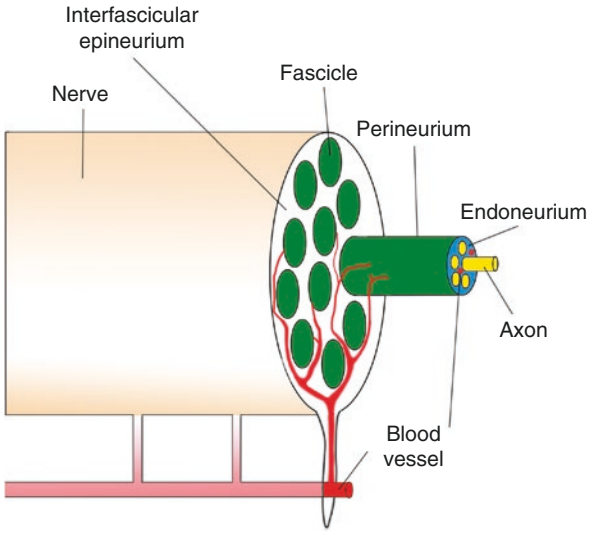

Fig. 14.3 Vasa nervorum (Reproduced with permission, © Bernard Naafs 2021. All Rights Reserved)

are attached to each other by tight junctions forming a semipermeable barrier, impermeable to large molecules such as proteins [1, 3].

14.6 Vasa Nervorum

The nutrient arteries to the nerves arise from the nearest large arterial trunk (Fig. 14.3). They bifurcate into descending and ascending branches and penetrate into the epineurium, thereafter entering the endoneurium at regular intervals obliquely through the perineurium. The venules follow the same routes (see Chap. 21, Fig. 21.11) [1, 17]. Only arterioles, capillaries and venules are found within the endoneurium; there is no lymphatic system.

The endothelial cells of the endoneural vessels, like the endothelial cells of the central nervous system, are connected by tight junctions forming a barrier like the cerebral blood-brain barrier. In contrast, epineural vessels have open junctions and may be fenestrated [1].

14.7 Pathology of Nerve Damage

14.7.1 Axonal Degeneration

When an axon or part of an axon is separated from the cell body due to compression or direct injury, this peripheral part will degenerate [17, 18]. Damage to a myelinated axon will lead to secondary changes in its myelin sheath. However, when the myelin sheath is primarily damaged, the axon is usually spared at the beginning. Damage may result from direct physical injury, compression or immunologic

factors, or be the result of ischaemic, toxic, or metabolic injury [17, 18]. The myelin sheath is, among other things, the actual protection of the axon, and if damaged, repair is started immediately. A damaged axon itself may also start to regenerate. The living proximal part of the axon sprouts to the denervated target organs. This is known as collateral sprouting. The damaged axon can sprout from its proximal portion, which is known as terminal sprouting. Axonal sprouting may cover 1 mm/day.

14.7.2 Damage to Schwann Cells

In normal situations, myelin turnover is controlled temporally and spatially [19] by an event described as adaptive myelination, in which the 'old' myelin is replaced to modulate neural circuits [20, 21]. Damage to Schwann cells may lead to demyelination, i.e. loss of myelin from nerve fibres leaving the axon intact. This demyelination may occur due to toxic or metabolic damage, compression and ischaemia, due to the complement system (MAC), or to macrophages stripping away the myelin [17, 18]. This may be segmental; i.e. some internodal segments are affected, while others are spared. Usually, the Schwann cell body remains viable, proliferates and may take part in remyelination. Remyelination requires the activation of the damaged Schwann cells, which dedifferentiate, reprogram and generate molecular events that culminate in the repair of neural tissue [22]. The repairing role of Schwann cells in peripheral nerves involves their myelinophagic activity, in addition to the positive regulation of neurotrophic factors [19]. Once this process has been successful, Schwann cells must return to their normal phenotype [22]. Usually, one internode is replaced by two or three, leading to slower stimulus conduction. The myelin sheath usually remains thinner than before.

14.7.3 Neuronal Degeneration

When axons or dendrites are lost, the damage is definitive, as is seen in many other neuropathies, e.g. amyotrophic lateral sclerosis (ALS), syphilis and alcoholism. However, the Schwann cells may form Büngner bands, which can direct the growth of interrupted axons [16, 19]. However, in leprosy, the smaller nerves are often so scarred that hardly any re-enervation can occur.

14.7.4 Perineurial Pathology

This is usually accompanied by an increased number of lamellae of perineurial cells, with at a later stage fibrosis and loss of perineurial cells. The main cause is inflammation, which may be due to infection or to metabolic or toxic stimuli. Thickening leading to compression may be due to increased collagen and often concomitant oedema. The former may occur in lepromatous leprosy, and the latter during leprosy reactions [17, 18].

14.7.5 Leprosy Neuropathy

In leprosy, the Schwann cell seems the primary target. *M. leprae* can be found in the nervous system as high as the sensory dorsal root ganglia but not higher up in the spinal cord or brain [23]. Major involvement is at cooler sites, at areas of movement and angulation. According to Stanley and the late Weddell (personal communications), this movement and angulation, leading to microtrauma, are prerequisites for establishing endoneural infection. Functional impairment of cutaneous nerves due to the infection and the host response is a very early feature of leprosy. Sensory abnormalities are already present in the earliest diagnostic clinical lesions; there is loss of thermal perception and sensitivity to pain, followed by an increase in the tactile and pressure detection threshold [24]. The cutaneous branches of peripheral nerves are affected in a single or multiple pattern (mononeuropathy or multiple mononeuropathy) [25]. The major question however is how the mycobacterium enters the nerve to infect Schwann cells.

Fite stated in 1943 that no leprosy existed without nerve damage [26]. In 1977, Shetty et al. showed that even clinically uninvolved nerves could show some pathology [27]. Antia hypothesized that contacts might also show some nerve involvement (personal communication). In 2017, during the Brazilian leprosy congress, Diogo Fernandes dos Santos from the group of Goulart (Uberlândia, MG, Brazil) reported demyelination in contacts of leprosy patients, both in PGL-1 positive as well as in PGL-1 negative [28]. They treated these contacts, but it would have been interesting to see if all these 'patients' would have developed leprosy. At the World Leprosy Congress (Manilla 2019), Glauber Voltan and Marco André Frade revealed to have found enlarged nerves in contacts using ultrasound (personal communication). The enlargement was related to the amount of exposure.

Thus, in small subcutaneous nerves, abnormalities, though minimal, are even detectable after only contact with *M. leprae* alive or dead [29]; nevertheless, when the disease develops, the process is a chronic one, with a natural course over years or even decades and histological evidence of nerve fibre degeneration and regeneration with collateral sprouting of axons and dendrites. If not interrupted by treatment or spontaneous healing, the end results of *M. leprae* infection and the host response in nerves are demyelination, nerve fibre degeneration and fibrosis [23], leading to functional deficits, when more than 20% of the nerve fibres are damaged.

The first essential step in understanding leprosy neuritis is the presence of *M. leprae* and/or its antigens in peripheral nerves. The original description of an ascending infection was extrapolated to propose that *M. leprae* initially bind to exposed Schwann cells in the dermis, and then move up proximally within the nerve, 'swimming like fish upstream' [23, 30]. Until recently, this was a well-known concept, although not generally accepted because it is inconsistent with several basic features of the biology of *M. leprae* and of peripheral nerves; e.g. how would this nonmotile mycobacterium navigate between Schwann cells, within the endoneurium with its absence of lymphatic vessels?

However, recent studies of peripheral nerves in experimentally infected armadillos and nude mice suggest strongly that *M. leprae* infects nerves from the

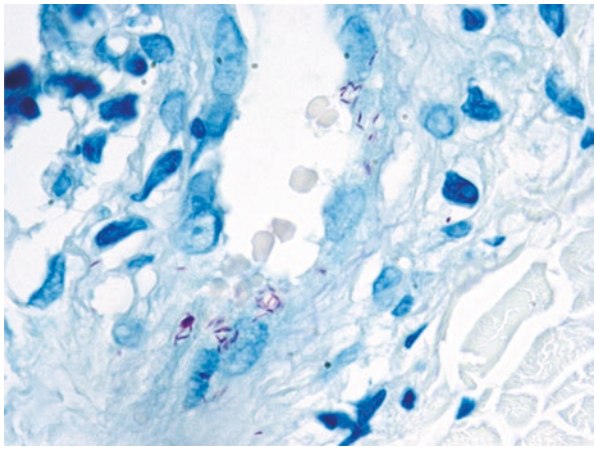

Fig. 14.4 *M. leprae* in endothelial cells (Fite-Faraco stain) (Reproduced with permission, © Enrico Nunzi 2021. All Rights Reserved)

outside, first being present in the epineurium. Initially, *M. leprae* is present in the lymphatics and blood vessels of the epineurium and most likely enter the endoneural compartment through its blood supply [31]. The perineurium is impermeable for the bacterium and there are no lymphatics in the endoneurium. This view, according to Scollard, gives new significance to old observations of substantial *M. leprae* infection of endothelial cells (Fig. 14.4) which have been largely overlooked during recent decades. He adds that 'in addition, it is likely that the characteristic perineural inflammatory infiltrates of leprosy are the "footprints" tracking the route of infection of the nerves' [30]. The mechanisms responsible for the apparent selectivity of *M. leprae* for the vasculature of peripheral nerves are not known, but are topics of current research [30]. As mentioned before, Weddell suggested that microtrauma could be an important cause, and Stanley was only able to get an infection into nerves when some trauma had occurred (personal communications). More recently, magnetic resonance imaging (MRI) has allowed detailed evaluations of peripheral nerves in leprosy, highlighting nerve thickness showed as hyperintense signals on T2-weighted images[1] [32]. T2 hyperintensity and nerve enlargement are attributed to myelin loss, inflammation and microtrauma [32, 33]. Trauma may result in adhesion molecules on the endothelial cells of the vessels in the endoneurium and allow bacterial and antigen-loaded macrophages to enter by diapedesis [34].

The entry of *M. leprae* into the Schwann cell has also been a matter of debate. At the beginning, the Schwann cell was seen as a phagocytic cell by itself (Fig. 14.5); later the role of *M. leprae* was considered to be more active. It was shown that *M. leprae* binds to the G domain of the laminin α2-chain which is expressed on the Schwann cell surface. Consequently, αβ-dystroglycan (DG) in the basal lamina of the Schwann cell then acts as a receptor for the laminin α2-*M. leprae* complex [35]. Other receptors may also be involved in the *M. leprae*-Schwann cell interaction,

[1] T2-weighted image (T2WI) is one of the basic pulse sequences in MRI. The sequence weighting highlights differences in the T2 relaxation time of tissues.

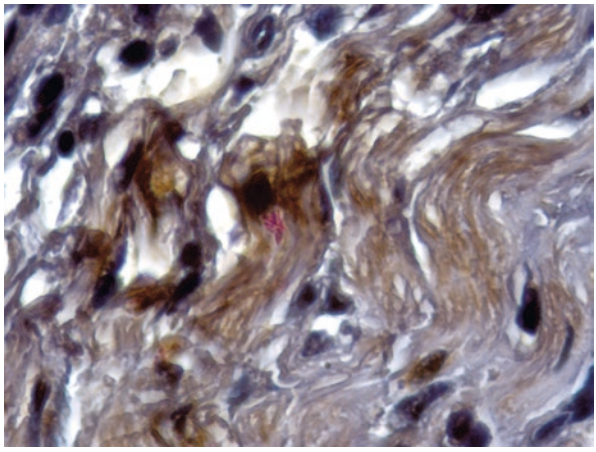

Fig. 14.5 *M. leprae* in Schwann cell. Double staining of human peripheral nerve: Schwann cells (S100 protein) are detected by immunohistochemistry and *M. leprae* with Fite-Faraco staining (×1000 oil) (Courtesy of A. Clapasson)

since blocking of the DG complex does not completely inhibit adhesion of *M. leprae* [31, 36]. Schwann cell colonization was shown to be achieved by the internalization of *M. leprae* via ErbB2/B3 receptors (tyrosine kinase B2/B3 receptor), present on the surface of Schwann cells [37]. The phosphorylation of ErbB2/B3 induces the hyperactivation of ERK1/2 (extracellular signal-regulated kinases), perpetuating a differentiated and proliferative phenotype in Schwann cells [38].

The next question to be answered is: Which receptor on *M. leprae* binds to laminin-2? This turned out to be a 21 kDa histone-like protein. This laminin-2-binding protein, LBP21, is one of the major surface antigens of *M. leprae*, probably serving as an adhesin for its interaction with peripheral nerves [36, 39]. This ML-LBP21 surface protein was identified as a *M. leprae* protein responsible for its interaction with Schwann cells [40], along with the specific terminal trisaccharide phenolic glycolipid-1 (PGL-1) [41].

However, this protein, LBP21, shows extensive homology with histone-like proteins of other mycobacteria. Additional evidence indicates that this mechanism of binding to the surface of the Schwann cell via a laminin-α2 is not unique to *M. leprae*. Other mycobacterial species, including *M. tuberculosis*, *M. chelonae* and *M. smegmatis*, have been shown to express a laminin-α2-binding capacity [42]. However, the terminal trisaccharide of the surface-exposed *M. leprae*-specific phenolic glycolipid-1 (PGL-1) has also been shown to bind to α2-laminin. Thus, PGL-1 may also be involved in the invasion of Schwann cells through the basal lamina in a laminin-α2-dependent pathway [37, 40]. It may act as a second receptor on *M. leprae*, in which the combined action of LBP21 and PGL-1 appears to provide sufficient binding to ensure entry of *M. leprae* into the Schwann cell. Díaz Acosta et al. [43] noted that during *M. leprae* entry, PGL-1 induces the expression of the mannose receptor (CD206) in infected cells, which activates the PPARγ receptor (peroxisome proliferator-activated gamma receptor), increasing the production of prostaglandin E2 (PGE2) and the recruitment of lipid droplets (LDs) [43]. In infectious diseases, the production of LDs supports the survival of intracellular pathogens such as *M. leprae* [44].

This is still not the whole answer. Other studies have also demonstrated the ability of myelin protein zero (P0, MPZ) to bind *M. leprae* [45], and another histone-like, laminin-binding protein (Hlp/LBP) expressed by *M. leprae* has been identified that also could play a role in the binding to Schwann and other cells [46]. More important is that *M. leprae* is also found to bind to ErbB2/B3, a Schwann cell receptor for NRG1, which seems to play a key role in the Schwann cell-axon interaction [39]. Infection of Schwann cells with whole, viable *M. leprae* does not seem to cause loss of Schwann cells, and even appears to favour Schwann cell survival rather than apoptosis [39]. However, human Schwann cells express toll-like receptor 2 (TLR2) both in vitro and in vivo. Binding of a *M. leprae*-derived lipoprotein to TLR2 on the Schwann cell has been reported by Oliveira et al. to result in apoptosis. Those investigators also identified Schwann cells that had undergone apoptosis in biopsies of human lesions, contrary to the earlier assumption [47].

Several pathological mechanisms have been proposed for nerve damage in leprosy: the interference of *M. leprae* cell wall proteins with host cell metabolism, an immune-mediated inflammation triggered by T-cell/SC interactions and a 'bystander' type of nerve injury due to the large influx of cells, cytokines and oedema during the course of immune and inflammatory responses to *M. leprae* [48, 49]. The resultant immunopathological changes manifest clinically in the form of skin lesions, ocular changes and peripheral nerve enlargement.

Save et al. noted, in addition to segmental demyelination, evidence of paranodal demyelination and atrophy, a reduction in axon calibre [50]. In subsequent studies of nerve biopsies from leprosy patients, they observed abnormal phosphorylation of neurofilament proteins in nerves from both treated and untreated patients independent of classification. Hypophosphorylated neurofilament proteins seem to be more susceptible to proteolytic degradation, and loss of these proteins may explain the observed reduction in axonal calibre. Dephosphorylation of neurofilament proteins has been recognized in a variety of other neurological disorders as well, but the mechanisms responsible in them, as in leprosy, are poorly understood [50]. Probably, up- or downregulation of genes due to infection or inflammation may be responsible.

As per one hypothesis, *M. leprae* enters a host cell and once inside manipulates the cell to create the environment in which it can survive and multiply. This mechanism has been described for viral as well for bacterial infections [51]. Whether Schwann cells have to transform into stem cell-like cells, as Rambukkana and colleagues have proposed [38, 52], may be that is going too far [53]. To identify this reprogramming of a host cell may be an early method to diagnose leprosy as a disease. *M. leprae* appears to have little effect on intact, mature Schwann cell-axon units, but does, according to Hagge et al., alter the expression of a small number of genes in Schwann cells (GFAP, TGFb1, NCAM, ICAM, N-cadherin and L1), according to their preliminary study [54]. To further evaluate the effect of *M. leprae* infection on primary human Schwann cells, Scollard quoting Diana Williams, a microarray analysis of approximately 15,000 genes has been performed, and significant changes (up- or downregulation) have been observed in several hundred genes [39]. This up- and downregulation may, on the one hand, be the mechanism through

which *M. leprae* survives in its preferential host; on the other hand, it may also be responsible for apoptosis and consequent demyelination [55].

As mentioned before, experimental studies in vitro have shown that *M. leprae* may reprogram adult Schwann cells by altering host gene expression, with the bacterially reprogrammed cells resembling progenitor or stemlike cells with mesenchymal traits. These stemlike cells acquire migratory and immunomodulatory characteristics; release chemokines, cytokines, and growth/remodelling factors; and disseminate the bacterial infection without being detected by immune cells. These reprogrammed cells possess the ability to attract macrophages, suggesting a potential role of the innate immune response in the initiation of neuropathogenesis during early *M. leprae* infection [55].

Sciatic nerves of nude mice (NU-*Foxn1*^{nu}) infected with *M. leprae* were evaluated after 6 and 9 months. In vitro results demonstrate downregulation of Krox-20 and Sox-10 along with the increase in p75NTR-immunolabelled cells. Concurrently, sciatic nerves of infected mice showed a significant decrease in Krox-20 and increase in p75NTR. These results corroborate previous findings on the interference of *M. leprae* in the expression of factors involved in cell maturation, favouring the maintenance of a non-myelinating phenotype in Schwann cells, with possible implications for the repair of adult peripheral nerves [56].

The complement system is a key component of the host defence against pathogens. Complement activation results in the cleavage of C3, followed by the cleavage of C5 and the formation of the membrane attack complex (MAC), which causes perforation of eukaryotic cell membranes, resulting in lysis of the target cell [57]. A recent study has shown that in nude-mouse sciatic nerves, intraneural injections of *M. leprae* sonicate and its components – particularly lipoarabinomannan (LAM)—result in MAC deposition, myelin loss and axonal damage [58]. Human studies have reported significant serum complement consumption by *M. leprae* [59]. In addition, MAC deposits have been observed on damaged nerves of lepromatous but not tuberculoid patients [59], suggesting that complement activation—and specifically the MAC—may function as a disease modifier during the early events of leprosy neuritis.

Nerve damage due to immunological reactions is the subject of a separate chapter (i.e. Chap. 21).

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The Leprosy Neuropathy

15

Lizia Reni, Salvatore Noto, and Pieter A. M. Schreuder

15.1 General Picture of the Leprosy Neuropathy

Most of the disabilities occurring in leprosy are sequelae of damage to the peripheral nervous system. *Mycobacterium leprae* (*M. leprae*), the causative organism of leprosy, has a peculiar affinity for the Schwann cell, where it multiplies and leads to damage of the nerve and its functions. In borderline leprosy, peripheral nerve damage is often acute and occurs during type 1 leprosy reaction (T1R); in lepromatous leprosy, it develops over years and has acute exacerbations during episodes of erythema nodosum leprosum (ENL) reaction, also called type 2 leprosy reaction (T2R). The distribution of nerve involvement is related to the position of the patient in the leprosy spectrum (cell-mediated immunity). In the hyperergic forms, tuberculoid and borderline tuberculoid leprosy, nerve damage is early and affects one or a few nerves asymmetrically. In the hypoergic or anergic forms (mid-borderline, borderline lepromatous, and lepromatous leprosy), nerve involvement tends to be late, widespread, and symmetric.

In diagnosis, it is useful to distinguish the peripheral neuropathies as mononeuropathy when affecting one nerve, multineuropathy when affecting more nerves, and polyneuropathy when nerve involvement is bilateral and symmetric. It starts as a demyelinating process and evolves to axonal damage. All three components of the peripheral nervous system are affected: sensory, motor, and autonomic. Sensory damage often occurs earlier than motor damage and, at the beginning, tends also to

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be more pronounced than motor deficit. Neural deficit may be clinically silent until 30% of the nerve fibers of the nerve are damaged [1]. Sensory loss causes anesthesia (loss of touch sensation), analgesia (loss of pain sensation), and inability to discriminate hot and cold. Motor deficit causes muscle weakness, up to complete paralysis and atrophy. Damage to autonomic nerve fibers impairs sweating and causes dry skin. Sensory, motor, and autonomic nerve damages are directly (or primarily) caused by leprosy. The complications that result from this direct nerve damage (often grouped as "anesthetic deformities" because of their relation to unprotected use of insensitive hands and feet) are called secondary impairments, e.g., ulcers, contractures, bone destruction, and shortening of fingers and toes. Secondary impairments may lead to a variety of disabilities and handicaps. In extreme cases, the leprosy patient may suffer de-habilitation and destitution [2].

15.2 Patient History and Physical Examination

15.2.1 Patient History

Patient history regarding symptoms, e.g., time of onset, type of onset (acute or chronic), parts of the body involved, if symptoms are associated with weight- bearing activities, medications used, and other medical conditions, may provide important information. The patient may refer symptoms of acute neuritis such as tingling sensation (paresthesia), or sometimes pain along the course of the nerve. He may refer numbness or motor deficit. Often, he may present already with history of advanced nerve damage, for example, weakness in closing eyes and using hands or feet, or the appearance of blisters or ulcers on hands and feet, which have occurred unnoticed.

15.2.2 Testing for Loss of Tactile Sensation on Skin Lesions

Testing for loss of sensation on skin lesions is relatively simple and, when positive, confirms diagnosis of leprosy [3]. Quietness in the environment or in the room where it is performed is important. Both the patient and the examiner must be positioned comfortably while examining. A fine, pointed wisp of cotton wool is used to touch the part to be tested. The process is first explained to the patient and then demonstrated while he watches and points carefully to the exact spot touched. When he comprehends fully, testing continues at various sites inside and outside the lesions, but with the patient's eyes covered. Only touch should be applied, not brushing across the skin. The patient with closed eyes can either point with one finger to the exact spot where the cotton wool touched the skin or he can confirm the exact place verbally when he feels the touch. Patient reliability should be tested by asking for the point of contact when not touching the skin at all. If he feels it but cannot point to the exact spot, it is called "misreference," the earliest sign of hypoesthesia [4]. Inability to identify the point stimulated at all denotes loss of sensation

to the stimulus used. Cotton wool may be too delicate to test for sensory loss in lesions on thickened skin of palms and soles; here, monofilaments or nylon bristles can be used.

15.2.3 Palpation of Peripheral Nerves

Palpation of nerves at "sites of predilection" is performed during physical examination of the patient. This is a fundamental procedure in diagnosis and follow-up of both leprosy and leprosy reactions. Nerve palpation is performed gently using the pulp of the fingers, not the fingertip or fingernail. The person's face should be observed to make sure that unnecessary pain is not caused when touching the nerve. The tenderness (spontaneous or when palpating), consistency (soft, hard, irregular), and size (enlarged, normal, small) of the nerve are evaluated. Tenderness when palpating the nerve or spontaneous nerve pain is a sign of reaction. It is essential to know the normal limits by constant practice in palpating nerves. During examination, one should compare nerves on the opposite site of the body. Enlarged nerves should be differentiated from tendons, blood vessels, or lymph nodes. In leprosy all peripheral nerves may be enlarged. Cutaneous branches associated with a skin lesion may be enlarged as well. The two most commonly affected are the ulnar nerve and the common peroneal nerve. The following paragraphs describe how to locate and palpate the peripheral nerves of predilection in leprosy. They will be described systematically, starting from the head, then those of the upper limbs, and finally those of the lower limbs.

15.2.3.1 Supraorbital Nerve

An enlarged supraorbital nerve is palpable as it passes upward out of the orbit. To palpate it, run your index finger across the forehead from the midline laterally (Fig. 15.1).

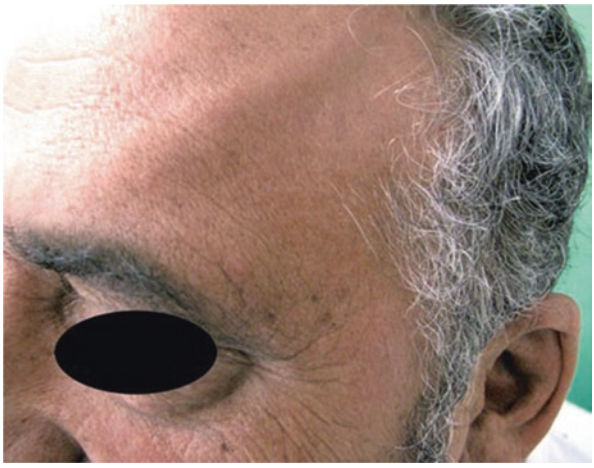

Fig. 15.1 Supraorbital nerve: enlarged (Reproduced with permission, © Enrico Nunzi 2021. All Rights Reserved)

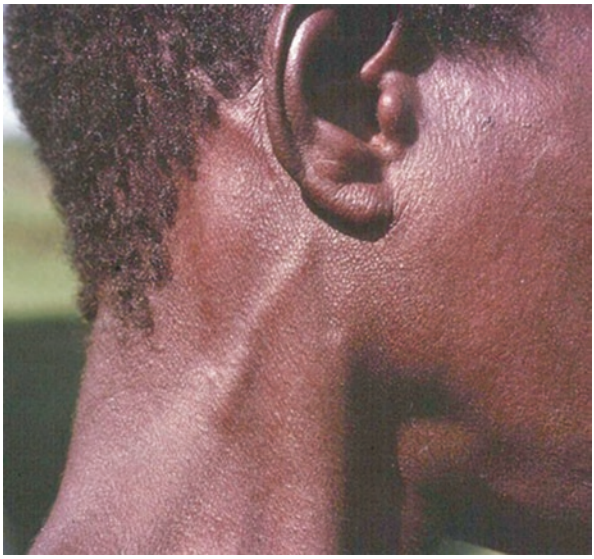

Fig. 15.2 Great auricular nerve: enlarged

15.2.3.2 Great Auricular Nerve

The great auricular nerve (Fig. 15.2) can be seen and palpated in the neck, emerging from the posterior border of the sternocleidomastoid muscle. The patient turns his/her head to one side, thus stretching this muscle. The great auricular nerve courses anteriorly and superiorly across the muscle toward the earlobe.

15.2.3.3 Ulnar Nerve

The forearm of the patient is bent at 90–110° over the arm. The examiner uses his left hand to palpate the right ulnar nerve and his right hand to palpate the left ulnar nerve. The nerve can be palpated first at the elbow in the olecranon groove, between the olecranon and the medial epicondyle of the humerus. Then, it can be felt and evaluated immediately above the groove. In comparing right and left ulnar nerves, it is useful to ask the patient to put his hands on the examiner's shoulders; in this case, the bending is about 135° (Naafs, 2010, personal communication). Alternatively, the patient may hold his own hands in front of his body.

15.2.3.4 Radial Nerve

The radial nerve is difficult to palpate. It can be appreciated and rolled against the humerus at approximately halfway down the lateral side of the arm, below the insertion of the deltoid muscle and anterior to the lateral head of the triceps muscle.

15.2.3.5 Radial Cutaneous Nerve

The radial cutaneous nerve branches from the radial nerve at the elbow. It is palpated at the lower third of the forearm. It can be rolled under the tips of the examiner's fingers as it crosses the lateral border of the radius just proximal to the wrist

and courses onto the dorsum of the hand. For diagnosis of leprosy, no other clinical or laboratory test has the same high sensitivity and specificity [5].

15.2.3.6 Median Nerve

The median nerve is felt in front of the wrist when the wrist joint is semiflexed, proximal to the flexor retinaculum. It is often easier to see than to palpate due to the presence (if present) of the tendon of the palmaris longus muscle.

15.2.3.7 Common Peroneal Nerve (Lateral Popliteal Nerve)

The common peroneal nerve is the continuation of the lateral component of the sciatic nerve, from which it separates in the upper part of the popliteal fossa; it then runs behind the head of the fibula and obliquely around its neck. It continues for about 10 cm in contiguity with the bone and enters the fibular tunnel to reach the muscles of the leg. While palpating the common peroneal nerve, the patient has the knee joints semiflexed and the examiner kneels in front of him; alternatively, the patient and examiner are seated in front of each other. The nerve is palpated in the popliteal fossa, just medial to the biceps femoris tendon and as it passes round the neck of the fibula.

15.2.3.8 Superficial Peroneal Nerve

The superficial peroneal nerve (also called dorsalis pedis) can be palpated on the dorsum of the foot and of the ankle.

15.2.3.9 Posterior Tibial Nerve

The posterior tibial nerve anatomically is the continuation of the medial portion of the sciatic nerve. It can be palpated as it passes posteriorly and inferiorly to the medial malleolus to supply the sole of the foot. It is difficult to palpate due to tendons and blood vessels which also pass this location.

15.2.3.10 Sural Nerve

The sural nerve can be palpated along the midline of the back of the lower leg, in the mid- to lower part of the leg, where calf muscles join to the Achilles' tendon.

The sural nerve can also be palpated as it runs down behind and under the lateral malleolus and along the lateral side of the foot.

15.2.4 Tinel's and Phalen's Signs

Tinel's sign is evocated by manually squeezing the nerve in areas of possible "entrapment." Examples are the ulnar nerve at the olecranon groove, the median nerve at the wrist, the lateral popliteal nerve (common peroneal) at the head of the fibula, and the posterior tibial nerve at the medial malleolus. When positive, the patient refers a small shock (transient paresthesia) propagating distally to the area supplied by the tested nerve. Phalen's sign applies to the median nerve only; when

positive, dorsal hyperflexion of the wrist with hands in opposition causes paresthesia or pain in the first three fingers.

15.2.5 Neurological Examination

Clinical assessment of the leprosy patient includes accurate neurological examination. We have already discussed testing for loss of tactile sensation on skin lesions and palpation of nerves. These are followed by neurological examination targeting exclusion of sensory, motor, and autonomic deficits. The evaluation of both sensitivity and muscular strength needs full collaboration of the patient, particularly when the deficit is bilateral and no comparison is possible. Lack of communication skills, a confused patient, suffering, old, or child may involuntarily alter the results.

The sensory system is evaluated first. Proprioceptive sensations (which arise from muscles, ligaments, bones, tendons, and joints) are studied by gently moving the patient's first toe and asking him to define its position in space (sense of motion and position), or placing a tuning fork over a bony prominence and asking the patient about perception of vibration (sense of vibration or pallesthesia).

Another maneuver for assessing deep sensitivities is Romberg's test: The patient is asked to stand erect with eyes closed and feet together; swaying, sometimes irregular swaying, and even toppling over are suggestive of deficit of these sensitivities. Study of superficial sensations (or exteroceptive sensations) includes three types: pain, temperature, and tactile; this has greater importance in leprosy, particularly for establishing diagnosis and the region and nerve affected. For tactile sense, light touch sensation may be assessed by gently touching the suspected region with the examiner's finger pulp or with a piece of cotton wool (see testing for loss of tactile sensation). Thermal sense, the first sensation to be lost, can be tested using two test tubes, one containing hot water and the other cold water. A pinprick test may be used to test pain sensation. Qualitative and quantitative assessment of sensation is performed by use of graded nylon monofilaments. Once a sensory deficit is appreciated, it is necessary to determine its topography. Circumscribed areas of hypoesthesia or anesthesia, particularly in correspondence with cutaneous lesions, indicate damage of cutaneous nerves. On the contrary, when the sensory deficit reproduces the distribution of a peripheral nerve, a lesion of the relevant nerve trunk is suspected even without motor deficit or muscle atrophy. In the next section, we describe the consequences of nerve damage for each nerve of predilection affected in leprosy.

Assessment of motor deficit is done by evaluating deep tendon reflexes, and muscular bulk and power. Bilateral comparison helps in appreciating unilateral deficit. Deep tendon reflexes, differently from other neuropathies, are commonly preserved in leprosy. Evaluation of muscular bulk should take into account patient gender and age, as well as the panniculus adiposus and physical activity; forced rest reduces muscular bulk also without peripheral nerve lesions. Muscle hypotrophy can be minimal, moderate, or serious (atrophy). While evaluating muscular bulk, the presence of fasciculations should be noted. These are random, spontaneous twitchings of a group of muscle fibers visible through the skin (better under sidelight),

commonly absent in leprosy neuropathy; their presence suggests other pathologies. When an important motor deficit is caused by nerve damage, as is the case in leprosy neuropathy, both muscular power and bulk should be decreased; otherwise, other diagnoses should be considered (e.g., diseases of the central nervous system or tendons/lesions). For evaluating motor strength, it is customary to use the 0–5 scale proposed by the British Medical Research Council. Grade 5 is normal power; grade 4 is active movement against moderate resistance; grade 3 is active movement against gravity; grade 2 is active movement with gravity eliminated; grade 1 is trace of contraction; and grade 0 is no contraction.

An isolated sensory deficit is common at the onset of leprosy neuropathy, while an isolated motor deficit is rarer; the latter is commonly accompanied by the former and by autonomic deficit.

Evaluation of the clinical picture is based on the topography of the sensory, motor, and autonomic damage. To determine the specific nerve affected, all parameters (bulk, power, sensation, and hydration) should be consistent; for example, a lesion of the ulnar nerve at the elbow clinically causes muscle weakness and hypotrophy, sensory deficit, and dryness of the skin in the medial part of the hand. Evaluation of the affected nerve is easier in the case of mononeuropathy, but leprosy tends to produce multineuropathy, and the clinical picture may be complicated, particularly in advanced cases due to damage to several nerves.

Gait in leprosy can be seriously altered. In the "stepping gait," the patient has to lift the affected leg high during walking in order to keep the foot clear of the ground (as if he or she is climbing up steps) [2]. It is caused by paralysis of the tibialis anterior and peroneal muscles that are responsible for dorsiflexion and eversion of the foot.

There may also be inability to walk on the heels.

15.3 Clinical Features

Symptoms and signs of neuritis may appear acutely, during reactions, with nerve enlargement, nerve pain or tenderness, loss of sensation, dry skin, and paralysis. More often, they appear slowly with gradual loss of sensory functions, decreased muscular power, and muscular hypotrophy. Neuritis can also be "silent" for weeks and months. In this case, the patient reports no symptoms, and, without clinical and electrophysiological investigations, undue delay in diagnosis and management of nerve damage may occur. Virtually, all peripheral nerves may be involved, but those that supply the face, eyes, hands, and feet need major attention and systematic evaluation.

15.3.1 Supraorbital and Great Auricular Nerves

Enlargement of these nerves represents an important diagnostic sign of leprosy, but their involvement produces minimal clinical damage to the patient. The supraorbital

nerve can rarely cause headache and pain in the orbital cavity, while the great auricular nerve has virtually no relevance to the patient (Figs. 15.1 and 15.2).

15.3.2 Facial Nerve

In leprosy, the facial nerve (cranial nerve VII) can be damaged at different levels. When it is affected only in its upper (or zygomatic) branch, there is weakness or paralysis of the orbicularis oculi muscle. This causes inability to close the eyelid, a condition called lagophthalmos, which may lead to a serious secondary keratoconjunctivitis. When paralysis of cranial nerve VII is complete, one may find Bell's palsy, where frontal, orbicularis oculi muscles and the muscles of the lower portion of the face are all affected (Fig. 15.3).

15.3.3 Ulnar Nerve

In leprosy, the ulnar nerve can be affected at the arm (Fig. 15.4), elbow, and wrist. Damage at the elbow is more common, precisely at two sites: posteriorly to the medial epicondyle and in the cubital tunnel. The cubital tunnel is a rigid anatomical

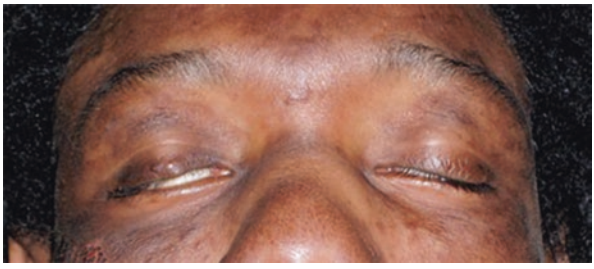

Fig. 15.3 Facial nerve: bilateral lagophthalmos

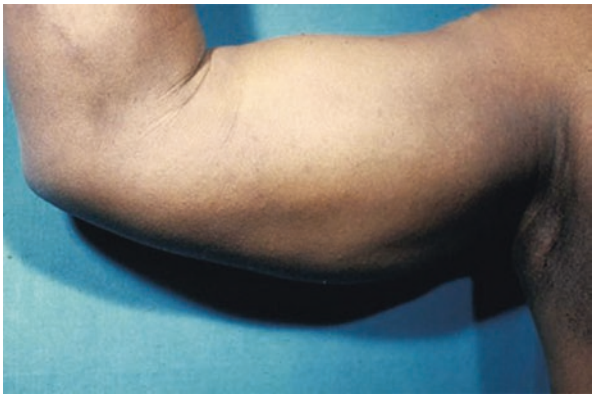

Fig. 15.4 Ulnar nerve: enlarged at the arm

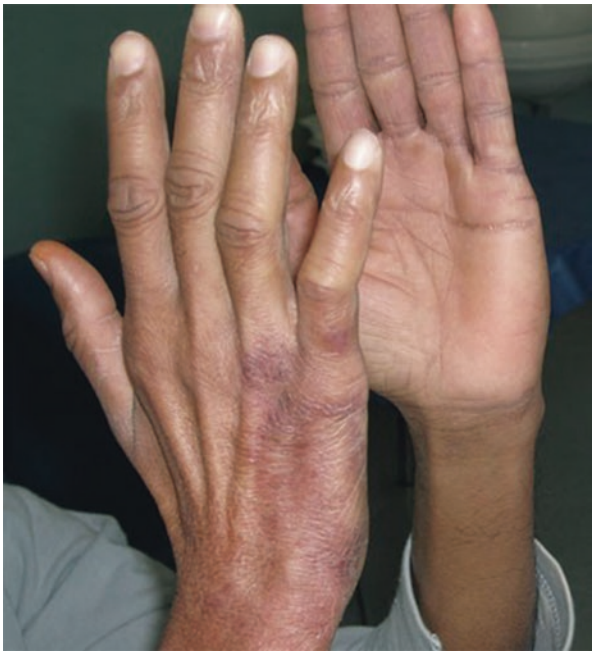

Fig. 15.5 Ulnar nerve: the right hand shows an erythematous skin lesion in the medial region, hypotrophy of the hypothenar region, hollowing of the interosseous spaces, extension of the fourth and fifth metacarpal phalangeal joints, and flexion of the proximal interphalangeal joints

structure whose roof is made up of an aponeurosis and the fibers of the flexor carpi ulnaris muscle, while the floor is made up of the medial ligament of the elbow and other muscular fibers. During leprosy reactions and neuritis, the enlarged nerve will be entrapped between those rigid structures with worsening of the neural lesion. Finally, the ulnar nerve can be affected in the Guyon canal as it passes through the wrist. Lesions at the elbow or at the wrist cause similar symptoms and signs. There may be tenderness or pain at the affected points and positive Tinel's sign. Sensory impairment is present at the fifth finger, at the medial region of the fourth finger, and at the medial region of the hand. Motor damage affects the intrinsic muscles of the hand. There is deficit of abduction of the fifth finger, and hypotrophy of the hypothenar and interossei muscles with creation of four grooves between the metacarpals. The weakness of the interossei muscles that bend the first phalanx bone and extend the last two causes the deformity called "claw fingers." Damage of the autonomic fibers causes dryness of the skin in the medial region of the hand (Figs. 15.4, 15.5, and 15.6).

15.3.4 Median Nerve

The median nerve is frequently affected in leprosy, alone or together with the ulnar nerve. It is rarely affected at the arm, elbow, or forearm. In leprosy, most commonly, the damage is just before the wrist or at the wrist where it runs within the carpal tunnel; here, the clinical features are related to the amount of compression. Sensory

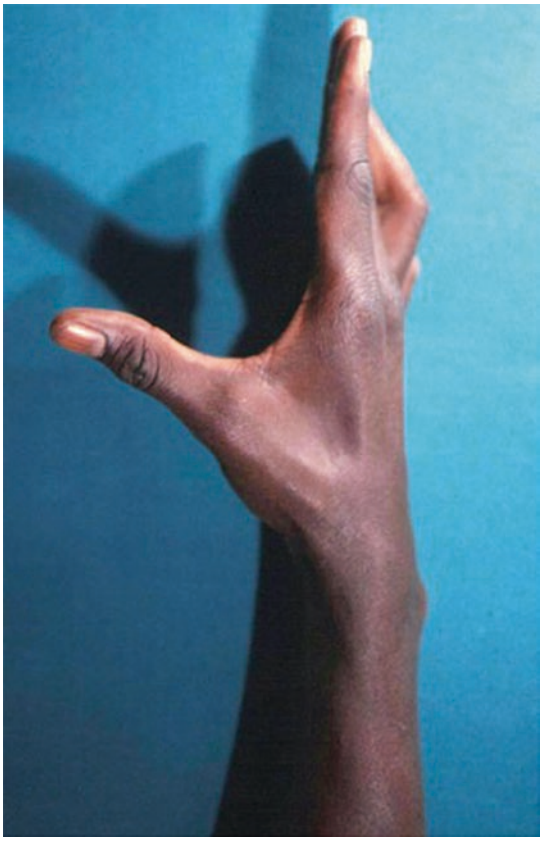

Fig. 15.6 Ulnar nerve: hollowing of the first interosseous space. Flexion of the proximal interphalangeal joint of the fourth and fifth fingers

deficit is almost always present and similar to that produced by lesions at more proximal sites. There are paresthesia, hypoesthesia, and even anesthesia involving the first three fingers, the lateral region of the fourth finger, and the lateral region of the hand, with sparing of the skin above the thenar muscle (the latter being affected only when the lesion is proximal to the carpal tunnel). In advanced damage, there is atrophy of the thenar, abduction and opposition of the first finger are compromised, and there is clawing of the second and third fingers. The skin in the area of the hand supplied by the nerve is dry. The diagnosis is based on evidence of sensory, motor, and autonomic deficit, pain at the wrist, and positive Tinel's and Phalen's signs. In case of carpal tunnel syndrome, electro-neurographic (ENG) studies show increased distal latency for motor fibers and, for sensory fibers, decreased conduction velocity in the length finger–wrist sparing of the proximal segments. Frequently, median and ulnar nerves are both affected, forming the picture of complete claw hand and causing severe hand deformity (Figs. 15.7, 15.8, 15.9, 15.10, and 15.11).

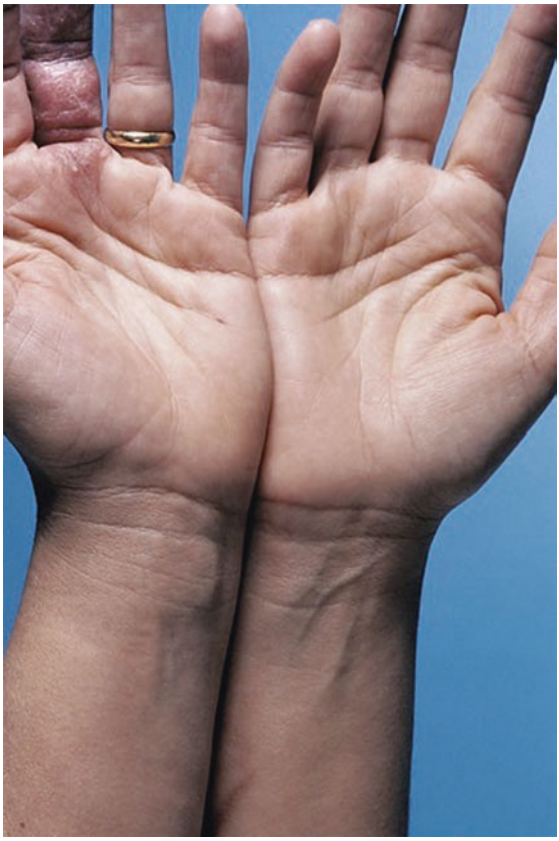

Fig. 15.7 Median nerve (left): enlarged at the wrist

15.3.5 Radial Nerve

The clinical picture depends on the site of the lesion. In leprosy, the radial nerve is rarely damaged at the upper arm (the triceps muscle is normally spared). Also, lesion just below the elbow (posterior interosseous nerve syndrome) is uncommon.

Commonly, in leprosy, motor abnormalities involve the brachioradialis muscle and the extensor muscles of wrist and fingers; the result may be a severely disabling wrist drop. Sensory disturbance is in the dorsomedial region of the hand (Fig. 15.12).

15.3.6 Radial Cutaneous (Superficial Radial) Nerve

When enlarged, the radial cutaneous nerve is very useful for diagnosis of leprosy. Its involvement does not cause important clinical problems to the patient other than an area of loss of sensation over the dorsum of the hand (Fig. 15.13).

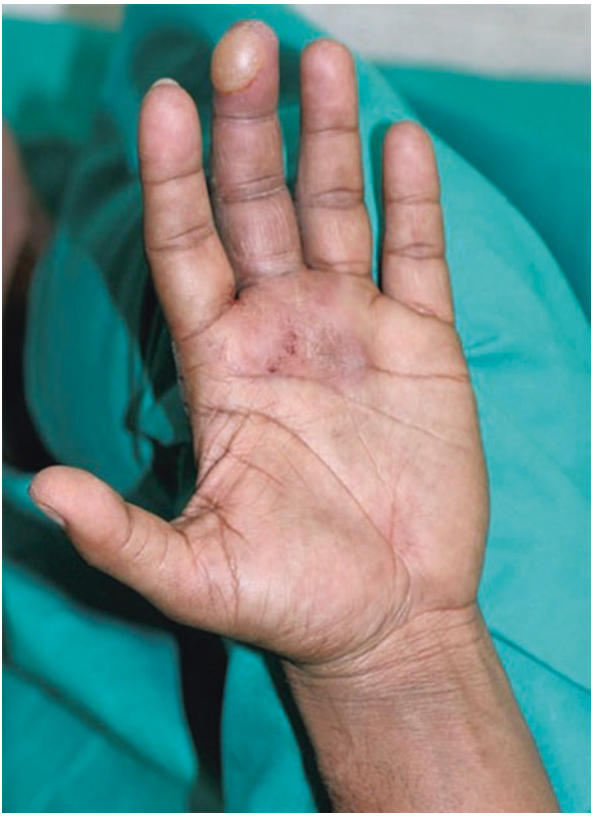

Fig. 15.8 Median nerve: sensitive damage, bulla on the third finger; the patient inadvertently burnt himself while cooking

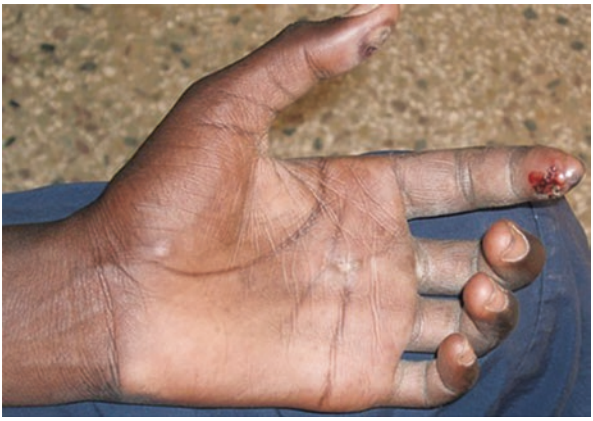

Fig. 15.9 Median and ulnar nerves: sensory median nerve damage with traumatic wounds to the insensitive first and second fingers; motor ulnar nerve damage with hypotrophy of the hypothenar muscle. Dry skin over the palm (autonomic damage)

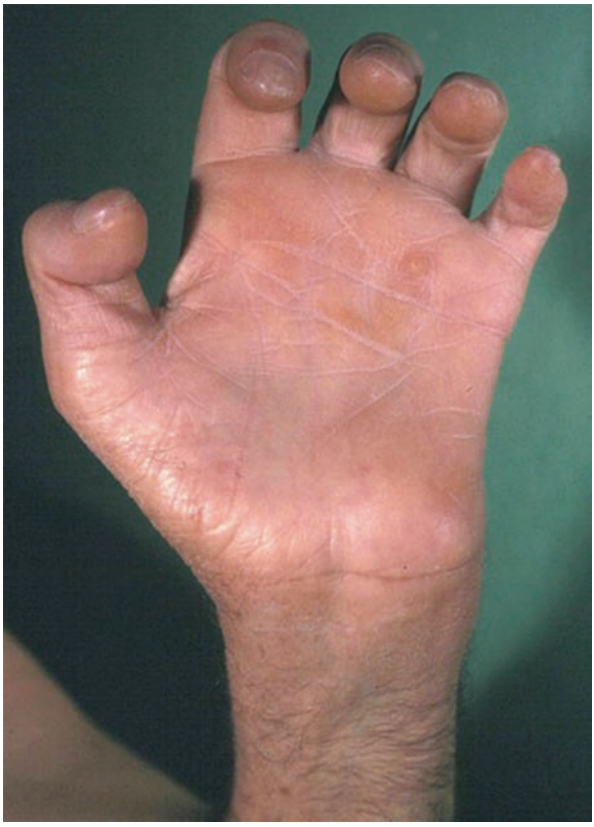

Fig. 15.10 Median and ulnar nerves: sensory, motor, and autonomic damage of both nerves, claw fingers, calluses on the tips of the fingers

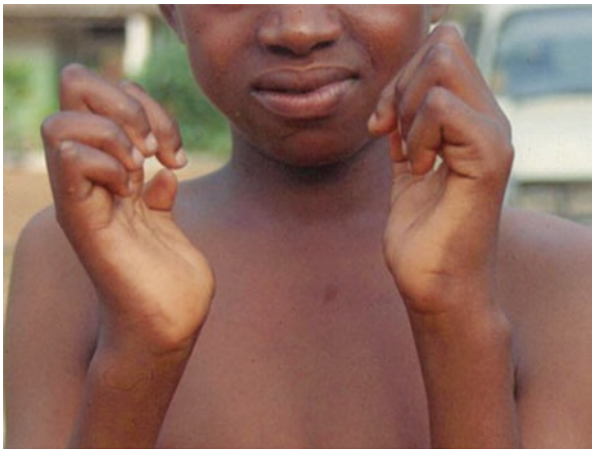

Fig. 15.11 Medial and ulnar nerves: bilateral damage

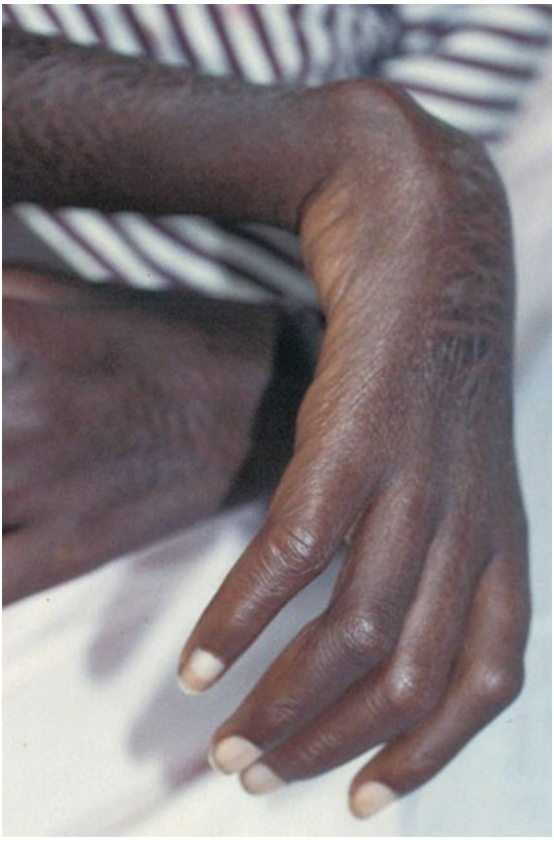

Fig. 15.12 Radial and ulnar nerves: wrist drop and hypotrophy of the hypothenar muscle

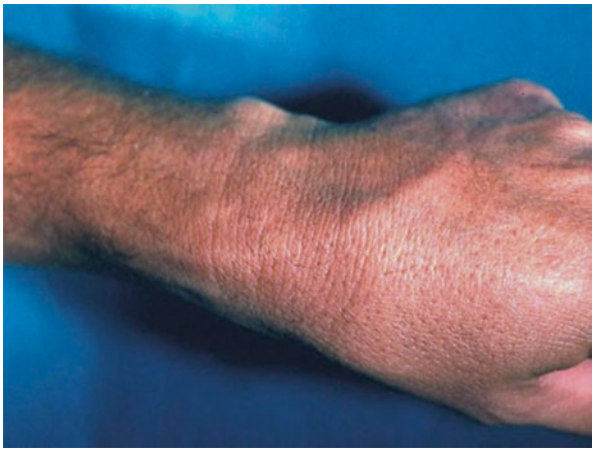

Fig. 15.13 Radial cutaneous nerve: enlarged at the lower part of the forearm, wrist, and dorsum of the hand

15.3.7 Sciatic and Common Peroneal Nerves

In leprosy, the common peroneal (syn.: lateral popliteal) nerve is commonly damaged in the popliteal fossa or proximally and around the neck of the fibula. The patient may complain of pain or tenderness along the area of the nerve. There is loss of sensation in the anterior and lateral aspects of the lower leg, dorsum of the foot, and toes. The motor deficit is in the anterior and lateral muscles of the leg. In the early stage, there is difficulty in dorsiflexion and eversion of the foot against resistance. Complete damage causes paralysis of the foot in ventral flexion (foot drop) and in inversion, with a stepping gait. In case of a focal lesion of the peroneal nerve, ENG study shows nerve conduction slowing or "blocking." If the ENG test is normal, a proximal lesion should be suspected, namely, a L5 radiculopathy (clinically producing low back pain) or a partial lesion of the sciatic nerve (more difficult to diagnose). In these cases, electromyography is useful for detecting subclinical abnormalities in muscles not innervated by the common peroneal nerve such as gluteal muscles (in L5 radiculopathy) and the short head of the biceps femoris in a lesion of the lateral trunk of the sciatic nerve. Correct localization of the site of the lesion will guide further diagnostic or therapeutic interventions (Fig. 15.14).

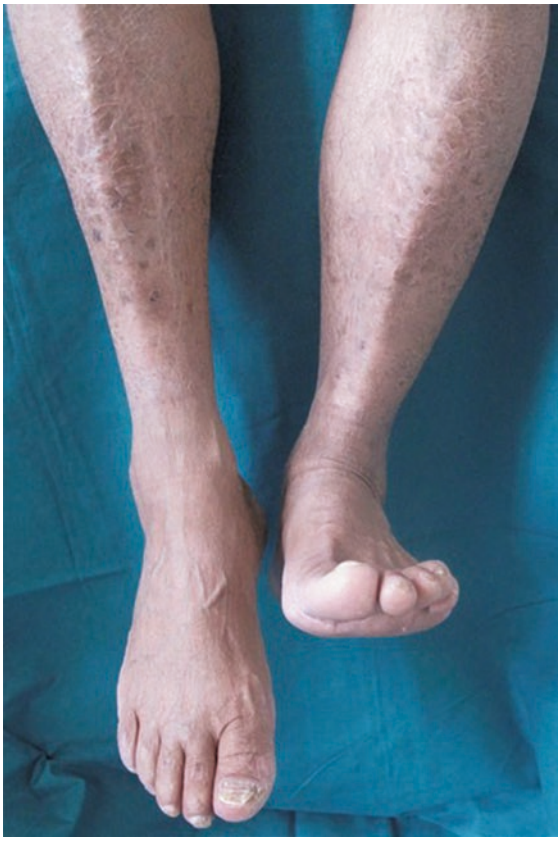

Fig. 15.14 Common peroneal nerve: the patient is not able to dorsiflex the right foot. Hypotrophy of the right anterior tibial muscle. Dry skin

15.3.8 Posterior Tibial Nerve

In leprosy, the posterior tibial nerve is frequently affected just proximally or within the tarsal tunnel. Early damage results in pain in the region of the medial malleolus radiating to the leg and foot, and paresthesia on the sole of the foot. The importance of the sensory deficit is in relation to the intensity of the damage and the affected branches (medial and lateral plantar and calcaneal). Advanced damage at the tarsal tunnel results in anesthesia of the sole, with sparing of two small areas, a medial one served by the saphenous nerve and a lateral one served by the sural nerve. If the lesion affects the calcaneal nerve, the sensory loss will be at the heel; if the medial plantar nerve is affected, loss of sensation will be at the medial region of the sole and at the first, second, third, and part of the fourth toe; when the lateral plantar nerve is affected, loss of sensation will be at the lateral region of the sole, fifth toe, and part of the fourth toe.

Motor deficit is difficult to assess clinically in early stages; when advanced and added to damage of the common peroneal nerve, it results in paralysis of the intrinsic muscles, clawing of the toes, and collapse of the arch of the foot. Deficit of the autonomic system causes loss of sweating and dryness of the sole. Damage to the posterior tibial nerve is the most common and most important cause of injury to the feet in leprosy [4]. Combination of common peroneal and posterior tibial nerve involvement causes devastating deformities to the feet (Fig. 15.15).

A distal lesion of the plantar nerves in the region of the metatarsal heads with pain in the sole irradiating to the second and third toe produces Morton's syndrome, sometimes also associated with sensory deficit.

15.3.9 Sural Nerve

The sural nerve is a sensory nerve producing, when damaged, an area of hypoesthesia confined to the lateral region of the foot, with minimal clinical consequences for the patient. ENG has demonstrated high incidence of lesions of this nerve in leprosy, as in other peripheral neuropathies. It is the preferred site for diagnostic nerve

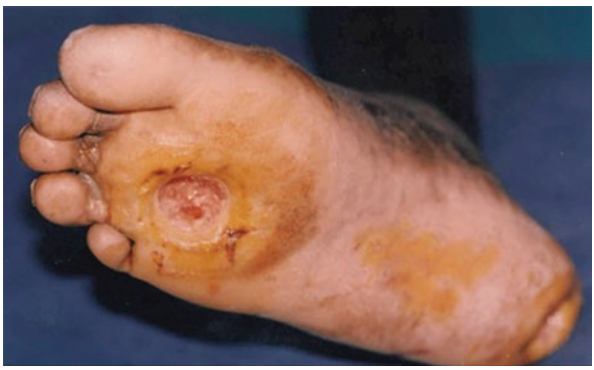

Fig. 15.15 Posterior tibial nerve: damage to this nerve results in anesthesia of the sole of the foot and plantar ulcers

biopsy because it lacks motor fibers and is easily accessible at the lower third of the back of the leg. When possible, the biopsy should be preceded by ENG, with abnormal ENG confirming the utility of proceeding with biopsy.

WHO classification of grades of disability in leprosy [6]		
Grade	Hands and feet	Eyes
0	No disability found	No disability found
1	Loss of sensation	The eyes are not given a grade of 1
2	Visible damage or disability is noted	Lagophthalmos, obvious redness of the eye, visual impairment, or blindness

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Primary Neural Leprosy

16

José Antonio Garbino, Wilson Marques Jr, and Bernard Naafs

16.1 Introduction

Leprosy is a multisystem disease that mainly affects tissues originating from the embryonic ectoderm: skin and peripheral nerves. It is clinically diagnosed by three cardinal signs: loss of sensation in skin lesion, enlarged nerves, and positive skin smear [1]. Two out of these three signs are needed to make a definite diagnosis. However, leprosy without skin lesions and with a negative skin smear, showing nerve lesions only, does exist, being known as primary neural leprosy or pure neural leprosy. Primary neural leprosy (PNL) is well known from the Indian subcontinent, where it constitutes 2–10% of newly diagnosed patients, depending on the area, the quality of the leprosy program, and the interests of leprologists [2–5]. During the past decades, it has been diagnosed outside India as well, partly due to increased awareness, in 1–4% in Brazil [6–8], 1–2% in Senegal, and 1–2% in the Netherlands.

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Table 16.1 Signs and symptoms of primary neural leprosy in the region of nerve distribution

• Loss of sensation
• Loss of muscle strength
• Loss of sweating
• Neuropathic pain: paraesthesia, paroxysmal pain
• Dysesthesia and allodynia
• Painful nerves
• Enlarged nerves
• Visible nerves

Table 16.2 Differential diagnoses of enlarged nerves

Acquired neuropathies
• Leprosy
• Acquired amyloidosis
• Chronic inflammatory demyelinating neuropathy and its variants
• Multifocal motor neuropathy
• Acromegaly
• Nerve tumours
Inherited neuropathies
• Hereditary motor and sensory neuropathies (Charcot–Marie–Tooth (CMT)1A, CMT1B, CMT1E neuropathy, Dejerine–Sottas neuropathy, hereditary neuropathy with liability to pressure palsy (HNPP))
• Refsum disease
• Inherited amyloidosis (TTR, PRPN)
• Neurofibromatosis

16.2 Diagnosis

The diagnosis is not easy and is often missed when not suspected [7–9]. PNL should always be considered in differential diagnosis when there are signs of neuropathy in patients who have lived in or come from endemic countries. The manifestations of pure neural leprosy are those found in most neuropathies, including loss of sensation, loss of muscle strength, loss of sweating, and enlarged and/or painful nerves (Table 16.1), although in the early phase of the disease, small fibre pathology is predominant. Though an enlarged nerve is one of the major criteria to diagnose leprosy, there are other conditions mimicking leprosy in this particular aspect (Table 16.2).

There are no reliable laboratory tests to diagnosis leprosy, let alone PNL. Complete skin and nerve examinations are mandatory, and electrophysiology [6–9] and biopsy may be of great help (Fig. 16.1, Table 16.3) [6, 7, 9]. In some instances, fine needle aspiration with polymerase chain reaction (PCR) may help to establish the diagnosis. The most common presentations of PNL are mononeuropathies and multiple mononeuropathies. When a neuropathy is established, history-taking becomes extremely important, since the aetiology can be diverse (Table 16.4). Careful neurophysiological evaluation may be of help. Some of the neuropathies including leprosy may be accompanied by neuritis. This is a general term for inflammation of peripheral

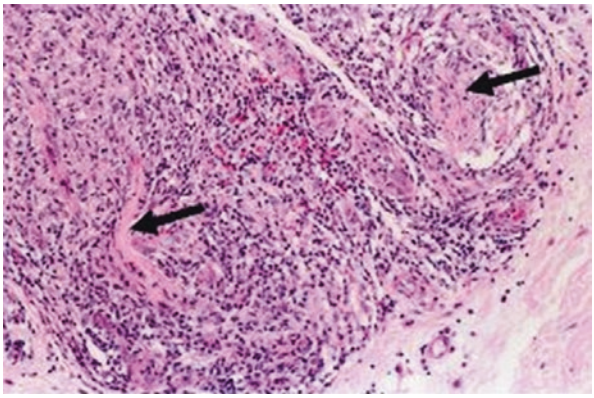

Fig. 16.1 Tuberculoid granulomas compromising neural branch and neural fragments inside (arrows). Epithelial macrophages are surrounded by lymphocytes expanding and destroying neural structures (HEx20)

Table 16.3 Histopathological spectrum of neural leprosy

- Tuberculoid pattern
- Borderline pattern
- Multibacillary pattern
- Multibacillary leprosy pattern with endoneural hyalinization/fibrosis
- Nonspecific oedema: nonspecific inflammatory endo- and perineural infiltrate (mycobacterial antigenic determinants present)
- Demyelination: nonspecific inflammatory infiltrate and demyelination (mycobacterial antigenic determinants present); complete demyelination, endo- and perineural hyalinization/fibrosis
- Nonspecific endoneural hyalinization/fibrosis
- No changes

Table 16.4 Causes of peripheral neuropathy

- Systemic illness (diabetes, uraemia, sarcoidosis, myxoedema, acromegaly): polyneuropathy or multiple mononeuropathy
- Autoimmunity (inflammatory demyelinating polyradiculoneuropathies a.o Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy): polyneuropathy or polyradiculoneuropathy
- Vasculitis (connective tissue diseases): multiple mononeuropathy
- Infections [leprosy, diphtheria, Lyme disease, human immunodeficiency virus (HIV), herpes zoster]: multiple mononeuropathy or polyneuropathy
- Cancer (paraneoplastic neuropathy): polyneuropathy or neuronopathy
- Nerve tumours; neuroma and neurilemmoma
- Paraproteinemic neuropathies (myeloma, cryoglobulinemia, monoclonal gammopathy neuropathy of unknown significance): polyneuropathy
- Nutritional deficiencies and alcoholism: polyneuropathy, neuronopathy
- Compression, posture, and trauma: mononeuropathy
- Toxic industrial agents and drugs: polyneuropathy
- Hereditary sensory (and autonomic) neuropathies: polyneuropathy
- Hereditary motor and sensory neuropathies: and especially hereditary neuropathy with liability to pressure palsies (HNPP): polyneuropathy, polyneuronopathy
- Hereditary neuropathy with liability to pressure palsies (HNPP): mononeuropathy, multiple mononeuropathy, or polyneuropathy

nerves. The symptoms depend on the nerves involved, but may include pain, paraesthesia ("pins and needles"), hypoesthesia, anaesthesia, paresis, paralyses, and wasting of muscles. Nerve palpation may show tenderness, and a positive Tinel sign may be observed. When PNL is diagnosed, it must be treated. If the clinical and neurophysiologic pictures are those of a mononeuropathy, it might be considered as paucibacillary (PB). If they are of a multiple mononeuropathy, multibacillary (MB) leprosy must be diagnosed [7–9]. Since *M. leprae* is occasionally found in the nerve biopsy (Fig. 16.1), some consider that all PNL should be treated as MB. Moreover, some PNL patients develop skin lesions at a later stage [4], often during reactional episodes. However, some patients may show PNL having gone through a stage with skin lesions, which due to their harmless appearance have been missed.

PNL may be considered as a primary presentation of PB or MB leprosy. When a patient is diagnosed with mononeuropathy, multiple mononeuropathy, or even polyneuropathy, leprosy should always be among the differential diagnoses. Permanent nerve damage can be avoided when the patient is treated in a timely and adequate fashion.

16.3 Discussion

In 1977, Shetty and Antia published that nerves in early leprosy and in leprosy contacts showed signs of demyelination in nerve conduction studies and in histopathology [10]. Diogo Fernandes dos Santos presented during the Brazilian Leprosy Congress in 2017 that using nerve conduction studies demyelination in contacts was seen, with and without a positive anti PGL-1 serology [11]. During the Manila World Leprosy Congress in 2019, Glauber Voltan and Marco André Frade said they had found enlarged nerves in contacts using ultrasound (personal communication). The enlargement was related to the amount of exposure. In these studies, no clinical symptoms due to nerve dysfunction of the investigated nerves was demonstrated. It is generally assumed that clinical dysfunction only can be detected when more than 20% of the nerve fibres are not functioning. These observations, published on the Leprosy Mailing List in February 2020, raise the question of whether the abnormalities found can still be called leprosy, let alone PNL. There are no sensory or other clinical defects, but there are histopathological and nerve conduction abnormalities [12]. The only way to diagnose PNL may be when live *M. leprae* can be detected by fine needle aspiration using reverse PCR; otherwise, the damage could be due to contact with *M. leprae* antigenic determinants from the environment and it may be not a straight infectious disease.

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Chronic Neuropathic Pain in Leprosy

17

José Antonio Garbino, Bernard Naafs, and Wilson Marques Jr

17.1 Introduction

At present, neuropathic pain (NP) is a well-recognized sensory manifestation of leprosy that may affect up to 78.9% of the patients [1]. Despite its high prevalence, this is still a difficult diagnosis, what may be partially explained by the necessary use of specific tools as the DN4 screening scale to differentiate neuropathic from nociceptive pain that is also present in leprosy [1, 2]. According to the International Association for the Study of Pain (IASP), nociceptive pain is that pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors, while neuropathic pain results from a lesion or disease of the somatosensory nervous system, both deserving appropriated investigation and management (https://www.iasp-pain.org/terminology) [2]. The main manifestations of neuropathic pain are paraesthesia, dysesthesia, allodynia, burning sensation and paroxysmal pain [3].

Depending on immunity, leprosy patients may develop distinct forms of the disease, namely, polar tuberculoid (TT), borderline tuberculoid (BT), midborderline (BB), borderline lepromatous (BL) and polar lepromatous (LL) leprosy. Two types of reactions are recognized, intense inflammatory phenomena involving nerves: type 1 leprosy reaction (T1R) or reversal reaction, which occurs in borderline (BT,

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BB and BL) patients who have cell-mediated immune reactivity against *M. leprae* antigenic determinants, and type 2 leprosy reaction (T2R) or erythema nodosum leprosum, which is predominantly immune-complex mediated and occurs only in BL, subpolar and polar-LL leprosy. Deterioration in nerve function and pain occur mainly during reactional episodes, which can happen before, during and after anti-mycobacterial treatment. Reactions in nerves respond to treatment with steroid regimens; neuropathic pain can persist after the reaction for months and, in many cases, for years.

17.2 Pathophysiology

The inflammatory processes that accompany the leprosy reactions, T1R and T2R, start with acute oedema which increases demyelination and frequently leads to important axonal loss. Clinically, the nerves may enlarge, be tender and present pain and loss of function. The acute inflammation which accompanies reactions provokes acute pain, mediated by inflammatory cytokines. In T2R, not only the nerves but also the skin, joints, eyes, lymph nodes and testicles can be affected; however, these pains are nociceptive, not considered to be neuropathic. In T1R, pain as a major symptom can be observed mainly in nerves. Most of these pain symptoms in nerves show good response to steroid treatment [3]. Chronic neuropathic pain, a self-maintained, post-inflammatory process, i.e. post-reactions, may be caused by disarrangement of peripheral nerve sensory pathways. Several theories have been postulated to try to clarify the mechanisms underlying NP [4]. Most of them are based on complex neurochemical models [5] which are difficult to translate into clinical practice. The most plausible and convincing theory for understanding NP is the assumption of ectopic generation of impulses in mechano-insensitive C-fibres which leads to neuropathic symptoms including pain [6]. Immediately after a nerve lesion, some patients may develop changes in Na+ ion channels which results in axonal hyperexcitability and symptoms of pain. Indeed, NP may be relieved by anti-epileptic, anti-hyperexcitability agents such as carbamazepine and gabapentin, which block Na+ channels [7]. These mechanisms can be present at the same time in different nerves, which is called pain of mixed origin. This situation can be found in relapse and during reactions but is most commonly seen after release from antibacterial treatment. Recently, it was proposed that Remak Schwann cell disruptions result in neuropathic pain even in the absence of nerve injury [8].

17.3 Clinical Presentation and Examination of Chronic NP

The most frequent manifestations observed in leprosy patients with NP are burning sensation of squeezing, pressure, electric shock, stabbing, pain provoked by brushing, pressure or cold, pins and needles and tingling (Raicher et al. [1]). Interestingly, the same study demonstrated that pain provoked by brushing and cold are much less

frequent in NP related to leprosy than in NP not related to leprosy, and the inverse occurs with pain provoked by pressure.

Clinical evaluation should include a visual analogue scale (VAS), in which 0 represents no pain and 10 represents unbearable, incapacitating pain [1], pinprick test for pain, graded sensory testing with Semmes–Weinstein monofilaments (STSW), nerve palpation and voluntary muscle testing (VMT), to define and classify the spontaneous pain as well as the nerve topography [8]. Moreover, verbal descriptors used by patients with pain complaints and questionnaires such as the DN4 [2] should be used. Dermatological examination to classify leprosy according to the Ridley–Jopling criteria and routine laboratory tests are recommended. Involved nerves or skin areas with a convincing neuroanatomical distribution confirm involvement of a specific peripheral nerve. These findings, together with the results of clinical neurological sensory and motor tests and/or electrophysiological investigations, fulfil the criteria for NP [8]. There is some indication that the presence of A-waves correlates with pain complaints of neuropathic characteristics, especially in those with reactions. Probably, such response shares similar mechanisms with the small-fibre dysfunction seen in these patients with NP, such as demyelination, intraneural oedema and axonal sprouting [8, 9].

17.4 Treatment

Several interventions are identified that might help in the relief of neuropathic pain in leprosy, such as conventional antipain medication, physical therapy, surgery and psychological approaches [3]. Although some advocate a rational treatment based on clinical manifestations, there is no evidence-based study justifying such kind of choice [2, 3, 9]. Following the general rules for the neuropathic pain treatment should be used in leprosy patients presenting NP, respecting the individual characteristics of each patient. Additionally, we should always consider that in a given patient, nociceptive and neuropathic pain may be both present. Most studies investigating the drug treatment for neuropathic pain have been done in patients with diabetes [10], and in the absence of a dedicated study, these are the rules that should be followed. The first-line therapies are as follows:

17.4.1 Tricyclic Antidepressants

(i) Amitriptyline: 10–150 mg/day
(ii) Nortriptyline: 10–150 mg/day

It is advised to start treatment, when there are no contraindications, with either amitriptyline or nortriptyline as a first-line drug. Patients with narrow-angle glaucoma, prostate enlargement and heart disease should be treated with caution. The drugs are started at low dose, 10–25 mg in the evening; this dose is increased in steps of 10–25 mg every 3–7 days to an adequate level for pain relief, with

maximum dosage of 150 mg/day. If this is not effective, the combination of items (b) and (c) is proposed. Nortriptyline is preferred over amitriptyline in the elderly patient. The initial choice depends on drug availability and the clinical condition of the patient.

17.4.2 Calcium Channel α2δ Ligands

(i) Gabapentin: 900–3,600 mg/day, divided over three doses
(ii) Pregabalin: 300–600 mg/day, divided over two doses

Gabapentin is administered three times a day, and the target dose in the treatment of pain is 900–3,600 mg/day. The initial dose is 300 mg at bedtime, and the dose can be increased by 300 mg in 1–3 days. Pregabalin is started with 75 mg at bedtime and increased in steps of 75 mg after 3–5 days. The maintenance dose in neuropathic pain is usually 450–600 mg/day, divided into two doses when a slow-release preparation is used or three when an ordinary preparation is used. Blood count, sodium and transaminase levels should be monitored, at least at the start of treatment. Gabapentin and pregabalin are better tolerated than carbamazepine in the elderly patient. Both, however, can potentially interact with alcohol, anaesthetics, barbiturates and sleep medications.

17.4.3 Serotonin Norepinephrine Reuptake Inhibitors (SNRIs)

(i) Duloxetine
(ii) Venlafaxine

Duloxetine should be initiated with 30 mg once a day, titrating up to 60 mg twice a day. Venlafaxine immediate release should be started with 37.5 or 75 mg once daily, titrating up to 225 mg once daily. The most important side effects are nausea, increased sweating and increased blood pressure.

17.4.4 Topical Analgesics

Together with oral medication, for painful skin areas in the distribution of a peripheral nerve, called “terminal neuritis” with dysesthesia and/or allodynia, a topical gel or cream with capsaicin may be useful at dilutions of 0.0125–0.075%, three to four times a day. Topical analgesics are another option in this situation: lidocaine at 5% dilution can be applied to painful skin patches or skin areas, two to three times a day. Based on personal experience, topical application of 2% menthol/3–5% phenol cream or ointment may also be beneficial [9, 11].

17.5 Conclusions

In leprosy, chronic NP may occur during the disease and often continues after the patient has been released from treatment. It may cause severe limitations and decrease quality of life. The sooner NP is identified, and treatment is started, the earlier abusive use of steroids, given because leprosy reactions are considered, can be stopped and diminished quality of life can be controlled. These patients, after release from antibacterial treatment, should be properly followed up and not released from care.

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Electrodiagnostic Studies in Leprosy

18

Lizia Reni

18.1 Introduction

Electrodiagnostic studies include electromyography (EMG), electroneurography (ENG), and the study of late response. They assess peripheral nerve function but do not test small myelinated or unmyelinated fibers that carry information about pain and temperature. EMG is a diagnostic methodology that, using needle electrodes, studies the electric activity of the muscle at rest and under voluntary contraction. ENG (or sensory and motor nerve conduction studies) measures how fast the nerve conducts impulses. It allows evaluation of sensory and motor fiber activity. Late responses (F wave and H reflex) are late compound potentials that can provide information about lesions situated in proximal nerve segments or in the anterior roots of the spinal cord.

Information obtained with these techniques is independent of the degree of attention and compliance of the patient; they are reproducible and allow judgment about the type and site of the neural lesion. These studies are helpful in identifying and distinguishing muscle versus nerve disorders, mononeuropathy and multiple versus polyneuropathy, and axonal neuropathy versus demyelinating neuropathy.

18.2 Principles of Electroneurography

Electroneurography uses bipolar stimulation and recording electrodes that are placed on the surface of the skin or introduced as needles. Recording electrodes are distinguished into active and reference electrodes. In motor ENG, an active electrode is positioned on the belly of the muscle and a reference electrode on the tendon; nerve is then stimulated at two or more points. Needle electrodes are

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necessary to test the sensory component of some nerves (i.e., sural nerve and posterior tibial nerve). In ENG, the potential evoked by electric stimulation is a biphasic wave of which four parameters are studied: morphology, amplitude, duration, and latency. The morphology is the shape of the electrical response recorded by the active recording electrode. The normal waveform includes a negative deflection (upward), a larger positive deflection (downward), followed by a negative rebound back to baseline. The amplitude is the distance from the baseline to the negative peak or between the negative and positive peaks of the motor or sensory response. It represents the approximate number of healthy muscle fibers or axons available. The duration is calculated from the beginning to the positive or to the negative peak, or to the rebound back to baseline. The latency is the time between the onset of stimulus and the onset of response (initial deflection from baseline). Distal latency is calculated by stimulating the more distal part of the nerve near to the recording location (e.g., in the median nerve, the most distal localizable spot is at the wrist). The distal latency is made up of the time needed by the stimulus to travel from the stimulation point to the terminal axons including the neuromuscular transmission time too.

18.2.1 Motor Conduction Velocity

This is one of the two parameters studied by ENG. To measure motor conduction velocity, it is necessary to eliminate the neuromuscular transmission time. This is done by calculating the difference between the two latencies of potentials obtained by stimulating the nerve at two or more points and dividing by the distance between the two points. The result is the conduction velocity expressed in m/s. Stimulating the nerve at several points allows calculation of the segmental conduction velocity. In some cases, incremental stimulation by small segments is needed; this is necessary, for example, to detect lesions of the ulnar nerve at the elbow or of the common peroneal nerve at the head of the fibula.

18.2.2 Sensory Conduction Velocity

The second parameter studied in ENG is the sensory conduction velocity. For this study, the orthodromic and antidromic techniques are used. Routine recording of the sensory conduction velocity can be done using surface electrode. This noninvasive technique produces sufficient and reproducible information. In some cases, for example, when studying the sensory component of the posterior tibial nerve, use of needle electrodes is necessary. The amplitude of the sensory potential, which has high interindividual variation, is recorded from the baseline to the negative peak or from the negative to the positive one.

The conduction velocity of both motor and sensory fibers is dependent on various factors such as age, nerve length, and temperature. During infancy, nerve conduction velocity increases progressively together with the myelinization process; at

the age of about 30–40 years, it starts decreasing imperceptibly, and in a more marked way after the age of 60 years. Longer nerves conduct more slowly that shorter nerves; accordingly motor and sensory fibers of the lower limbs conduct slower than those of the upper limbs. Nerve conduction is faster at higher body temperature; it is advisable to perform the test at 34 °C.

18.3 Indications of Electroneurography

Electroneurography confirms the diagnosis of neuropathy, and detects the affected nerve as well as early nerve involvement, prior to clinical evidence. It allows the study of the site and type of the neural lesion: demyelinating segmental neuropathy versus axonal degeneration. Localization of the site of the lesion is necessary to facilitate further investigations such as ecography and nuclear magnetic resonance (NMR), or surgical procedures. Nerve damage may be diagnosed in a timely fashion if the nerves of predilection of leprosy supplying the hands and the feet, namely, the ulnar, median, common peroneal, and posterior tibial nerves, are all examined, independently of their clinical status. ENG should study the entire nerve; studying only the more frequently affected regions (e.g., the wrist for the median nerve or the elbow for the ulnar nerve) may result in mistakes.

18.3.1 Axonal Versus Segmental Demyelinating Neuropathy

Axonal damage or prevalently axonal damage causes decrease in the amplitude of the action potential of the muscle or of the nerve without important changes in the conduction velocity in spared fibers. Segmental demyelinating neuropathy causes decrease in the conduction velocity along the entire nerve or in part of it. Demyelinating neuropathy is diagnosed when the decrease in conduction velocity is 60% or more of the normal value.

18.3.2 Conduction Block

The presence of a so-called conduction block [1] corresponds to a more severe demyelinating lesion. It reveals the impossibility of propagation of the action potential through a segment of the nerve. It is documented by a significant reduction of the area and amplitude of the potential evoked by proximal compared with distal stimulation. To evaluate a conduction block, it is necessary to perform a group of stimulations in the area suspected of being demyelinized. A total block is found in the absence of an answer when the nerve is stimulated proximally, while the answer to distal stimulus is preserved. A partial block is found for a decrease of the amplitude of at least 20–30% or by some authors 50% of normal value, particularly in cases when proximal stimulation gives rise to technical problems (Fig. 18.1).

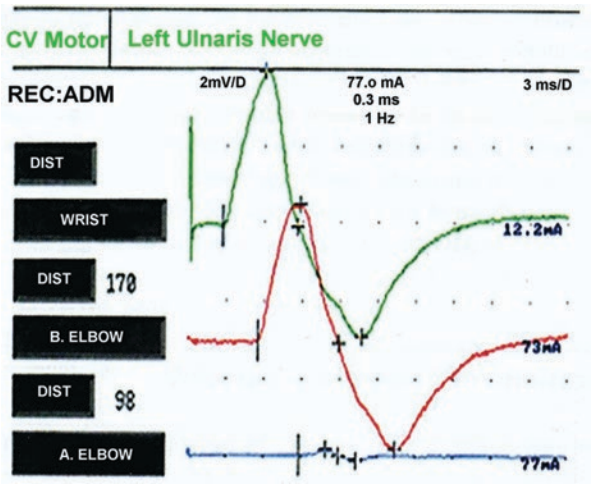

Fig. 18.1 Ulnar nerve conduction study with segmental stimulation at the wrist, below elbow, and above the elbow. Traces show an abrupt drop in amplitude above the elbow (50%), indicating a partial conduction block

18.4 Electroneurography Findings in Leprosy

Early leprosy neuropathy is characterized by ENG signs of mainly demyelinating damage, namely, decreased conduction velocity, conduction block, and increased distal latency. Late leprosy neuropathy produces different ENG signs in relation to the seriousness of the clinical picture and to the sequelae of previous lesions. Persistence of the decreased conduction velocity and decreased amplitude of the potential may be present, the latter being an expression of axonal damage. The ability to distinguish between early and late neuropathy has important treatment implications.

18.5 Contraindications for Electroneurography

There are few contraindications to ENG. If the patient has a pacemaker or defibrillator, the advice of a cardiologist and switching off the electronic device are necessary, as the electric stimulation of the test may interfere with it. When using needle electrodes, it is necessary to use caution in the presence of ulcers, skin lesions, or lymphedema. Needle electrodes are not to be used in subjects on anticoagulant therapy due to the risk of hematoma.

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Imaging in Leprosy

19

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Leila Oppezzi, Alberto Tagliafico, and Carlo Martinoli

19.1 Introduction

Electrophysiology, which still represents the clinical gold standard for nerve assessment, does not always allow to assess the exact location, cause and extent of a nerve lesion and the concurrent disease of surrounding tissues. Given these limitations, diagnostic imaging is increasingly used to evaluate peripheral nerve abnormalities, such as inherited disorders, entrapment syndromes, dysimmune neuropathies, traumas and tumours, thus influencing the diagnosis and clinical care of patients with peripheral neuropathies [1–3]. Both high-resolution US and MR imaging can provide effective depiction of nerves in the limbs and extremities offering a precise morphologic correlation for functional data. With last generation equipment, a variety of new imaging techniques, such as MR neurography, diffusion tensor imaging and fibre tractography, seem to make MR imaging of nerves increasingly detailed and advanced. On the other hand, despite an inherent operator dependency and a longer learning curve, high-resolution US provides speed of performance and important advantages over MR imaging, including a higher spatial resolution that makes it possible to demonstrate very small distal branches (even <1 mm thick), the ability to explore long nerve segments in a single sweep and, last but not least, its dynamic capabilities with real-time scanning during joint motion or muscle contraction. In the last years, the advent of ultra-high-frequency US with sophisticated focusing in the near field and matrix technology has further improved imaging resolution making the evaluation of nerve fascicles more precise and detailed [4]. Based

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on the literature, both imaging modalities have been shown to be able to provide relevant information in patients affected by leprosy [5–10]. Until now, however, too few clinical studies are available in the literature to compare the effectiveness of either imaging modality and draw guidelines on which one should be used in the first line [11]. Similarly, the role of imaging in the diagnostic workup of patients with leprosy has still to be defined.

19.2 Basics of Nerve Imaging

US is able to image nerves directly and demonstrate the fascicles as hypoechoic tubular structures embedded in a hyperechoic background [12] (Fig. 19.1). Peripheral nerves are flexible structures and may change in shape from round to oval depending on the width of their anatomic passageways and the nature of perineural structures. Across joints, nerves traverse osteofibrous tunnels that redirect their course [1]. At these sites, they may assume a more homogeneous hypoechoic appearance due to a tight package of their fascicles [13]. In normal states, the perineural and intraneural vasculature is inconspicuous, and only scanty coloured flags may be observed at Doppler imaging owing to a too low blood volume and slow flow velocities. Apart from direct nerve evaluation, US may also demonstrate muscle abnormalities in the territory of innervation, such as loss in bulk and fatty replacement as a result of a denervation process. The intraneural architecture of nerves can also be depicted on MR imaging [13]. With this technique, the use of a high matrix, a relatively small slice thickness and a limited field of view is mandatory to optimize spatial resolution. On short-axis T1-weighted images, nerves are characterized by multiple small rounded hypointense dots corresponding to the fascicles and surrounded by high intensity signal related to the epineurium [14]. On T2-weighted images, they become isointense to mildly hyperintense as compared

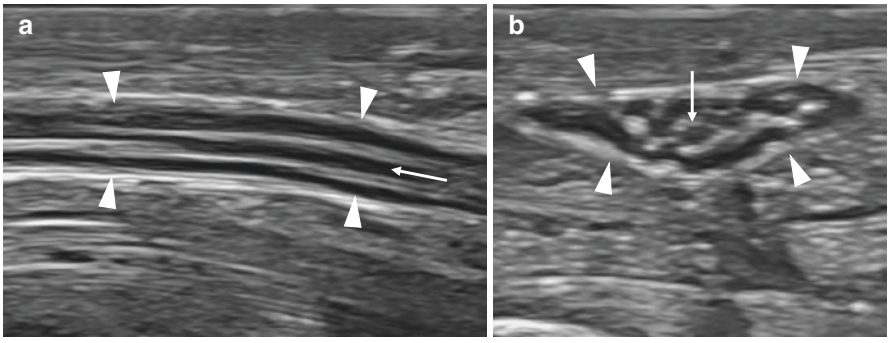

Fig. 19.1 US appearance of normal nerves. Long-axis (**a**) and short-axis (**b**) 22-8 MHz US image of the median nerve at wrist. (**a**) The nerve (arrows) is composed of multiple hypoechoic parallel bands (arrow) related to the fascicles, separated by the hyperechoic epineurium. (**b**) The nerve (arrows) is characterized by a honeycombing appearance made of rounded hypoechoic fascicles (arrow) embedded in a homogeneous hyperechoic background related to the epineurium

to normal muscle. Swollen nerve fascicles with high endoneurial fluid content may exhibit slightly higher signal intensity than the surrounding fat. The major disadvantage of T2-weighted images without fat suppression is that the signal intensity of the epineurium is similar to the one of nerve fascicles. This may obscure intraneural abnormalities that usually result in a high signal intensity [14]. Because of the integrity of the blood-nerve barrier, normal nerves show no enhancement after gadolinium administration. Some specific improvements in the MR protocol have recently developed to obtain more detailed depiction of the internal architecture of nerves. With MR neurography, for instance, high-resolution phased-array coils and tSE T2-weighted images with fat and flow suppression are used to remove the signal from non-neural structures and obtain a pure nerve delineation [15]. Nerve fascicles show high signal intensity on MR neurography, whereas the epineurium and all the structures surrounding the nerve are characterized by a low signal intensity. Signal intensity changes can be observed in denervated muscles as a result of neurogenic oedema (due to increased muscle blood volume and extracellular fluid) and atrophy with fatty degeneration. While acutely and subacutely denervated muscles show high signal intensity on T2-weighted images, related to oedema, and normal signal intensity on T1-weighted images, Chronically denervated muscles exhibit increased signal intensity on T1-weighted images and loss of muscle bulk resulting from fatty infiltration and atrophy. Consequently, acute and subacute muscle denervation is depicted on T2-weighted images, whereas chronic muscle denervation is better evaluated on T1-weighted sequences [13, 15]. Some functional MR imaging techniques have been introduced to image nerves, such as diffusion tensor imaging and tractography. However, the description of these very advanced techniques is beyond the scope of this chapter [13].

19.3 Nerve Imaging in Leprosy

In the early stages of leprosy, large nerves exhibit a normal appearance on US and MR images [6]. This can be explained by the fact that the nerve damage mostly occurs at intradermal level and does not affect the main trunks extensively [10]. In the limbs and extremities, the occurrence of nerve shape changes, and echotextural abnormalities do not correlate with the disease duration, but seem to have a direct relationship with the number and severity of acute reactional episodes (reversal reactions) that the nerve has suffered during the course of the disease. In general, the more and most severe episodes of reversal reactions undergone in the past, the more extensively deranged the nerve echotexture. On the other hand, patients with long-standing disease who did not undergo reversal reactions show normally appearing nerves. No significant correlation has also been found between the nerve abnormalities visualized on US and MR imaging and the two main disease poles—paucibacillary and multibacillary: this indicates that the clinical types of leprosy cannot be separated based on imaging features. In fact, both paucibacillary and multibacillary forms present borderline histopathologic features that are often overlapping. Abnormally swollen nerves are often found within or in proximity to osteofibrous tunnels, such as the cubital tunnel

for the ulnar nerve, the carpal tunnel for the median nerve, the area of the fibular head and neck for the peroneal nerve and the tarsal tunnel for the tibial nerve [6, 7]. In most instances, the abnormal segment of a nerve is more extended longitudinally than one can expect in case of a compressive neuropathy and is typically located proximal and not distal to the tunnel. The nerve swelling can be quantified by measuring the nerve cross-sectional area or the calibre when the nerve size is too small for a reliable calculation of the area. Even if measurements obtained at specific levels are a valuable means to obtain better standardization, the nerve cross-sectional area should be sampled, in logical terms, at the site where the nerve is maximally enlarged and the histopathologic changes are, therefore, most relevant. The nerve area can then be used as a baseline reference to better perceive nerve size changes during follow-up studies. Two methods are used to measure the nerve cross-sectional area: the indirect method, based on calculation of the nerve diameters by calipers and application of the ellipse formula (transverse diameter x anteroposterior diameter x $\pi/4$), and the direct method, based on manual tracing and automated calculation of the area [16, 17]. Both methods can be used depending on the examiner's preference and the equipment software available on US and MR devices. Abnormally swollen nerves may exhibit a preserved echotexture with fascicles that are enlarged but still visible as individual structures (Fig. 19.2). If severe intraneural derangement occurs, the nerve assumes a more homogeneous pattern with loss of the fascicular structure on both US and MR imaging [6, 7]. No specific imaging signs exist to differentiate compression-related damage from histopathologic derangement pertaining to the underlying disease. The presence of an abrupt nerve calibre change at the entrance of an osteofibrous tunnel is the only finding that may suggest a concurrent compressive syndrome. In some instances, nerve abnormalities can be encountered in patients with normal neurophysiological findings, implying the concurrence of abnormal morphology with preserved nerve function in leprosy patients [7]. Recently, sonoelastography has been tested in

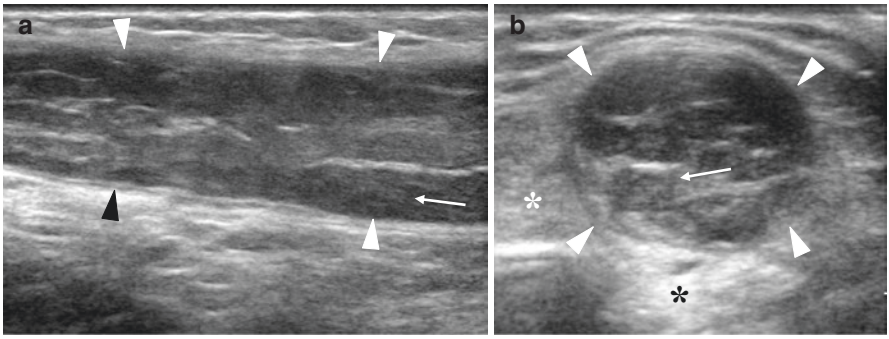

Fig. 19.2 US examination of the ulnar nerve at the elbow in a 32-year-old patient with borderline tuberculoid leprosy, long-standing symptoms and recent worsening of the nerve function. Long-axis (**a**) and short-axis (**b**) 17-5 MHz US images demonstrate diffuse swelling of the ulnar nerve (*arrowheads*) which is characterized by markedly swollen fascicles (arrow) and poor visualization of the hyperechoic bands of the interfascicular epineurium. Reactive extraneural changes with increased echogenicity of the perineural fat (*asterisks*) are observed

leprosy neuropathy with promising results, even if further validation on larger series of patients is required [18].

19.4 Reversal Reactions

From the histopathological point of view, reversal reactions lead to an increased intraneural pressure due to oedema and massive infiltration of inflammatory cells. This produces a microcompartmental syndrome inducing compression on nerve filaments and Schwann cells so that their function is rapidly compromised with demyelination and axonal loss [19]. In this phase, US and MR imaging can demonstrate marked fusiform swelling of the nerve, fascicle thickening and epineurial abnormalities. It is conceivable that these findings may reflect severe nerve involvement by cellular infiltrates, granuloma formation and, in chronic long-standing disease, intraneural fibrotic changes. During the course of acute reversal reactions, the involved nerves may show marked T2 hyperintensity [6]. Some authors have suggested that demyelination causes an increase in the endoneural T2 signal due to a decrease in myelin-bound water and a concomitant increase in extracellular water [20]. This process is emphasized in the course of reversal reactions as a result of concomitant nerve cell damage. It should be noted, however, that the increase in T2 signal intensity is the result of changes related to inflammatory infiltrates, oedema (intraneural free extracellular water) and hyperaemia. In other terms, the high T2 signal intensity should be basically regarded as a nonspecific finding encompassing many different histopathological phenomena that cannot be distinguished as individual entities. On the contrary, Doppler systems and Gd-enhanced MR imaging are more reliable indicators of the intraneural hyperaemia that accompanies reversal reactions [6]. In these patients, Doppler imaging is able to demonstrate increased blood flow signals in the perineural and intraneural microvasculature based on the higher blood volume and flow velocities associated with neoangiogenesis (Fig. 19.3).

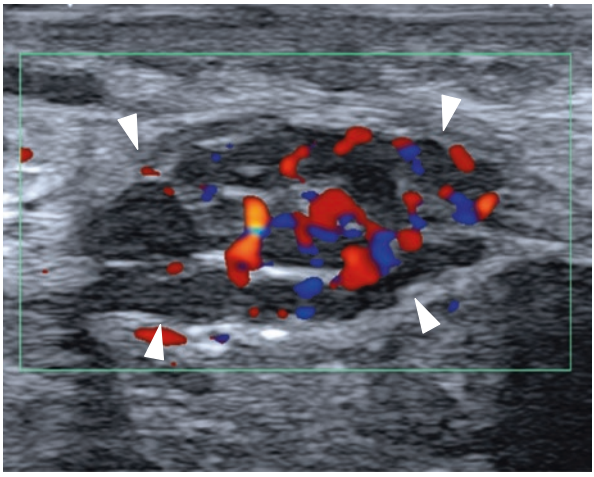

Fig. 19.3 Reversal reaction. Short-axis 24-8 MHz colour Doppler US image of the median nerve (*arrowheads*) at the distal forearm in a 25-year-old patient with borderline tuberculoid leprosy and sudden onset of acute symptoms and nerve palsy. Marked intraneural hyperaemia is observed in the abnormal nerve. Note the selective location of colour flow signals in the interfascicular epineurium

Similarly, acutely inflamed nerves exhibit gadolinium enhancement involving both fascicles and surrounding epineurium. The increased uptake of contrast medium in the nerve substance seems to depend on disturbances in the microvasculature and venous stasis and on alteration of an effective nerve-blood barrier during reversal reactions (Sugimoto et al. [20]). The inflammatory process causes abnormal permeability then resulting in leakage of contrast medium into the nerve tissue. This hypothesis agrees with previous ultrastructural studies that report severe damage to the endothelial cell structure of intraneural vessels during acute reactions [21]. With the resolution of the acute phase, some reduction of the nerve size can be recognized owing to reabsorption of intraneural oedema and venous stasis. In longitudinal studies, US seems to be superior to MR imaging to quantify such nerve size changes, even if they are subtle and limited to the fascicular level. A parallel decrease in vasculature can be appreciated at Doppler imaging. With time, the cumulative effect of cell damage and formation of epithelioid granulomas leads to irreversible intraneural changes with progressive deposition of collagen in the nerve tissue. This may lead to a hypoechoic nerve with loss of the fascicular echotexture, definite lowering of both T1- and T2-weighted signal intensities, a hypovascular intraneural pattern on Doppler imaging and poor to absent gadolinium enhancement.

Intense immune-mediated reactions, most often encountered in tuberculoid-type leprosy, may activate such a strong inflammatory process which can lead to necrosis of nervous tissue and formation of intraneural abscesses (Fig. 19.4). Distorted and disrupted fascicles around the abscess and intense hyperaemia are associated findings. At US, abscesses look anechoic and can be compressed only partially by the probe [5, 22] (Fig. 19.5). With the use of gadolinium-enhanced sequences, MR

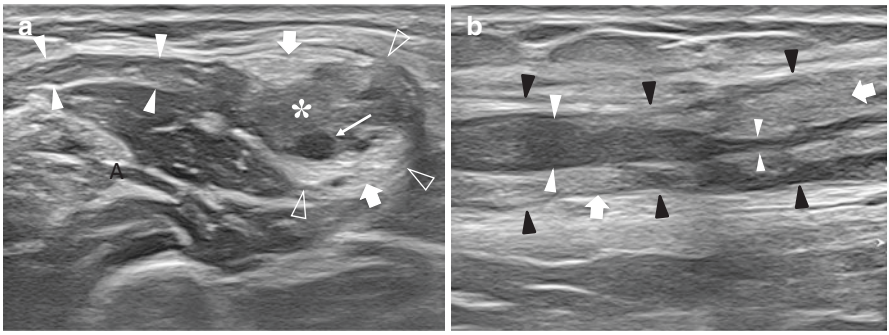

Fig. 19.4 Intraneural changes in the ulnar nerve of a 28-year-old patient with borderline tuberculoid leprosy who underwent repeated episodes of reversal reactions. (**a**) Short-axis 22-8 MHz US image of the ulnar nerve (void arrowheads) reveals profound derangement of the intraneural architecture with fascicles (*thin arrow*) reduced in number and spaced out by irregular hyperechoic (*large arrows*) and hypoechoic (*asterisk*) intraneural areas. Note the extraneural extension (white arrowheads) of the abnormal hypoechoic area along the fascial plane indicating the path of a previous abscess. (**b**) Long-axis 22-8 MHz US image of the ulnar nerve (black arrowheads) demonstrates its abnormal echotexture made of irregular fascicles with alternating thinned and thickened segments (*white arrowheads*). A thickened echogenic epineurium (*white arrows*) is also seen displacing the fascicles

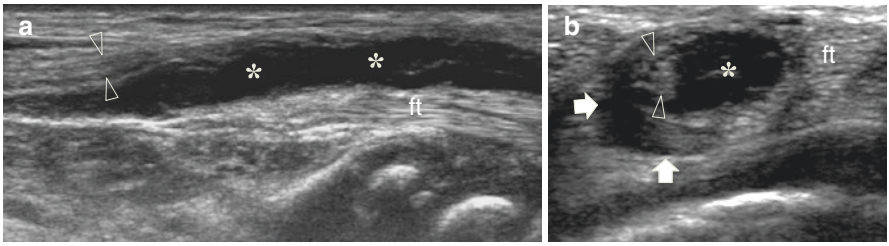

Fig. 19.5 Intraneural abscess in a 9-year-old boy with borderline tuberculoid leprosy. Long-axis (**a**) and short-axis (**b**) 12-5 MHz US images of the median nerve at wrist show extensive intraneural anechoic areas (*asterisks*) causing peripheral dislocation of the fascicles (*arrowheads*). Diffuse perineuritic signs (*white arrows*) are also demonstrated. ft, flexor digitorum tendons

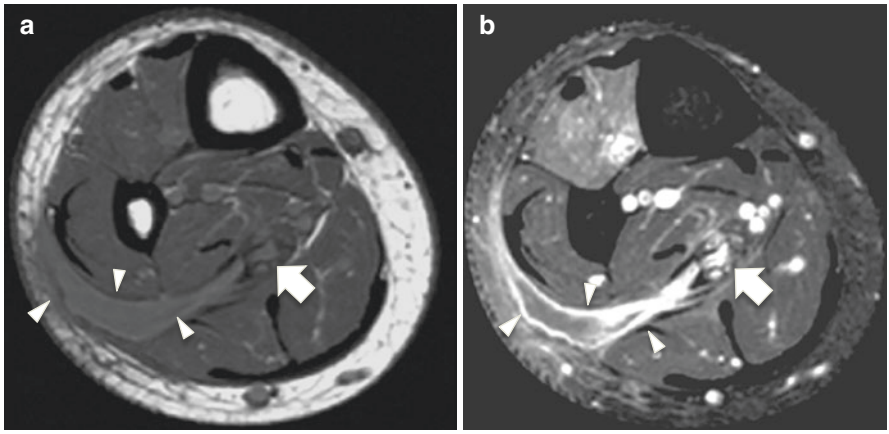

Fig. 19.6 Extraneural spread of a nerve abscess in a 34-year-old patient with tuberculoid form of disease. Axial (**a**) T1w- and (**b**) fat-suppressed tSE T1w MR images after gadolinium administration demonstrate a swollen tibial nerve (*arrow*) characterized by swollen fascicles and increased signal intensity in the postcontrast acquisition. The nerve is in continuity with a crescent-like collection (*arrowheads*) located along the fascial planes between the soleus, the peroneal muscles and the flexor hallucis longus

imaging is able to distinguish an intraneural abscess from other focal intraneural abnormalities by showing absent uptake of gadolinium in the fluid collection and lack of surrounding intra- and perineural inflammatory signs. Occasionally, the abscess may migrate outside the outer epineurium, discharging necrotic material along fascial planes even far from the nerve of origin (Figs. 19.4 and 19.6). A further type of nerve abnormality can be seen in patients affected by multibacillary leprosy who have had episodes of erythema nodosum leprosum. These patients have nerves that are less enlarged than patients with a history of reversal reactions, but often characterized by profound structural abnormalities with absence of any discernible fascicular echotexture. In advanced disease, the low T1 and T2 signal intensities and the presence of a few atrophied fascicles are consistent with the progressive

deposition of collagen and some contraction of the endoneural area [6]. It's in fact known that episodes of erythema nodosum leprosum promote intraneural deposition of collagen and can cause epineurial sclerosis secondary to vasculitis and lysosomal digestion of immune complexes by granulocytes.

19.5 Role of Imaging in Patient Management

In case of suspected neuropathy, US should be regarded as the first-line imaging modality to be used as complement of clinical examination and neurophysiology. Compared to MR imaging, US has the advantage to check multiple sites for possible nerve lesions and to evaluate long nerve segments in a single study. The examination must be systematic and should include the entire nerve course with a special focus on the osteofibrous tunnels. In accordance with clinical and neurophysiological data, US depiction of an abnormal nerve at these tunnels may reinforce the indication for nerve release surgery (possibly associated with neurolysis) to prevent or delay the progression of nerve damage by relieving the effects of microtrauma and focal compression. In this field, US may help the selection of nerves in which decompression has a higher probability of cure. The nerve cross-sectional area, along with T2 signal, intraneural hyperaemia and Gd enhancement, is the most useful parameter to evaluate the results of therapy suggesting a possible role of imaging to monitor the inflammatory process in longitudinal studies. This seems particularly relevant when clinical findings do not help to determine whether a patient is actually in remission. In this setting, US can inform about the status of the involved nerve and can provide a more rational basis for the decision to maintain the antireaction therapy or to better adjust the treatment approach. During steroid therapy, doses are usually adjusted depending on the patient's response as assessed on clinical findings. This assessment may be inaccurate, however, because clinical abnormalities may normalize despite persistent intraneural inflammation. We believe that US can give a more reliable assessment of the status (progression/regression) of the inflammatory process at intraneural level and this may better contribute to adjust the medical treatment appropriately and in a more personalized way. In this field, US seems opening interesting perspectives to guide local injection therapy around the abnormal nerve segment and perform percutaneous nerve biopsies in atypical cases and suspected disease [23, 24]. Finally, US is also effective to assess the status of decompressed nerves after tunnel release or transposition. Concerning the outcome, preliminary experience also suggests that imaging has potential to predict reversal reactions by depicting increased hyperaemia and high intraneural T2 signal even in the absence of clinical evidence of an ongoing acute episode [6].

Comparing the effectiveness of US and MR imaging, it must be noted that both techniques show similar results in demonstrating nerve enlargement, endoneural structural abnormalities and compressive signs [6]. In a routine clinical setting, selection of the appropriate imaging technique may depend therefore on accessibility and cost-effectiveness. Although high-end MR equipment provides precise imaging of nerves and higher sensitivity in detecting reversal reactions, this

technique is not easily accessible and very expensive. Because leprosy is not a major health problem in the western world, but it is endemic in developing countries, especially in tropics and subtropics, the use of MR imaging in the large scale is often precluded owing to its high costs. US is much more convenient, and reasonably accurate in this field. Its application for evaluating nerves in leprosy has been introduced 10 years ago. However, it is evident that economic issues and poor acceptance of this technique that requires a long learning curve and high-end machines have probably impaired its effective use in many countries until now. Apart from direct nerve imaging, radiological modalities can also contribute to assess the soft tissue and skeletal abnormalities that may occur in the follow-up of patients with nerve function impairment, such as ulcers and subsequent osteomyelitis or neuro-osteoarthropathy [8].

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Differential Diagnosis of Leprosy: Nerves

20

Lizia Reni

20.1 Differential Diagnosis: Nerves

Leprosy is mainly a disease of the peripheral nerves and of the skin. When the first signs appear on the skin, the diagnostic pathway is completed by careful examination of the peripheral nerves and by slit-skin smear examination. On the contrary, when the clinical picture is dominated by involvement of nervous trunks, looking for cutaneous signs and symptoms will define the diagnosis. In the pure neural form of leprosy, only accurate study of the pathology of the peripheral nerves, with neurological differential diagnosis, will help to reach the correct diagnosis. Histopathology is performed in some cases on some easily accessible sensory nerves (sural and superficial radial nerves).

20.2 Radiculopathies

Radicular compression. Compression of spinal nerve roots with motor or sensory fiber damage produces motor and sensory deficits involving usually one limb. The pain is characteristically irradiating from the cervical or lumbosacral spine to the ipsilateral limb. Motor and sensory damages extend to the area supplied by the radicular plexus, not to the area of the nerve. Motor involvement may produce fasciculations, usually absent in leprosy.

Conduction velocity is normal or only moderately altered, while the F wave and the H reflex may be altered. These parameters, respectively, indicate involvement of the more proximal segments of the nerve and/or of the roots of the spinal cord.

Electromyography confirms the denervation activity with characteristic topography due to radiculopathy. The diagnosis is confirmed by nuclear magnetic resonance (NMR).

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Postherpetic paralysis. In postherpetic paralysis, which sometimes follows herpes zoster infection, both pain and motor deficit have radicular distribution, and there is history of a relevant cutaneous eruption.

20.3 Pathology of the Brachial Plexus

Complete brachial plexus lesion produces a flaccid paralysis, reduction or loss of reflexes, and sensory disorders of the upper limb.

Partial brachial plexus lesion produces clinical pictures related to the affected regions. The causes are often traumatic, compressive, or by infiltration. Parsonage—Turner syndrome and actinic brachial neuropathy must also be mentioned.

The pathogenesis of Parsonage–Turner syndrome is partially unknown, but an autoimmune mechanism is considered. The clinical picture is frequently preceded by infections, surgical interventions, or vaccination. It begins with intense pain referred to the shoulder, interscapular region, followed after some days by tiredness, muscular hypotrophy, and sensory deficit to part or all of the upper limb. It differs from the leprosy neuropathy because pain is localized more proximally; nerves are not painful, tender, or enlarged. Electromyography shows signs of denervation of muscles or groups of muscles, whose distribution is related to the plexus not to the nerve. Electroneurography may present decreasing amplitude of the evoked potential but no significant slowing of the conduction velocity. The F wave may be absent or altered, confirming pathology of the roots or of the proximal nerve segments. Actinic brachial neuropathy may follow, even after years, radiotherapy in the axillary region.

20.4 Inflammatory Acute Polyradiculopathy (Guillain–Barré Syndrome)

This syndrome affects both sexes and all ages; it is often preceded by febrile infection of the respiratory tract or by *Campylobacter jejuni* infection. It produces sensory signs and motor involvement at the extremity of the limbs; the evolution of the motor involvement is centripetal; in 1 or 2 weeks, it may affect all limbs and the cranial region. There are loss of deep tendon reflexes and autonomic disturbances. Involvement of the respiratory muscles with necessity for mechanically assisted ventilation is not rare. In its typical forms, electrophysiology shows demyelination with prolongation of distal latency, loss or alteration of F wave, and considerable slowing of conduction velocity [1]. Early axonal damage is linked with rapid and severe evolution of the disease and with incomplete recovery. Cerebrospinal liquor examination is normal in the first days and then shows increasing proteinorachia with a peak about the fifth week. Therapy is with plasmapheresis or intravenous immunoglobulins. Death occurs in 3–5% of cases; in 10% of survivors, there are sequelae with disabilities.

20.5 Mononeuropathies

Involvement of a single nerve gives particular diagnostic problems. Compressive or entrapment mononeuropathy in a leprosy patient might have a different etiology, for example, rheumatoid arthritis. Some conditions may facilitate the development of compressive neuropathies, namely, reduction of the adipose panniculus (exposing the nerve to trauma), wrongly applied plaster of Paris, chronic compression and repetitive movements, use of vibrating tools, coma, or heavy sleep as in alcohol or drug abusers. In these cases, the more affected nerves are the radial nerve at the arm or forearm, the median nerve at the wrist, the ulnar nerve at the elbow, and the common peroneal nerve at the head of the fibula. Entrapment mononeuropathy presents an electrophysiology pattern similar to that observed in leprosy focal neuropathy. In some rare genetic neuropathies, the nerve is more susceptible to compression.

20.6 Multineuropathies

In leprosy, several nerves may be affected contemporaneously or one after the other. In these cases, the differential diagnosis should be with neuropathies of different etiology. The majority of multiple mononeuropathies are caused by systemic vasculitis of vasa nervorum: polyarteritis nodosa, Churg–Strauss syndrome, rheumatoid arthritis, systemic lupus erythematosus, and progressive systemic sclerosis.

In polyarteritis nodosa, the involvement of the peripheral nerves is similar to that of leprosy: pain to one or more nerves followed by motor and sensory deficits. This neuropathy may be without systemic symptoms: fever, arterial hypertension, abdominal pain, and arthralgia. The differential diagnosis with leprosy is facilitated by presence of high level of erythrocyte sedimentation rate (ESR) and other serologic alterations, with the biopsy of relevant nerves showing necrotizing arteritis.

In Churg–Strauss syndrome (also called allergic granulomatosis), peripheral neuropathy is frequently painful. It is often preceded by asthma, rhinitis, cutaneous vasculitis, and increase of blood and tissue eosinophils. Rheumatoid arthritis (RA) may present entrapment neuropathies. It can be distinguished from leprosy by the enlargement of tendons and joint alterations. More severe forms of neuropathy may be present in advanced stages of RA when serology is clearly positive. Also the neuropathy of systemic lupus erythematosus is present in advanced stages of the disease, when clinical and serologic findings are evident. In sarcoidosis frequently the cranial nerves are affected, particularly the facial nerve, with damage to all its branches (Bell's palsy). Diagnosis is facilitated by the presence of typical pulmonary alterations.

Sjögren's syndrome may be complicated by a peripheral neuropathy. It may appear as a superficial and proprioceptive sensory deficit in limbs of old-aged women with xerostomia and keratoconjunctivitis sicca. Schirmer's test, lip biopsy, and serum antibodies (SS-A and SS-B) are useful for diagnosis.

Cryoglobulinemia (mixed essential) can be associated with vasculitic mononeuropathy multiplex or to a more generalized polyneuropathy. Diagnosis is simpler

when it is in association with glomerulonephritis, arthralgia, and purpura, but more difficult when the neuropathy is isolated.

The neuropathy of Lyme disease is classified as multineuropathy. It appears in different forms in 10–15% of patients affected by this disease. Typically, it appears 13 weeks after the tick bite or the appearance of erythema chronicum migrans. It presents paralysis of the cranial nerves, radiculitis, and aseptic meningitis. The neuropathy can present also months after the infection with multiple mononeuropathies, lumbar or brachial plexopathies, and sensory polyneuropathy sometimes associated with moderate encephalopathy; rarely it is associated with Guillain–Barré syndrome. In the diagnosis, positive history of tick bite, arthralgias, and cutaneous rash are considered.

20.7 Polyneuropathies

The polyneuropathies present bilateral and symmetric nerve involvement and enter in the differential diagnosis of advanced borderline lepromatous and lepromatous leprosy [2].

Polyneuropathy associated with paraproteinemias. The polyneuropathy associated with alterations of serum immunoglobulins is a sensory and motor polyneuropathy that can be found in benign monoclonal gammopathy, multiple myeloma, plasmacytoma, and Waldenström macroglobulinemia.

Benign monoclonal gammopathy. In benign monoclonal gammopathy, electroneurography shows demyelination damage; there is an increase in serum mono-/polyclonal immunoglobulins, mostly of the IgG, IgM, and IgA types. In this condition, it is possible to identify several specific antimyelin antibodies, which react against a myelin-associated glycoprotein (MAG) or against sulfatidic components of myelin (SGPG, SPLPG). The cerebrospinal fluid typically shows an increase of the proteins.

Multiple myeloma. In multiple myeloma, there is involvement of the peripheral nervous system (in about 13% of cases) and, significantly, in the osteosclerotic form. In serum, there is frequently excess of an atypical monoclonal c-globulin: “k” in multiple myeloma, or “k” in the osteosclerotic variant. A small group of patients affected by osteosclerotic myeloma presents a sensory and motor polyneuropathy called polyneuropathy, organomegaly, endocrinopathy/edema, M-protein, and skin abnormalities (POEMS).

20.8 Immunomediated Demyelinating Neuropathies

Chronic inflammatory demyelinating polyneuropathy (CIDP). CIDP, similar to the acute inflammatory polyneuropathy (Guillain–Barré), is a diffuse polyradiculopathy, but it starts insidiously and progresses slowly, continuously, or by subacute flares. ENG shows typical demyelinating pattern: increased distal latency, slowing

of the conduction velocity, and partial conduction block. The differential diagnosis with leprosy neuropathy is based on examination of cerebrospinal fluid, which shows increased proteins with rare or absent cellularity [1].

Multifocal motor neuropathy. This recently characterized peripheral neuropathy presents similarities to CIDP. It shows male prevalence, commonly beginning as a subacute painless mono- or multiple motor neuropathy. There is no sensory deficit, and electrophysiology shows partial block of motor conduction. It is differentiated from the leprous neuropathy by absence of sensory deficit, both clinically and electrophysiologically; there is no pain, and in the affected segments, fasciculations are present, not seen in leprosy. An IgM anti-GM1 antibody directed against a ganglioside component of peripheral myelin is present in serum.

20.9 Metabolic Neuropathies

We discuss here those related to diabetes, uremia, nutritional deficiency, and alcohol abuse.

Diabetes. Diabetes mellitus is the most common cause of polyneuropathy in general clinical practice and can present in several forms [3]. There can be acute, ischemic, and isolated involvement of a cranial nerve (typically III), but any other major peripheral nerve can be affected. There can be multiple mononeuropathies and radiculopathies; these appear during uncontrolled periods of the disease or during weight loss and affect the lower limbs. Clinically, there is severe pain in the lumbar area, or at the hip and extending to the limb, followed by weakness and hypotrophy of the proximal muscles and hypo- or areflexia. Sensory and motor polyneuropathy is the most common form of diabetic neuropathy. It complicates type I and II diabetes, is more frequent in older age, and is related with disease duration. It appears insidiously with sensory deficit in the distal segment of the limb, evolves slowly, and produces plantar ulcers similar to those caused by leprosy. Clinically, diabetes polyneuropathy differs from leprosy neuropathy because it is limited to the lower limbs, and the motor deficit is limited and often subclinical; there are often symptoms of autonomic deregulation such as diarrhea, atonicity of the gastrointestinal tract, bladder dilation, impotency, and postural hypotension.

Uremia. Polyneuropathy represents one of the most common complications of chronic renal failure; often it complicates the terminal stage before dialysis treatment is started. It is a sensory and motor neuropathy that affects the lower limbs first and then the upper limbs.

Nutritional deficiency and alcohol abuse. In conditions where nutrient intake is heavily reduced, it is still possible to observe a sensory and motor polyneuropathy related to lack of group B vitamins. Prolonged alcoholism causes a similar neuropathy; this begins subacutely at the lower limbs and then becomes chronic with motor and mainly sensory alterations, associated with pain; frequently there is involvement of the central nervous system. Anamnesis, B12, and folic acid levels allow diagnosis.

20.10 Infectious Polyneuropathies

We discuss here those neuropathies related to acquired immune deficiency syndrome (AIDS), hepatitis C, and diphtheria.

AIDS. AIDS may be complicated by different types of neuropathy. The more frequent is a prevalently sensory, symmetric bilateral, axonal polyneuropathy. A multiple mononeuropathy has also been described with pain, and an acute and chronic inflammatory polyradicular syndrome with demyelinating characteristic.

Hepatitis C. Liver damage associated with hepatitis C, and particularly with cryoglobulinemia, may produce a sensory and motor neuropathy of variable severity.

Diphtheria. The diphtheria exotoxin produces, within 15 days from infection, paralysis of the laryngeal and pharyngeal muscles, with dysphagia and rhinolalia. In this early phase, the differential diagnosis is with myasthenia gravis and botulism. The polyneuropathy appears after 2 or 3 months from the infection and produces motor and sensory deficit that characteristically affects contemporaneously the four limbs. Alternatively, it may present a descending course, from the upper to the lower limbs. The cerebrospinal fluid shows increase in proteins. It is a rare condition outside epidemics of diphtheria.

20.11 Iatrogenic Polyneuropathies

Several drugs may cause peripheral neuropathy. Often they are sensory forms that affect the four limbs, are dose dependent, and develop after very high dosages or prolonged therapies. Involved drugs can be chemotherapeutic drugs such as cisplatin, vincristine, thalidomide, antibiotics such as isoniazid and dapsone, and some cardiac drugs.

Dapsone and thalidomide are of specific interest in leprosy. Dapsone may cause a mainly motor neuropathy. Thalidomide is associated with a mainly sensory neuropathy; it commonly appears after a few months of treatment and gradually aggravates with prolongation of treatment. It affects both upper and lower limbs, sometimes with severe pain. ENG shows mainly axonal damage (reduced amplitude of potential, particularly the sensory one, without significant decrease in conduction velocity).

20.12 Toxic Neuropathies

Tricresyl phosphate (and other organophosphates) and other substances such as thallium and arsenic, rarely, cause a neuropathy that can be rapid (days) and fatal. The clinical picture includes signs of systemic poisoning. Chronic arsenic poisoning causes a neuropathy that evolves in weeks or months and is accompanied by systemic signs such as anemia, jaundice, cutaneous pigmentation, nail changes, and gastrointestinal symptoms. Lead produces peripheral neuropathies following

chronic exposure. Damage is mainly motor, particularly affecting the radial nerve. Suspicion is raised by anamnesis, and signs such as anemia, gingival alterations, and abdominal pain. Diagnosis is by laboratory investigation: basophilic dots in erythrocyte precursors, high level of blood lead, urinary excretion of lead, and coproporphyrins.

20.13 Paraneoplastic Polyneuropathy

A sensory and motor neuropathy may accompany a malignant tumor. In some cases, it may precede by months or years the appearance of the tumor. The superficial and deep sensitivities are affected with severe ataxia and a possible motor deficit. The clinical picture may be disabling. In some cases, anti-Hu antibodies are present.

20.14 Genetically Determined Neuropathies

Genetically determined neuropathies can be divided into Charcot–Marie–Tooth (CMT) disease, hereditary sensory and autonomic neuropathy (HSAN), familial amyloidotic polyneuropathies, and neuropathies related to hereditary disturbances of the metabolism.

Among them, those grouped in the CMT eponym are relatively more frequent. Signs of this group of diseases, even if slight, are present at early age, but sometimes the pathology is evident only in adulthood. The clinical picture presents bilateral and symmetrical sensory and motor alterations with a progressive course, involvement of upper and lower limbs, and areflexia. A characteristic sign is bilateral pes cavus. Diagnosis is suggested by the presence of the typical foot, by the familial nature of the condition (sporadic cases are also reported), and by the prolonged course of the disease. Electrophysiology differs in the different forms of the disease. Molecular diagnosis is possible in several forms. CMT1A, probably the more frequent, is transmitted as autosomal dominant; the genetic alteration is on the 17p11 chromosome, consisting of a duplication of the gene for the peripheral myelin protein (PMP22).

Hereditary neuropathy with liability to pressure palsy (HNPP). HNPP is an hereditary neuropathy, related to the CMT group, presenting thickening of peripheral nerves; it may enter in the differential diagnosis of leprosy. HNPP is transmitted as autosomal dominant; the genetic alteration, as in CMT1A, is on the gene for PMP22, in this case being a deletion. In its classical form, the peripheral nerves present abnormal susceptibility to compression. Plaster cast, excessively tight shoes, sleeping in the wrong position, sitting with legs crossed, and other forms of localized pressure on the nerves may cause acute peripheral paralysis. Electrophysiology shows nerve lesions mostly localized where the nerve is more exposed, such as the ulnar nerve at the elbow and the peroneal nerve at the head of the fibula. In HNPP, differently from the leprous neuropathy, there is never pain. The nerve is rarely as thickened as in leprosy.

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Part V

Reactions in Leprosy

Reactions in Leprosy

21

Bernard Naafs and Salvatore Noto

21.1 Introduction

Nerve damage leading to impairments and permanent disability is still the major problem in the course of a leprosy infection. Were it not for this damage, leprosy would be a rather innocuous skin disease, whereas even today it is one of the most feared diseases, often associated with severe social repercussions for the sufferer.

Despite the claim of the World Health Organization (WHO) that it would not be a public health problem anymore after the year 2000 (later extended to 2005), leprosy still remains one of the main causes of peripheral nerve damage.

The main reason is that there is a lack of awareness and knowledge, frequently leading to a major delay in diagnosis. Among others, the WHO elimination policy is therefore to blame.

Nerve damage may occur before antimycobacterial treatment, during this treatment, and even in patients who are released from treatment, labelled cured by the leprosy programme. It can be stated that there is no leprosy without nerve damage. This damage usually occurs during episodes of disturbances in the immune status of the patient, the so-called reactions. Reactions belong to the normal course of a leprosy infection. Leprosy treatment can prevent or precipitate them.

There are three types of reactions: type 1 leprosy reaction (T1R), also called reversal reaction (RR); type 2 leprosy reaction (T2R), also called erythema nodosum leprosum (ENL); and Lucio's phenomenon, a reaction occurring specifically in multibacillary patients from Mexico. The latter does not cause nerve damage and is discussed in another chapter.

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Type 1 leprosy reaction (T1R) occurs in borderline leprosy (BT, BB and BL). In these forms of leprosy, nerve damage occurs early in the course of the disease; it is usually rather gradual, taking weeks or even months to become irreversible, but, occasionally, severe nerve damage may occur overnight.

Type 2 leprosy reaction (T2R) occurs in lepromatous leprosy (BL, LLs and LLp). In these forms of leprosy, nerve damage occurs late in the course of the disease; it may take years to develop the damage; however, it may increase suddenly during an episode of T2R. Both reactions, T1R and T2R, can occur in BL leprosy, even at the same time.

Reactions must be diagnosed early and treated appropriately if permanent disability is to be avoided. Ideally, the reactions should not occur at all, being prevented by treatment. To achieve this, it is of utmost importance to understand the mechanisms behind the reactional states and the principles of management.

Much knowledge on immunology and pathology has been accumulated over the past 50 years; methods of detection have been introduced as well as adequate treatment for most of the patients. This chapter covers these aspects. It must be emphasized that the experienced clinician is still the centre of diagnosis and treatment of a reaction. A major problem is that this experience has disappeared due to the decentralization of the leprosy services and the dismantling of the vertical leprosy programmes.

21.2 Type 1 Leprosy Reaction

Many names have been attached to this type of reaction, which has led to fierce arguments among leprologists who did not understand each other's definition and terminology and hardly listened to each other's arguments. As a result, for quite some time, there was an Anglo–Saxon–French leprology, a Spanish–Portuguese–South American one and an Indian one. Some of the terms used are reversal reaction, borderline leprosy reaction, tuberculoid reaction, tuberculoid in reaction, active tuberculoid leprosy, downgrading borderline leprosy, upgrading versus downgrading reaction and Jopling type 1 reaction. Some of these terms overlap with one another partially, and others completely while being conceptually different. However, recently, leprologists have started to speak the same language and call it type 1 leprosy reaction (T1R).

21.2.1 Signs and Symptoms of T1R

T1R usually only involved the nerves and the skin, but it must be emphasized that liver and joints may be affected occasionally. Skin involvement frequently accompanies nerve involvement, but may also precede or follow nerve damage.

Clinically, a reaction may be suspected when in borderline patients there is increased inflammation of pre-existing skin lesions. Hypopigmented or only slightly erythematous macules become red and swollen, form plaques (Figs. 21.1, 21.2, 21.3, 21.4) and occasionally undergo ulceration. Crops of new lesions may

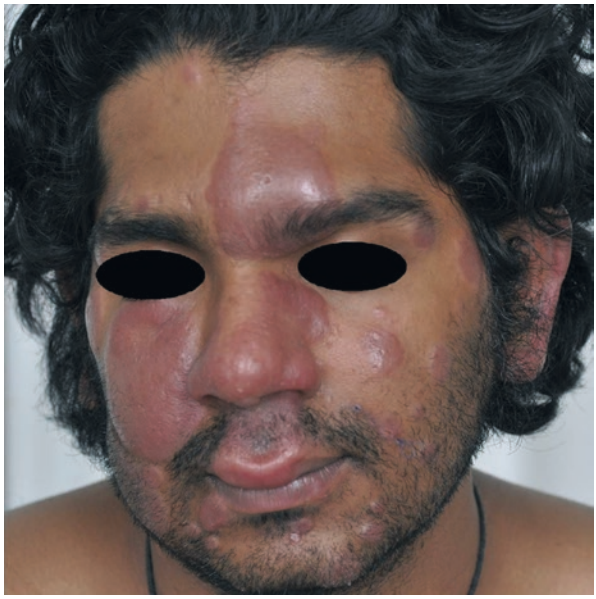

Fig. 21.1 BT leprosy in T1R. Erythematous, oedematous plaques on the face. (© Enrico Nunzi 2021)

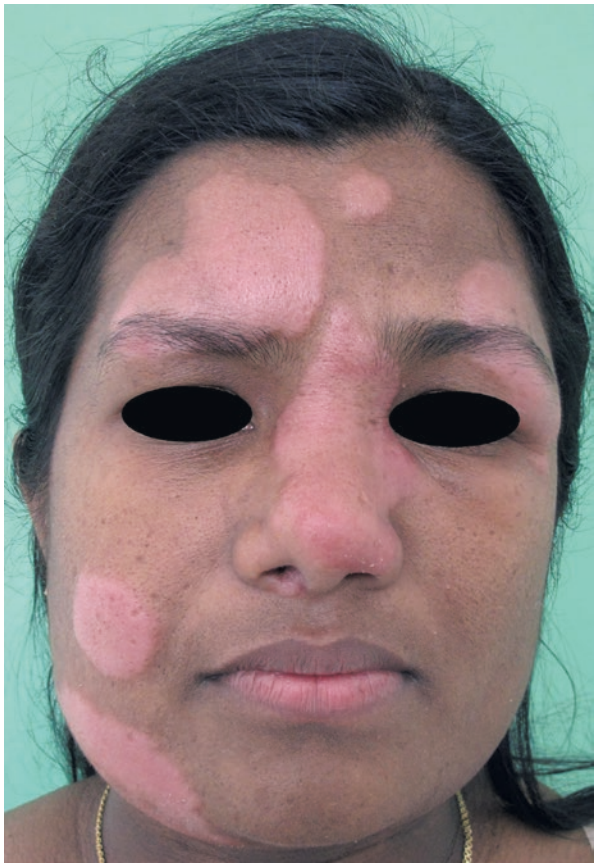

Fig. 21.2 BT leprosy in T1R. Erythematous, oedematous plaques on the face. (© Salvatore Noto 2021)

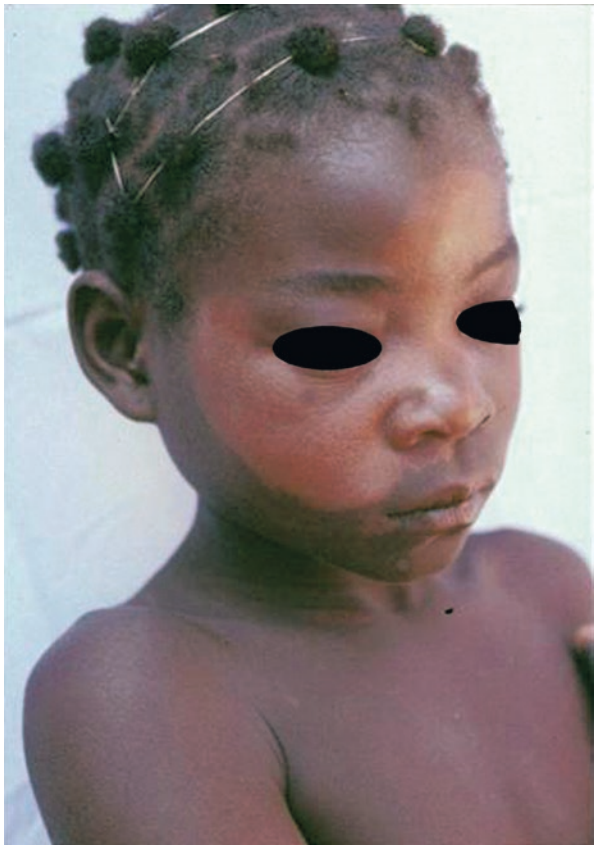

Fig. 21.3 BT leprosy in T1R. Large erythematous lesion on the face; on the right cheek, oedema is evident. (© Enrico Nunzi 2021)

suddenly appear in previously clinically uninvolved skin. Sometimes, extensive oedema of the extremities or face may be present, i.e. acroedema, in particular in BL patients (Fig. 21.5). Patients may complain of a burning, stinging sensation in the skin lesions and complain of aches and pains in the extremities or in the face and of loss of strength and/or sensory perception.

The peripheral nerve trunks at specific sites may become swollen and tender on palpation (Chap. 14). The Tinel sign may become positive, i.e. lightly tapping over the nerve elicits a sensation of tingling or "pins and needles" in the distribution of the nerve.

Loss of strength may involve the muscles serving eyelids, face, hands and feet. Patients may suddenly start to drop things from their hands or stumble when walking.

To diagnose a reaction early, one may ask the patient to close his eyes lightly, and to notice any, even minimal, movement of an eyelid or a slight gap in the closure (Chap. 14), which may herald further damage. It must be noted that, when a patient is asked to close his or her eyes firmly, such minimal damage will pass unnoticed. Loss of vision is one of the major disabilities and should be prevented.

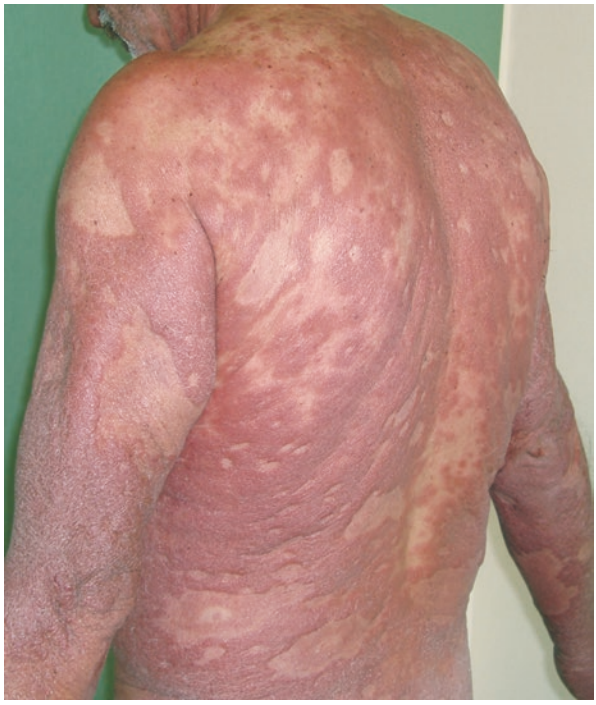

Fig. 21.4 BB leprosy in T1R. Erythematous and oedematous lesions all over the trunk and the upper limbs. The punched-out lesions and the immune areas are evident. (© Enrico Nunzi 2021)

Another early sign to note is whether hands and feet are sweating or have new dry areas. The appearance or an increase in size of dry areas is often a first sign of an incipient reaction. If sensory loss is severe, patients may injure their hands and feet and also may start to develop blisters without knowing the cause.

However, patients with T1R, contrary to patients with T2R, are not ill. Some have remarkably few complaints and symptoms; therefore, detection may be delayed or even missed.

Early diagnosis and treatment of these complications need objective clinical parameters. These consist of mapping (drawing) the lesions, which is tedious but certainly worthwhile, and of careful assessment of nerve functions by voluntary muscle testing (VMT) and graded sensory bristle test (GST) [1, 2]

21.2.2 Laboratory in Type 1 Leprosy Reaction (T1R)

Laboratory tests have little to contribute, such as follow-up of cytokines, tumour necrosis factor-alpha (TNF-α), interleukin, (IL)-1 or IL-2, measurement of the acute-phase response, the ratio of serum amyloid A-/C-reactive proteins and activation products such as neopterin, nor the presence of antibodies against specific *Mycobacterium leprae* (*M. leprae*) antigens (phenolic glycolipid 1 or LID 1) [3]. This also, but less, applies for more elaborate testing of cell-mediated immunity

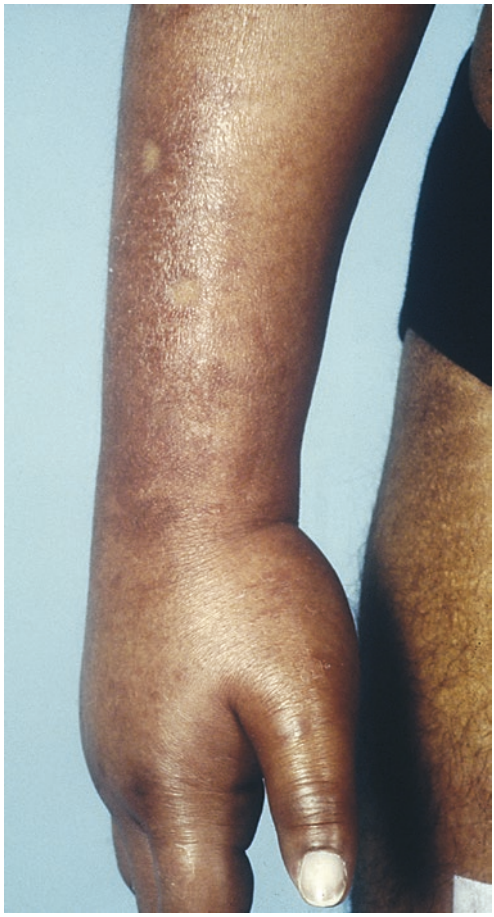

Fig. 21.5 Acro-oedema in T1R. (© Bernard Naafs 2021)

(CMI); functional tests such as lymphocyte transformation tests, migration inhibition tests and INF-y˙and IL-2 release essays.

A new development is that a transcriptomic signature of risk for T1R consisting of five messenger RNA genes (CCL2, CD8A, IL2, IL15 and MARCO) is identified based on cross-sectional comparison of their RNA expression. In addition, intra-individual longitudinal analyses of leprosy patients before, during and after treatment of T1R indicated that several IFN-induced genes increased significantly at onset of reaction, whereas IL15 genes decreased. Importantly, the prospective five-gene signature for T1R could predict a T1R at least 2 weeks before onset [4]. Thus, the transcriptomic biomarkers provide promise for in the future early detection of these acute inflammatory episodes and thereby help to prevent permanent neuropathy and disability in leprosy patients. But for the time being, it is still the experienced clinician who by careful observation and simple clinical tests has to detect the reaction. Pathology and immunopathology are of only limited help to confirm the diagnosis [5]. However, the above-mentioned investigations are very useful for research purposes.

21.2.3 Immunology and Pathology of Type 1 Leprosy Reaction (T1R)

Histopathologically, the lesions show all the characteristics of a delayed-type hypersensitivity reaction (Chap. 12). In the initial lesion, only mild extracellular oedema with some proliferation of fibroblasts may be seen, with an increased number of lymphocytes in the leprosy granuloma. Later, there is further increase in the oedema and a change in the cellular composition in and around the epithelioid cell granuloma, due to an influx of lymphocytes that are mainly of CD4 subtype, especially the Th1 class [6–9].

Using methods for detection of messenger RNA (mRNA), it was shown that, besides interferon-gamma (IFN-γ), production of IL-2 and TNF-α was increased, which confirms a shift to the Th1 subtype during a reaction [5–10]. Possibly due to this shift, humoral immunity during a T1R seems to be diminished [5]. However, there also may occur a shift to Th2 activity in the course of a reaction, since there is an increase in mRNA for IL-4 in some of the lesions [7, 8]. During a reaction and when it subsides, the relative number of CD8+ cells (suppressor/cytotoxic) increases.

The importance of cells with the CD4 marker is emphasized by the observation that leprosy and especially T1R may occur when human immunodeficiency virus (HIV)-infected patients are treated with effective antiretroviral therapy, the CD4+ cells increase and T1R pathology occurs as an immune reconstitution inflammatory syndrome (IRIS) [11].

It is still not known which antigens or antigenic determinants are responsible for T1R [5]. Neither is the orchestration of the cytokines and chemokines known. Moreover, the events may compartmentalize. What happens in the tissues may be different from what is found in the blood.

It has been shown that, during T1R, peripheral blood lymphocytes show increased immune response towards *M. leprae* antigens. This was demonstrated in vitro using leukocyte migration inhibition tests and lymphocyte transformation tests. When the reaction subsides, the immune response decreases. In vivo, such reactions may be seen after pregnancy [12], starvation and immunosuppressive therapy and as mentioned before after starting treatment for an HIV infection. However, which of the *M. leprae* antigens, let alone which antigenic determinants are involved, is still unknown. Heterogeneity has been shown, not only between different patients but also over time in one patient, when the maximum CMI response may change from one antigen to another. Since *M. leprae* is very difficult to find in paucibacillary leprosy patients, especially in those with T1R, autoimmune phenomena have been incriminated by some to play a role in the reactional process.

It has been shown that human nerve and skin have a number of antigenic determinants in common with *M. leprae* [13]. Many of those epitopes are on heat-shock proteins (HSPs) [14]. This can be demonstrated in particular in macrophages and epithelioid cells of granulomatous diseases such as sarcoidosis, necrobiosis lipoidica, and granuloma annulare [15]. In animal models, it has been shown by electron microscopy that *M. leprae*-primed macrophages attack Schwann cells [16], not only in the presence but also in the absence of detectable *M. leprae*. It was also

observed in vitro that T cells that reacted with *M. leprae* also reacted with components of Schwann cells [17]. In serology, it was already demonstrated a long time ago that most leprosy patients have antibodies against nerve components.

That the innate immunity is involved in a T1R can be seen because there is a marked signature in the blood, comprising genes mostly related to the innate immune responses, including type 1 IFN components, autophagy, parkins and Toll like receptors [18]. Leprosy reactions in general show increased Th17 cell activity and a reduced FOXP3+ Tregs with concomitant decrease in TGF-β and increase in IL-6 [19].

21.2.4 Up- and Downgrading Reactions

In the presulphone era, it was observed that, after an exacerbation—reactions are exacerbations—of the disease, patients became more or less bacilliferous or even "cured". As a result, they most likely suffered nerve damage. It was considered that, had they become more tuberculoid, the event had been upgrading, while had they become more lepromatous, downgrading had occurred [20]. The original publications mention regression, less bacilli and lepromatous transformation and more bacilli.

When sulphones became available, many noticed the occurrence of exacerbations or pseudo-exacerbations of the disease after introduction of treatment, after which the patients may have been more damaged but the bacillary load seemed to have diminished. The term "reversal reaction" was coined for these phenomena. One tried to prevent it to happen by introducing treatment with low-dose sulphones and thereafter slowly increasing the dose, but in fact paved the way for sulphone resistance.

Many discarded the concept of a downgrading reaction, since during effective antibacterial treatment, no bacterial multiplication was expected, and used only the term reversal reaction. The concept, however, was never abandoned entirely, since reactions still occurred in untreated patients and some pathologists had the strong impression that, when a reaction occurred, they observed, even in treated patients, the appearance of, or temporary increase in, the number of *M. leprae*, some of which were solid staining [20]. This concept became even more relevant with the introduction of WHO-advised multiple drug treatment (MDT). Reactions now did occur not only before and during treatment but also after antimycobacterial treatment. The latter often was very difficult to distinguish from relapse [20]. Moreover, an increase in the number of solid-staining bacteria could occasionally be observed, which only disappeared after the reaction settled. This was explained by assuming that this late reaction had been effective in clearing the bacillary load, thus being an upgrading reaction. After the decline of the number of bacilli with effective antimycobacterial treatment, enough of the cell-mediated immune resistance was restored to deal with the bacteria multiplying anew [20]. Interestingly, the same authors who noticed an increase in bacterial load during a reaction occurring when on dapsone

monotherapy and during late T1R hardly observed this phenomenon during multidrug treatment.

Initially, to explain the disappearance of bacilli during one type of reaction and not during the other, the concept of protective immunity and nonprotective delayed type of hypersensitivity was introduced [21]. When the reaction was directed against certain antigens, the bacteria were killed. When it was directed against others, only the tissue was damaged due to a bystander effect, but not the bacteria. However, this concept was increasingly challenged.

Another explanation that was proposed is that, during an upgrading reaction, immunity is directed against antigenic determinants that are essential for the bacterium to survive, and that during a downgrading reaction, the reaction is directed against antigenic determinants of secreted antigens and remnants of dead and dying bacteria, or even against antigenic determinants of the host that the host has in common with *M. leprae* [13].

A third concept, that in both upgrading and downgrading reactions the same antigenic components may be involved, is the most likely. There is competition between enhanced cell-mediated immunity, stimulated by certain antigenic determinants of the bacteria or determinants of the human host, and a suppressive effect induced by others.

It is not unlikely that the orchestration of the cytokines that result from the immunological events is responsible for the final effect of up- or downgrading. An observation supporting this concept is the finding that different antigenic determinants induce a different cytokine profile in different individuals depending on their genetic make-up and immunological history, including their contact with environmental microorganisms. It should also be realized that events may differ from site to site in the tissues and that peripheral blood does not necessarily need to mirror this [22].

21.2.5 Treatment of Type 1 Leprosy Reaction

Editor note: The treatment of reactions is dealt with herewith and in Chap. 28 "Medical Therapy". The chapters represent two approaches, both largely used, to the management of these complications.

Treatment should be based on the understanding of the immunopathology, namely, a harmful delayed-type hypersensitivity reaction against *M. leprae* antigens. A logical approach, therefore, would be to reduce the amount of stimulating antigens with chemotherapy while suppressing the cell-mediated immune response.

It is important to realize that dapsone, which is a major constituent of chemotherapy when given at dose of 50 mg or higher, on its own has suppressive effect on the occurrence and development of T1R. In some countries, the prevalence of T1R during treatment has reduced after the introduction of the WHO-advised multiple drug treatment (MDT), during which 100 mg dapsone is given daily.

For immunosuppression, prednisolone is the drug of choice, though azathioprine, cyclosporin, methotrexate (MTX) and some biologicals also have been shown to be effective. Prednisolone, however, has a triple action. It reduces the oedema immediately, is immunosuppressive and decreases post-inflammatory scar formation.

The duration of the immunosuppression should be long enough to cover the period that the antigen load is able to trigger the CMI response [23, 24]. For tuberculoid (TT, BT) patients, this may be 2–6 months, for mid-borderline (BB) patients 4–9 months and for some borderline lepromatous (BL) patients even up to 2 years.

The initial dose (particular at the lepromatous range (BL, LLs)) usually does not need to exceed 40 mg daily. A higher initial dose has, only at the beginning of the treatment, some positive effect on the oedema, but does not improve the outcome in the long run. The crucial starting dose seems to be between 25 and 40 mg, depending on the classification, higher in tuberculoid than lepromatous. After 1–3 months, 15–20 mg suffice, but this should not be tapered too quickly. When 10 mg is reached, the treatment can be discontinued within 1 month. Sensory testing and voluntary muscle testing can guide tapering. Graded sensory test has been shown to be the most sensitive.

Some programmes, wrongly, give only 2 months prednisolone at doses above 15–20 mg and taper within 1 month. Immediately after these 3 months, their results are excellent; however, 3 months later, most of the patients have nerve damage as before [23, 25].

It is important to check patients who will be treated with prednisolone for intercurrent infections, since infections may exacerbate during immunosuppressive therapy (especially worm infections should be taken care of). However, since the duration of therapy is relatively short, serious adverse effects of prednisolone are not frequently observed [26, 27].

When, during an otherwise effective anti-reaction treatment, one or two nerves are not responding, but other nerves do, it may be assumed that "venostatic oedema" is involved (explanation further on). A nerve decompression operation should be considered [28]. This should be done as soon as possible, but within 2–3 months at the latest. The operation should be performed under steroid cover as this prevents postoperative oedema and decreases postoperative scarring. It should be remarked that, though nearly all leprosy surgeons are convinced of the positive effect of nerve release in selected patients, some, usually not involved with surgery, consider the evidence to be insufficient.

21.3 Type 2 Leprosy Reaction (T2R)

The nomenclature of T2R, erythema nodosum leprosum (ENL), lepra reaction, lepromatous leprosy reaction or Jopling type 2 reaction is as confusing as its variations in clinical presentation.

21.3.1 Signs and Symptoms of T2R

The name erythema nodosum leprosum describes the most common manifestation of this reaction—an eruption of tender, red papules and nodules—which develops in a few hours to days and lasts a few days to weeks. The patient feels unwell, has pyrexia, may have granulocytosis and often has albumin in the urine.

The papules and nodules are red to purple in light-skinned patients (Fig. 21.6) and skin-coloured (Fig. 21.7) or dark blue–red nearly black in dark-skinned patients. When they resolve, they leave a greyish-blue lesion resembling a bruise in light-skinned patients (Fig. 21.6) and a deep blue–brown or black discoloration in dark-skinned patients. The resolving lesions usually desquamate. Active and fading lesions may be present at the same time. Occasionally, the lesions coalesce and become plaques. Both plaques and nodules may ulcerate (Orbaneja's necrotic nodules). Most frequently, the lesions occur along the extensor side of the arms and thighs, on the trunk and on the face, but may also occur elsewhere. They differ in their distribution from the erythema nodosum lesions that occur during sarcoidosis or tuberculosis, chlamydia, yersinia or streptococcal infection, which have a predilection for the shins. Sometimes, the lesions can be more easily palpated than seen.

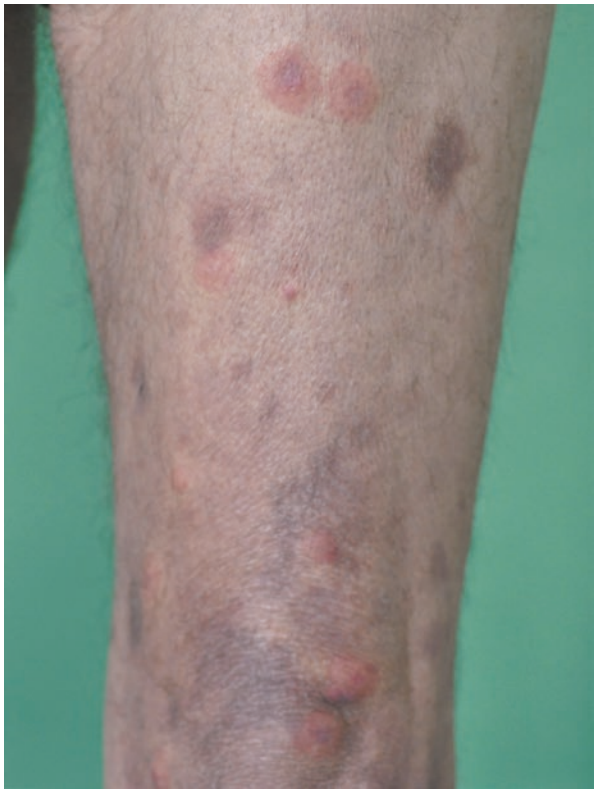

Fig. 21.6 BL leprosy in T2R. On the upper part of the thigh, erythema multiforme-like lesions. On the knee, erythematous nodules. The darker macules are old ENL lesions. (© Salvatore Noto 2021)

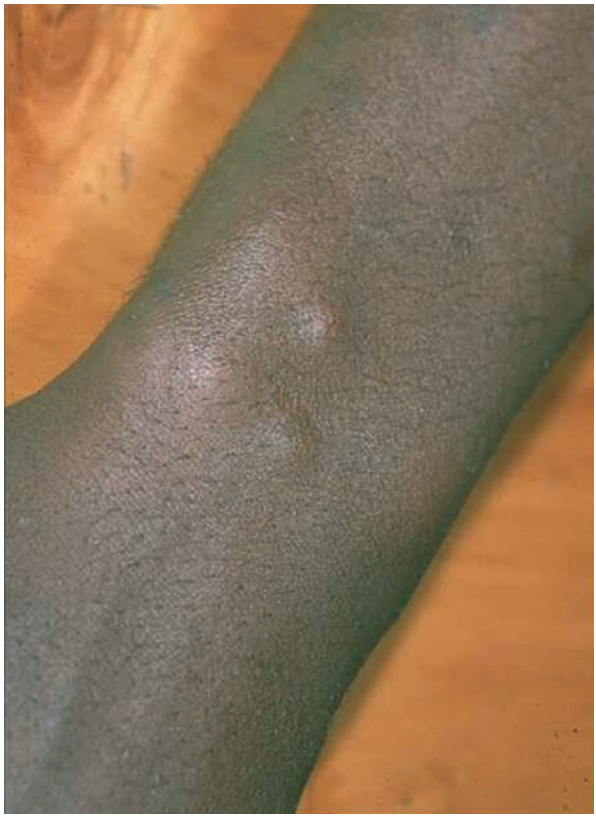

Fig. 21.7 T2R nodules on the dorsum of the wrist. (© Bernard Naafs 2021)

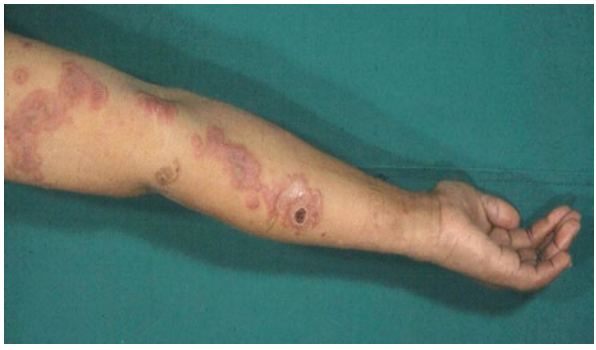

Fig. 21.8 BL leprosy in T2R. Erythema multiforme-like lesions on the upper limb. One lesion shows central necrosis. (Courtesy of E. Nunzi, 2020, All rights reserved)

They feel firm, and palpation is frequently painful to the patient. They often extend downwards to the deeper layers of the dermis and into the subcutaneous fat.

Also other clinical manifestations of T2R have been reported: the so-called erythema multiforme type often seen in Brazil, but with the increasing awareness reported from other parts of the world too (Fig. 21.8). Some patients display

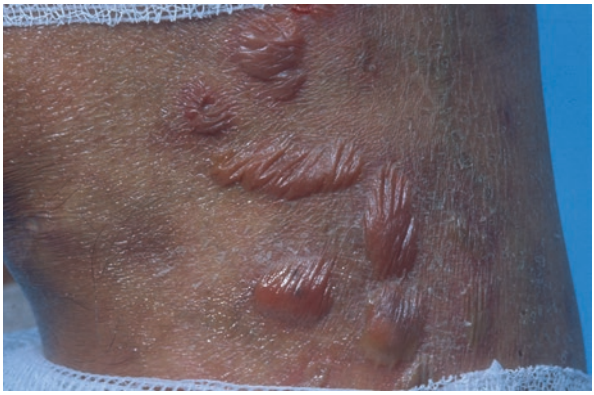

Fig. 21.9 Bullous T2R. (© Bernard Naafs 2021)

superficial bullous ulcerative cutaneous lesions (Fig. 21.9) associated with high fever, malaise and oedema. Histopathology of this bullous form demonstrates dermal oedema with mononuclear cell infiltrates and the presence of *M. leprae* within the capillary endothelium and neutrophilic infiltration in the dermis.

In T2R the skin is not the only organ involved. Painful enlargement of lymph nodes, the liver and the spleen may occur, as well as episcleritis and iridocyclitis with glaucoma. Involvement of lymph nodes may lead to oedema of the extremities, particularly the legs. This oedema should not be confused with that which occurs as a result of a nephrotic syndrome that however may be caused by chronic T2R. In men, epididymo-orchitis can be seen. Nerves as well as joints can become swollen and tender. Periostitis, tendovaginitis and myositis are observed. Glomerulonephritis can be present too, which also may lead to oedema. Even peritonitis has been noticed during abdominal operation and confirmed with histopathology.

In short, since lepromatous leprosy is a generalized disease, each organ or tissue may be involved in the T2R process, with the CNS as a possible exception.

T2R usually occurs in episodes, lasting from only a few days to 1 to 2 weeks. Over 95% resolve spontaneously within 1 month [29] (Fig. 21.10). Some patients may experience widespread and recurrent lesions which continue to appear for months or even years, and in a few patients, the condition may become chronic. Blindness and chronic T2R probably are the most serious complications of leprosy. Chronicity and treatment may even lead to death [30].

Though T2R occasionally occurs in untreated patients, a great number, sometimes over 50–60%, of the lepromatous patients under or after treatment develop one or more attacks. The frequency of the occurrence and the severity of T2R seem to be related to the progress of the disease before treatment is started. In programmes with early detection, only 10–15% of multibacillary patients may experience a T2R attack, which then is often mild. In most programmes, occurrences of 30–40% may be observed.

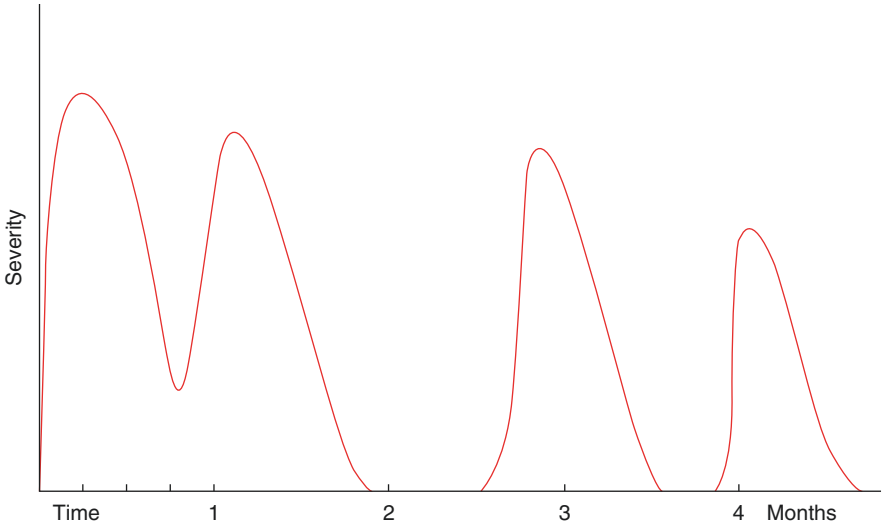

Fig. 21.10 Severity and duration of T2R. (© Bernard Naafs 2021)

21.3.2 Immunology and Pathology of Type 2 Leprosy Reaction

In the initial T2R lesions, there is, against a background of borderline lepromatous (BL) or lepromatous (LL) histopathology, a slight increase in the number of lymphocytes, especially perivascularly. The majority of these infiltrating cells are CD4+ Th2 cells [31]. When the reaction continues, the number of these cells increases further and exceeds the number of CD8+ cells that normally form the majority in a lepromatous leprosy lesion. This shift can be shown by an increase in mRNA for IL-4, IL-5, IL-13 and perhaps also IL-10 cytokines, which are indicative of a Th2 type of reaction.

It has been observed that, early during T2R, in the lepromatous granuloma in between the foamy cells, smaller cells—probably monocytes turned into active young macrophages—can be detected, and these may be responsible for destruction of inert foamy macrophages and, as a consequence, release of antigens. These can then be presented by fresh macrophages to the immune system and stimulate the CMI. The involvement of the CMI can be witnessed by the observation that the number of IL-2 receptors on the immune-competent cells increases, as does HLA-DR expression, not only within the infiltrate but also on the keratinocytes of the overlying epidermis [31].

It has further been shown that, within ENL lesions, the plasma cells, stimulated by the IL-4-producing cells, produce antibodies against *M. leprae* antigenic determinants. These antibodies then will combine with the omnipresent antigens and, when not engulfed by a macrophage, form immune complexes [31, 32]. These gives rise to complement activation and full-blown T2R (Fig. 21.11). Antigen, IgG, IgM, complement and IL-4 mRNA have been shown to be present in the tissues. Particular

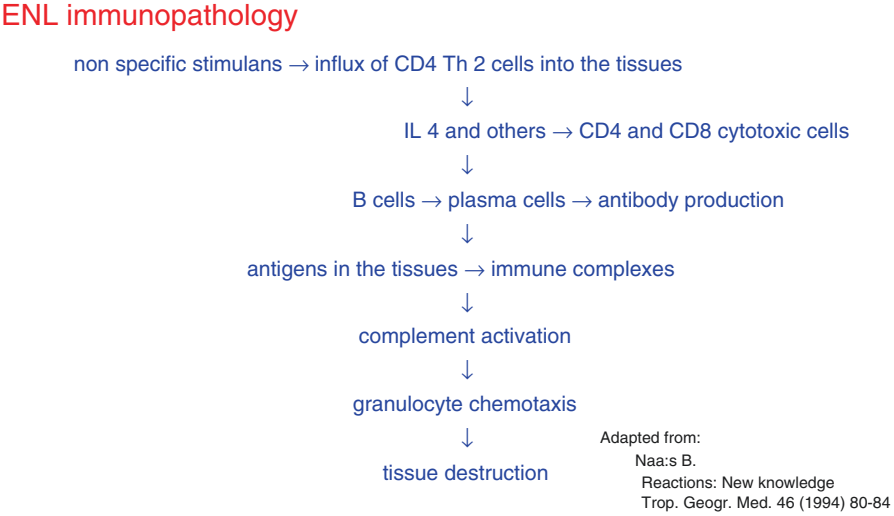

Fig 21.11 ENL immunopathology (Adapted from Naafs B. Reactions: New Knowledge. Trop Geogr Med 46 (1994) 80–84)

important is the IL-4, because it is known to be a B cell stimulator, increases the HLA-DR expression and is a growth factor for the mast cells.

When the T2R reaction is full blown, polymorphonuclear granulocytes dominate the picture (Chap. 12); a few leu7-positive (natural killer) cells can also be seen, as well as an increased number of mast cells.

Involvement of both immune complexes and cell-mediated immunity has also been shown in peripheral blood. During T2R there is, in vitro, an increase in the response of peripheral blood leukocytes to mitogens, indicating a generalized increase in CMI. Complement factor C3d is found to be increased in peripheral blood, which may indicate complement activation and is probably a spillover from tissues and not a sign of a classic Arthus phenomenon.

IL-4, IL-5 and TNF-α are, together with IFN-γ, the most prominent cytokines present; TNF-α is known to be a pyrogen and may be responsible for the increase in body temperature during T2R, and certainly will contribute to further tissue damage. There are some indications that autoimmunity might also play a role in tissue damage during T2R.

It was found using flow cytometry and immunohistochemistry that T2R patients showed significantly higher Toll-like receptor-9 (TLR-9) expression when compared with nonreactional lepromatous patients, both locally in the skin lesions and in circulating mononuclear cells. TLR-9 preferentially binds DNA present in bacteria and viruses, and triggers signalling cascades that lead to a pro-inflammatory cytokine response. The levels of endogenous and pathogen-derived TLR-9 ligands in the circulation of T2R patients were also higher. Furthermore, peripheral blood mononuclear cells (PBMCs) isolated from the T2R patients secreted higher levels of TNF, IL-6 and IL-1β in response to a TLR-9 agonist than those of the

nonreactional patients and healthy individuals [33]. According the authors, these data strongly indicate that DNA sensing via TLR-9 constitutes a major innate immunity pathway involved in the pathogenesis and evolution of T2R.

It was further noticed by Vieira et al. [34] that T2R patients showed a decrease in Tregs and increase in IL-17+ cells in biopsy of an active lesion. It must however be realized that in biopsy there is a compartmentalization and a visible ENL (T2R) lesion may already be in the progress to resolving.

21.3.3 Differential Diagnosis: Type 1 Versus Type 2 Leprosy Reaction

Sometimes, especially in BL and subpolar LL patients, it is difficult to distinguish T1R from T2R. They may even occur together, or one after the other. Some signs and investigations may be of help in differential diagnosis. T2R is a generalized disease in which, besides skin and nerves, other organs such as joints and lymph nodes may be involved. The patient may be ill (during T1R he usually is not), may have a raised temperature and erythrocyte sedimentation rate (ESR) and may even have protein in his urine.

The skin lesions in T2R are mostly tender, whereas in T1R they are not. Lesions in T1R may have sensory loss in comparison with surrounding skin, while in T2R this is usually not the case. Palpating the lesions, a T2R plaque consists of confluent papules and nodules, whereas in T1R the lesions are more homogeneous. Both T2R and T1R lesions may ulcerate, but a smear from a T2R lesion shows predominantly polymorphs, while that from a T1R lesion shows lymphocytes. Two old tests may be of help. The Ryrie test involves stroking the sole of the foot with the back of a reflex hammer, which in T2R elicits a burning pain which also may be noticed when watching the patient walk, as if on hot coals. Another test is the Ellis test, which involves squeezing the wrist; during T2R, this elicits a painful reaction, which does not occur during T1R unless the radiocutaneous nerve is tender. It has been described that neuro-electrophysiology also can be used to distinguish T1R from T2R. T2R may develop a conduction block, whereas T1R shows only temporal dispersion [35].

21.3.4 Treatment of Type 2 Leprosy Reaction

Since T2R is an episodic self-limiting disease, as was already shown by de Souza Araújo in 1929 [29], many drugs have been wrongly judged to be of therapeutic value [25].

Treatment of this reaction is less straightforward than that of T1R. Like in T1R, the antigenic load should be reduced, preferably with WHO MDT. Clofazimine (Lamprene), a normal constituent of this MDT, has been shown to suppress T2R, and since its introduction, the prevalence of T2R seems to have decreased.

It has been shown that clofazimine inhibits neutrophil mobility in vitro and the lymphocyte response to mitogens. It also appears to decrease C3d levels, suggesting that it interferes with the breakdown of C3.

21.3.4.1 Mild T2R

Mild T2R or ENL with only a few erythematous papules and no signs of involvement of other organs except the skin is usually not very damaging, although the patient may feel uncomfortable. In these patients, the symptoms can easily be treated with mild analgesic and anti-inflammatory drugs such as aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Although an attack will subside spontaneously, these prostaglandin suppressant drugs may help to alleviate the reaction.

21.3.4.2 Moderate T2R

When the reaction is slightly more severe and accompanied by pyrexia, leucocytosis and some involvement of other organs except nerves, eyes or testes, additional treatment is required. In the 1990s, chlorpromazine has been used by some leprologists. This drug had been shown to inhibit complement-mediated reaction in rabbits, and it actually prevented tissue injuries [36]. Promethazine has also been advocated. This has some inhibiting action on the complement cascade, and since the number of mast cells is increased during T2R, it may interfere with its mediators such as histamine and alleviate the reactional symptoms.

When a reaction involves joints (arthritis) or nerves with no obvious nerve damage, a combination of a NSAID and antimalarials (chloroquine or hydroxychloroquine) is frequently effective. Chloroquine stabilizes lysosomal membranes, preventing tissue destruction, and it modulates complement activation by immune complexes.

21.3.4.3 Severe T2R

In severe cases of T2R with orchitis, iridocyclitis with glaucoma or neuritis with deterioration of nerve function, corticosteroids or thalidomide should be considered, some give both, particularly when there is acute nerve involvement.

A high initial dose of prednisolone is often required. The action of this drug on T2R is complex and results in suppression of cell-mediated immunity, inhibition of release of lysosomal enzymes and cytokines, decrease of fluid leakage at the site of inflammation, decrease in the response of neutrophils to chemotaxis, inhibition of prostaglandin synthesis and response to prostaglandins.

Prednisolone therapy has been shown to be very effective, although at the high dose which is necessary, side effects are numerous, especially in patients with chronic or recurrent T2R. At present, steroid dependence seems to be a major problem in many leprosy control programmes; this may be induced by the more freely use of steroids.

It should be realized that part of the T2R is complement mediated [32] and that these types of reactions need a high dose of steroids. The usually given 20–30 mg

proves not to be enough and even does not prevent new reactions from occurring. Therefore, it is advisable to give a high dosage of steroids (60–120 mg) for a short period, a few days, and then taper off within 1 month, the natural duration of most T2Rs. When the reaction reoccurs during tapering, the initial dose should be restarted and the tapering started again.

At present, thalidomide seems to be the drug of choice [37]. Since it became more easily available, American, European and recently Indian leprologists resorted more often to this drug. It is extremely effective and better than prednisolone [38]. Thalidomide has a number of side effects that usually do not warrant discontinuation of the drug. Teratogenicity is well known and limits its use. Neuropathy may occur more frequently than is reported, because when it occurs, it is usually masked by the leprosy neuropathy.

The mode of action of thalidomide is still unclear. It has been shown to be effective in adjuvant disease in rats. It inhibits de novo synthesis of IgM. Since IgM and especially IgM rheumatoid factor(s) may play a role in perpetuation of T2R, this could be an important finding. It stabilizes lysosomal membranes. It inhibits granulocyte chemotaxis. It inhibits induction of ENL (T2R) lesion via immunomodulation, which results in a significant decrease in the observed CD4/CD8 ratio. It is agonistic to synthesis of IL-2 and it may be agonistic or antagonistic to synthesis of TNF-α.

In most papers, its action is thought to be mediated through TNF-α. But why then does it not work in T1R, where TNF-α seems also to be an important molecule? An anti-TNF-α biological was tried in T2R, and the reaction subsided, but it is not unlikely that this was the normal course of the reaction in these patients.

Thalidomide is given at the dosage of 100–300 mg daily for a period of a few days and then tapered to a dose that prevents new occurrence of T2R.

It has been shown that a combination of low-dose steroids together with thalidomide could be counterproductive, and therefore thalidomide alone should be given for prevention of new reactions, in general at the dosage of 50–100 mg, sometimes lower or higher as judged by the attending physician. However, in several countries, restrictive political legislations limit its use.

Colchicine, which inhibits vascular injury in experimental Arthus reaction by inhibiting chemotaxis of neutrophils, has been shown to have some effect on T2R, but the results are not as impressive as claimed in initial trials.

Cyclosporin A has been shown by some to be effective in severe T2R and may be a substitute for thalidomide, though thalidomide is more effective. However, in our hands, cyclosporin A was of little benefit in preventing T2R, indicating that it may be less effective in suppressing the Th2-type CD4 cells than the Th1-type CD4 cells involved in the reversal reaction (T1R).

A strong anti-T2R effect has been claimed for pentoxifylline at high dosage. Others however were not that impressed. It was tried because some investigators were of the opinion that TNF-α is of major importance in ENL and pentoxifylline is known to suppress its production effectively. However, as mentioned before TNF-α is also present in T1R, in which neither thalidomide nor pentoxifylline is of much help, though pentoxifylline also diminishes leukocyte adherence. Pentoxifylline

diminishes only the leg oedema during T2R more effectively than thalidomide, but this was to be expected. In a comparative trial, it was shown to be inferior to thalidomide [39].

21.3.4.4 Recurrent T2R

Presently, one of the main problems in management of T2R is the large number of patients who become steroid dependent. Often, clofazimine is used to diminish the severity and frequency of the reaction. It is given at the dose of 100–300 mg daily.

Recently, it has been shown that methotrexate is effective to wean patients off steroids provided steroids are only given when there is active T2R. In the period between T2R episodes, steroids should not be given, only the MTX.

It has been reported that immunotherapy with bacillus Calmette–Guérin (BCG) alone and together with *M. leprae* was able to reduce the frequency and severity of T2R. This was shown too for *M. vaccae*, M. "W" and the ICRC bacilli. More research should be directed at the mechanisms involved in this phenomenon, and controlled trials to study the clinical effect of mycobacterial immunotherapy should be done, especially since some patients with chronic severe recurrent T2R are not properly controlled with presently available therapies.

21.4 Nerve Damage

In leprosy nerve damage may occur at three levels:

At the level of the cutis (skin) where nerve endings are affected,
At the level of the subcutaneous nerves
At the level of the nerve trunks

The histopathology of reactional tuberculoid leprosy [40] shows granuloma formation high in the dermis and dermal papillae. The granulomatous infiltrate sometimes seems even to erode the epidermis, but obviously destroys the nerve endings in the papillae. It is not unlikely that the driving forces behind these damaging reactions are antigenic determinants in the epidermis and in the peripheral nerve endings which are similar to those of *M. leprae* antigens. The reaction could be an autoimmune phenomenon [13].

In borderline leprosy, the nerves of the lower dermis and especially those located around the adnexa are most often involved. Granuloma formation can be seen in and around these nerves together with a proliferation of Schwann cells in and around the perineurium. Damage can be attributed to compression and destruction of nerve fibres by the epithelioid granuloma. During the reactional episode, there is a further influx of immunocompetent cells with oedema formation and expanding granuloma. This contributes further to nerve damage, especially when extracellular oedema accumulates inside the thickened perineurial sheath, converting it into a rigid compressing tube compromising the axons inside [41].

The mechanisms that occur in nerve trunks and larger subcutaneous nerves are more complicated. At the tuberculoid end of the spectrum, these processes are similar to those in the skin, with massive granuloma formation with occasional colliquation and abscess formation. Further into the borderline range, these features are usually less distinct and often even absent. Frequently, only oedema is observed [41].

Damage to cutaneous and subcutaneous nerves causes loss of sensation in the affected areas and loss of autonomic nerve function such as sweating and regulation of vascular tone. However, it is the damage to the peripheral nerve trunk which is the major consequence of T1R. This damage is partly caused by immunological reactions, but mechanical factors are also involved [41] (Fig. 21.12). During T1R, inflammation and consequently oedema occur in the nerve, as it occurs in the skin. The reaction leads to oedema located within the interstitial tissues of the epi-, peri- and endoneurium. Unlike the skin, the nerve cannot expand without limit. The perineurium, which is largely impermeable to fluids, forms a rigid compressing tube around the expanding endoneurium. This results in an increase in pressure within the nerve. As a result, the axons in the endoneurium are compressed by the increased pressure [28] (Fig. 21.12). As a consequence, there is a loss of conducting nerve fibres and thus loss of muscular strength, peripheral sensation and autonomic functions. The intra-axonal flow which brings nutrients from the cell to the peripheral nerve ending is interrupted, and sooner or later, the peripheral nerve fibre dies off and is destroyed [28].

When the pressure and the tension along and within the perineurium increase due to an increase in pressure in the endoneurium, there is an increase in the pressure exerted on the blood vessels which transverse obliquely through the perineurium. These blood vessels are then compressed. The venules with relatively low pressure are compressed more than arterioles with higher pressure. The compression of the

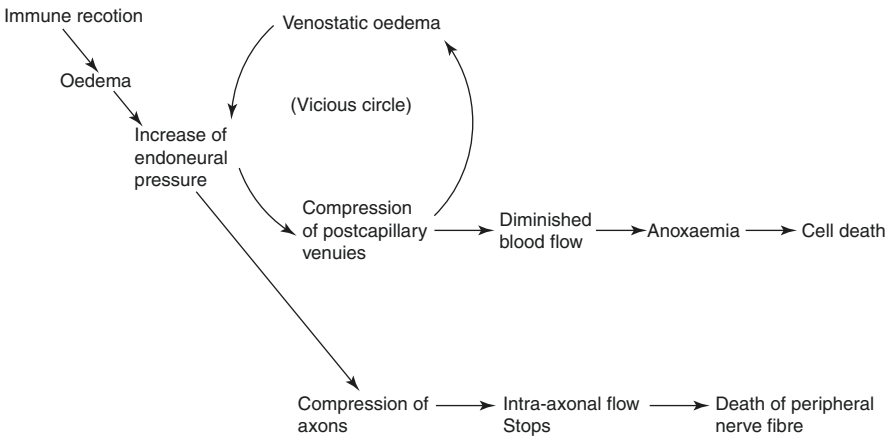

Fig 21.12 Mechanical nerve damage. (© Bernard Naafs 2021)

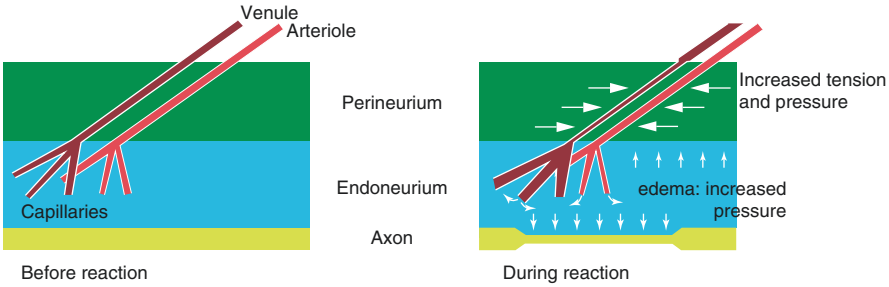

Fig. 21.13 Pathogenesis of "venostatic oedema" in peripheral nerves during reaction. (© Bernard Naafs 2021)

venules will lead to higher pressure in the capillaries of the endoneurium, which may start "leaking" and thus increase the pressure in the endoneurium. This "venostatic oedema" is able to maintain itself even when the immunological events subside (Fig. 21.13).

In the histopathology of nerve in T2R, an increase in neural cell adhesion molecules (N-CAM) can be observed, and N-CAM-positive CD8+ cells can be isolated from the tissues. In vitro it was shown that, during active T2R, when peripheral blood mononuclear cells (PBMC) were exposed to *M. leprae*, there was an increase in cytolysis of N-CAM-expressing Schwann cells by CD8+ N-CAM-positive cells. It is interesting to note that IL-15 is capable of inducing N-CAM expression and that IL-15 mRNA is increased in leprosy tissues [42].

In T2R, the mechanisms leading to tissue destruction, i.e. activation of granulocytes, also contribute to damage of nerves fibres and endings. It also has been shown that TNF-α, which seems to be a major cytokine involved in T2R, is able to demyelinize nerve fibres. Demyelination seems to be a major factor in nerve damage due to multibacillary leprosy as shown by nerve conduction studies, as described in Chapter 14. The damage in multibacillary leprosy can also be caused by lipoarabinomannan that on its own may lead to demyelination by complement activation and membrane attack complex (MAC) formation when in contact with the Schwann cell [43]. Moreover, in the large nerve trunks, the immunological processes may give rise to venostatic oedema with compression of axons as described for T1R [28, 41].

21.5 Voluntary Muscle Testing (VMT)

In the mid-1960s, a numerical system for use in leprosy was developed to assess muscle strength, i.e. voluntary muscle testing (VMT) [1]. When this test is regularly and carefully done, it assists in early detection of a reaction. The facial, median, ulnar and peroneal nerves should be assessed. Deterioration in VMT may precede more obvious clinical signs.

In the field, extensive VMT is out of reach. However, limited testing has been shown to be possible. Experience has been gained with VMT of the orbicularis oculi for the facial nerve, of the opponens pollicis brevis for the median, of the abductor digiti minimi for the ulnar and of the muscles of the anterior compartment of the leg for the peroneal nerve (dorsal flexion of the foot and/or the big toe). Since the use of only three grades, as is often done, is too crude, the five-point scale (Chap. 14) should be used even under field conditions, provided proper supervision and training are continuously provided. However, because the test is not sensitive enough, minimal damage may go unnoticed.

21.6 Graded Sensory Bristle Test (GST)

A more sensitive method, in particular for minimal and mild nerve damage, is the graded sensory bristle test (GST), which uses standardized nylon monofilaments developed by Weddell in the mid-1930s, nowadays usually called Semmes–Weinstein monofilaments. The test was later validated and used for follow-up of nerve lesions during leprosy reactions [2]. Over the years, it has proved to be a reliable and reproducible test, for which the filaments are now standardized (Fig. 21.14).

The graded bristle test can be done by mapping areas of sensory loss and grading this loss which is usually done by physiotherapists. However, due to its sensitivity and tediousness, it is prone to inaccuracy and can therefore only be used effectively by experienced investigators under quiet conditions.

For the busy clinicians, it is simpler to assess a small, defined area such as the thenar area for the median nerve, the hypothenar area for the ulnar nerve [2] or the plantar forefoot and heel for the posterior tibial nerve. Care must be taken not to assess within a skin patch when present. The same areas can be assessed in the field, provided again that proper supervision and training are continuously provided.

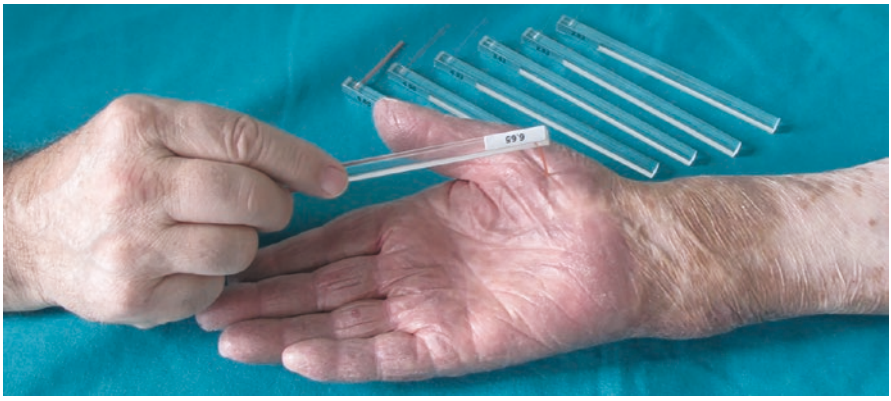

Fig. 21.14 The graded sensory bristle test using standardized nylon monofilaments. (© Enrico Nunzi 2021)

21.7 Two-Point Discrimination Test

Another sensory test which may be useful, especially for the foot, is the two-point discrimination test (moving or static) that is done by means of a paper clip bent to a calliper. It is less sensitive than the graded bristle test when used for mapping of the hand, but nearly as sensitive as graded bristles when used on defined areas such as the forefoot or heel to assess peripheral nerve function. Importantly, it is not time consuming.

21.8 Other Tests

The WHO has advised that ballpoint and pinprick testing are too crude, while graded sensory bristle testing (GST) is not.

It should be noted that more sophisticated physiological methods such as electromyography (EMG), sensory and motor nerve conduction velocity testing, evoked response testing and measuring of autonomic reflexes as well as ultrasound of nerves add little to early detection of T1R or T2R in the field, but they do in research centres and may even help to distinguish T1R from T2R [35].

21.9 Triggering of Leprosy Reactions (Focussed on Covid-19)

Because it is not certain what influence a Covid-19 infection or vaccination will have on the development of reactions in leprosy, it is necessary to extrapolate what we know in this regard.

Leprosy clinical course seems to be dictated by the CMI. The CMI is responsible for the T1R. When there are enough *M. leprae* antigenic determinants present, a rise in CMI may lead to a reaction.

The total available antigenic determinants of *M. leprae* for the cells responsible for the CMI against these determinants may increase because of the treatment of leprosy or other infections. Bacteria such as *M. tuberculosis*, other mycobacteria or nocardia and even streptococci (heat-shock protein 65) have similar antigenic determinants and early infection, or treatment may cause a T1R response. Advanced infections may suppress CMI.

A BCG vaccination increases the CMI and in this way can lead to a T1R. Immune reconstitution at the end of pregnancy, after initiation of treatment for human immunodeficiency virus (HIV) infection and after immunosuppressive treatment can also lead to a T1LR as well as recovery from any disease. This may also happen after a Covid-19 infection, particularly when this has been treated with immunosuppression.

The mechanism of a type 2 leprosy reaction (T2R) is not clear, but it is thought to be an immunocomplex disease in which neutrophils play an important role. Increase in antigen load is a major factor (relapse and resistance).

It is also known that T2R can be triggered by several events, ranging from psychological stress, pregnancy or anaemia to infections like tuberculosis and intestinal worm infestations. Stopping of clofazimine treatment seems also to play a role, like lowering the doses of steroids for T2R (this however could be the same previously suppressed T2R). It is generally accepted that every vaccination can trigger a T2lR.

Interesting is to realize that during Covid disease, there is a cytokine "storm" with influence on the neutrophils. Some think that it resembles T2R.

But till today, there are no well-described case reports with any leprosy reaction we are aware off.

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Lucio's Phenomenon in Leprosy

22

Mario Magaña

The name lazarine leprosy is linked to the fact that this particular form of the disease was identified by Ladislao de la Pascua when he was director of Saint Lazaro Hospital (*Hospital de San Lázaro*) in Mexico City in 1844; he described three forms of the disease: tuberous or nodular, anesthetic, and spotted or *manchada*.

The next and final director of that same hospital was Rafael Lucio, who also recognized those three forms, and studied in more clinical detail and fineness the spotted form; in association with Ignacio Alvarado, he published his observations in 1852 in a paper entitled *Opúsculo sobre el mal de San Lázaro o Elefanciasis de los Griegos*.

Lucio and Alvarado studied 41 patients (21 men and 20 women) with diffuse leprosy and observed that 13 of them (6 men and 7 women) developed peculiar painful red spots on the skin, which we now know as Lucio's phenomenon (LPh).

In 1948, Latapí and Chévez-Zamora studied the subject again and identified that the histopathological nature of the process was a vasculitis, coining the names erythema necroticans or Lucio's phenomenon; however, they were unable to determine the kind and size of the vessel involved, or which kind of vasculitis was occurring [1].

Clinically, patients with diffuse leprosy develop LPh, frequently with general symptoms: fever, arthralgias, myalgias, and appearance of very painful, red or purpuric macules, of irregular shapes, angulated or "stellar"; these lesions usually begin on the lower legs and continue progressing up to the thighs, hip, trunk, and upper limbs [2] (Figs. 22.1 and 22.2).

In a matter of days, these spots exhibit "dryness" and appear as authentic infarcts with detachment of the necrotic skin and showing ulcers (Fig. 22.1).

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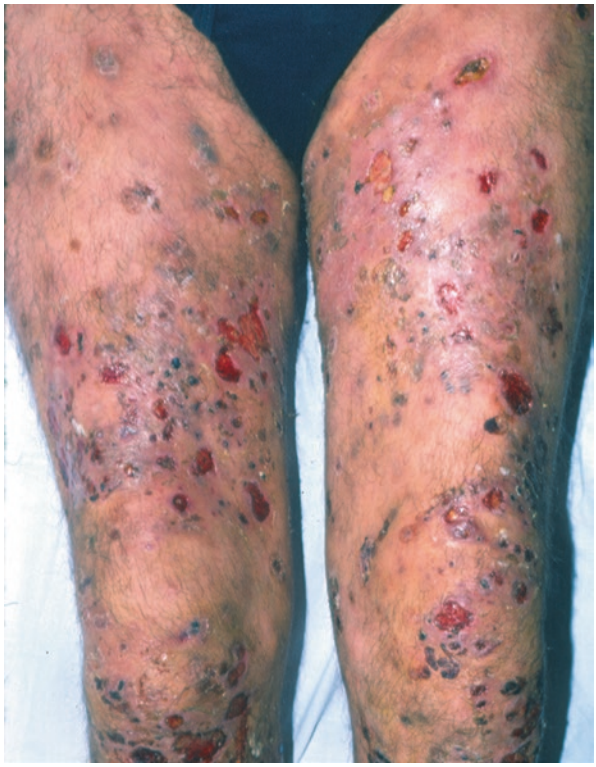

Fig. 22.1 A 40-year-old man with diffuse Lucio–Latapí lepromatosis who did not know about his disease until he developed Lucio's phenomenon; note the numerous infarcts

Histopathologically there is a necrotizing panvasculitis; epidermis shows foci of ischemic necrosis, frequently with detachment from the dermis with ulceration and hyperplasia [3].

In the dermis, it is possible to see the features characteristic of diffuse lepromatous leprosy, such as wide infiltration by foamy macrophages of Virchow cells, always with abundant acid-fast bacilli inside them (globi) or outside in the stroma in association with lymphocytes, around vessels, nerves, and adnexal structures including erector muscles (Fig. 22.3).

Blood vessels such as muscular arteries, arterioles, capillaries, venules, and veins of the skin (including hypodermis) are involved in LPh; the more superficial ones, usually venules, develop a leukocytoclastic vasculitis, on direct immunofluorescence showing IgG, IgM, C3, C4, and fibrin [3]. This is an acute reaction, in which the cellular immune response is not robust enough and the humoral response is exaggerated, with the host developing an immune-complex disease. This is a kind of Arthus reaction in which vasculitis occurs, mostly in venules.

The most outstanding changes in LPh are seen in the subcutaneous fat, where a lobular panniculitis develops, but in particular in medium-sized arteries, whose walls are widely infiltrated by macrophages containing plenty of bacilli, usually as globi but also as solitary units, and also outside of them in the stroma. Other vessels are infiltrated by macrophages too. This infiltration, by definition in classic

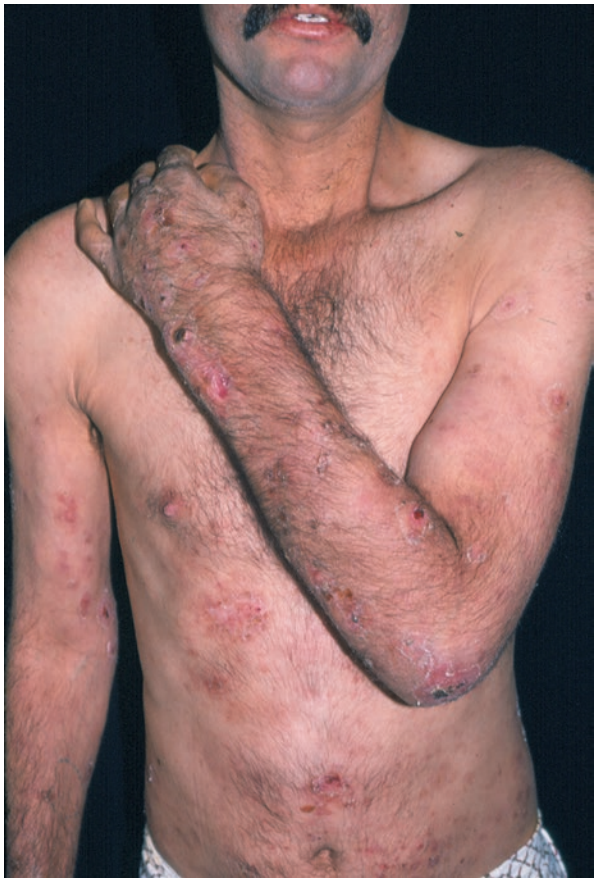

Fig. 22.2 Same patient as in Fig. 22.1; there is no loss of hair in any area; cutaneous infarcts involve also the trunk and upper limbs

pathology, is considered as granulomatous; it distorts the architecture of these vessels, with thickening and dissection of their walls with the consequent narrowing of their lumens, with occlusion and ischemic changes. Whether this infiltration by macrophages is passive or not remains unclear [3].

In support to our concept, that LPh is a panvasculitis, are the findings by Han et al., who demonstrated *M. lepromatosis* in units and in globi involving the lining of the lumen of veins, arterioles, and medium-sized arteries; these authors interpreted the involved cells as endothelial, but there are no markers of them and they very well may be already macrophages. We demonstrated the arterial involvement some years ago [3]; they also show a medium-sized artery occluded by the presence of macrophages plenty of bacilli infiltrating the vessel wall [4, 5].

Treatment is the same as for any multibacillary patient according to the World Health Organization: rifampin 600 mg/month, clofazimine 300 mg/month, dapsone 100 mg/day, and clofazimine 50 mg/day for 1 year and thalidomide 200–300 mg every day; thalidomide is not useful for this process; it is best to give prednisone 0.5 mg/kg every day until the acute phase is under control, if possible with the patient hospitalized.

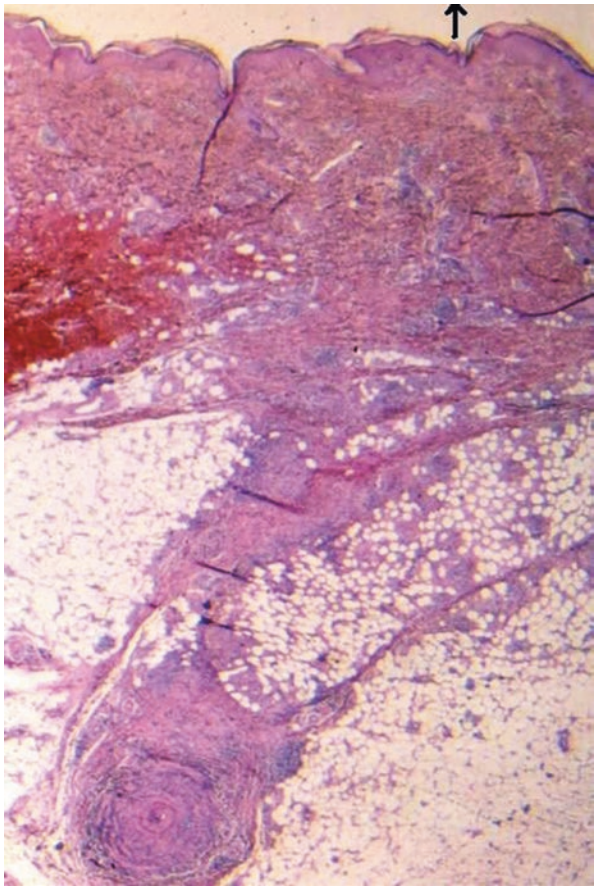

Fig. 22.3 Lucio's phenomenon showing a lobular panniculitis and a medium-sized artery; the lumen of this vessel is almost obliterated and the wall is thickened due to the presence of macrophages

Editor's Note: Over the last decade there have been reports about the presence of anti-cardiolipin antibodies in the patients with Lucio's Phenomenon (LPh) which is thus considered as "an anti-phospholipid syndrome in patients with polar lepromatous leprosy (LLp)" [6–8].

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Part VI

Leprosy: Systemic Involvement

Ocular Involvement in Leprosy

23

Susan Lewallen and Paul Courtright

23.1 Magnitude of Ocular Disease in Leprosy

Leprosy has long been recognized to affect the eyes, resulting in various ocular complications, visual impairment, and blindness. Efforts to determine exactly the magnitude of the problem, however, were hindered in the past by methodological problems with surveys and the use of inconsistent definitions of "ocular involvement." More recently, introduction of multidrug therapy (MDT) has changed the proportion of people with leprosy who will suffer ocular complications of the disease. An important issue in considering the magnitude is the need to differentiate complications of leprosy that are sight threatening from those that are not, and the fact that people with leprosy also suffer from the major causes of blindness that affect those without leprosy, but they may have significantly less access to eye services. We focus in this chapter on the eye conditions in people with leprosy that are likely to cause visual impairment or blindness.

23.2 Specific Ocular Conditions in Leprosy

The fact that *Mycobacterium leprae* grows best at temperatures of less than 37 °C explains its predilection to infect the anterior segment of the eye, and therefore the manifestations of leprosy in the eye. Ocular damage can occur as a result of direct infiltration of anterior segment structures by *M. leprae*, or it can result from nerve damage in the eyelids.

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The three most important causes of visual disability and blindness in leprosy patients are corneal opacification (usually secondary to lagophthalmos), uveal disease (in particular, chronic uveitis), and cataract.

23.2.1 Corneal Disease

The cornea can be affected in several ways; the most common and important is chronic exposure, which occurs when the lower eyelid becomes lax and rolls out (ectropion) or when the eyelids do not close completely (lagophthalmos). Chronic exposure causes the cornea to become dry and at high risk for ulceration, which result in scarring or sometimes in perforation of the cornea with loss of the eye.

Complicating the problem of corneal exposure is that people with leprosy may also have decreased sensation in the cornea, from damage to the fifth cranial nerve. This significantly increases the risk for unrecognized foreign bodies on the cornea, which adds to the chances of infection and ulceration.

The cornea may also be directly infiltrated by *M. leprae*, causing punctate keratitis (so-called avascular keratitis) visible on slit-lamp biomicroscopy. This is more a curiosity and an indication of massive numbers of bacilli in the body than it is a cause of decreased vision.

23.2.1.1 Treatment

Patients with opacification of the cornea and red eye may have recent or ongoing active keratitis or ulceration and need referral within a few days to an eye specialist. In the meantime, they can be given antibiotic ointment (without steroid), which will help lubricate the eye and may prevent further damage until definitive treatment can be provided. An ophthalmologist should treat the condition just like any other corneal ulcer or exposure-related corneal disease.

23.2.2 Lagophthalmos and Ectropion

The two conditions that predispose to corneal disease, lagophthalmos and ectropion, are of critical importance to understand and treat in order to avoid the corneal damage described above. Lagophthalmos and ectropion occur as a result of nerve damage in the zygomatic and, less frequently, temporal branch of the seventh cranial nerve.

Lagophthalmos is diagnosed when the eyelids do not close fully (Fig. 23.1). This may be obvious, but all patients should be checked by asking them to close the eyes gently, as in sleep, and examining from below with a torch light (Fig. 23.2). With normal eyelid closure, the eyeball itself cannot be seen. If any of the glistening sclera or cornea is visible, then the patient has lagophthalmos. If a patient has lagophthalmos in gentle closure, then he is asked to squeeze his lids forcefully; if lagophthalmos is still present, this indicates a more severe degree.

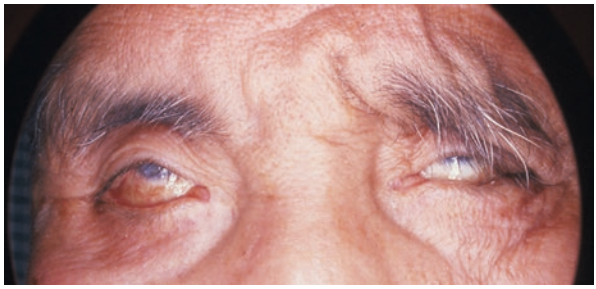

Fig. 23.1 This man has marked lagophthalmos when he tries to close his eyes. (The long eyebrow hairs are due to a skin/hair transplant, performed to give cosmetic relief to the loss of eyebrows and eyelashes seen in some people with leprosy)

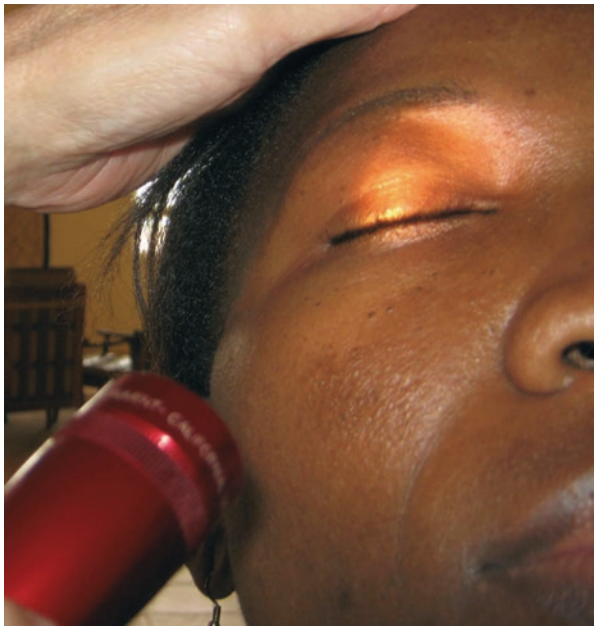

Fig. 23.2 The proper way to check for lagophthalmos is to shine a light from below and look for any show of the globe. This patient has no lagophthalmos

Interestingly, a high proportion of people diagnosed with leprosy who have a facial patch over the malar region or around the eye area will develop neuritis of the facial nerve (which innervates the orbicularis muscle of the eyelids) and subsequent lagophthalmos [1]. This is particularly seen in paucibacillary (PB) patients when type 1 reaction occurs in the patch. If lagophthalmos is recognized early (within about 6 months of occurrence), the patient should be treated with a course of systemic steroids, which will often lead to resolution of lagophthalmos [2]. Anyone with a facial patch should be followed up closely, so that reactions can be treated immediately.

Lagophthalmos in multibacillary (MB) patients may develop differently, not necessarily associated with facial patches. It is more likely to be bilateral and may be caused by simple proliferation of *M. leprae* in the facial nerves. MB patients who

have had prolonged inadequate treatment (people who were on dapsone for years, or MDT defaulters) are more likely to develop lagophthalmos [3, 4].

23.2.2.1 Treatment

Patients with long-standing lagophthalmos in gentle closure alone are instructed to try to build up the strength of the eyelid muscles and increase the wetting of their corneas by consciously squeezing the eyelids hard five to ten times, several times each day. The idea is for the patient to try to develop a "think blink" habit. They can force themselves to blink whenever they see a common object, such as a mango tree, a cow, or a motorcycle. If they exercise "think blink" for long enough, the action will become a habit. Other helpful actions include the following:

- Using eyeglasses and hats or caps to decrease the chance of a foreign body entering the eye.
- Covering the head with a cloth or bed sheet, or using a mosquito net while sleeping.
- Keeping the eyes clean and moist. Ocular lubricating drops such as sterile methylcellulose or artificial tears should be used several times a day if they can be purchased.
- Daily inspection of the eyes. This can be done by using a mirror or having a friend inspect for any signs of a foreign body, redness, or eyelashes turning into the eye.
- Vision should be self-checked daily, one eye at a time, by looking at the same fixed object at the same distance and noting whether its clarity has changed.

If lagophthalmos is present in forced closure and the cornea is exposed or there is decreased vision, the patient should be referred to an ophthalmologist to evaluate the need for surgery to correct the lid position. In the meantime, the measures above, along with lubricants, should be employed.

Obvious lagophthalmos, even if the cornea is not exposed, often constitutes a stigmatizing lesion for people with leprosy, and this is reason enough to suggest surgery if the patient desires it. Surgery that provides good cosmetic results is important.

23.2.3 Uveal Disease

The uveal tract includes the iris and structures behind it. Inflammation of this structure (uveitis, sometimes referred to as "iritis" when the iris is primarily involved) is another serious complication that may occur in leprosy. It takes two forms: acute and chronic.

Chronic uveitis is more common and occurs almost exclusively in MB patients. In fact, there is some debate, beyond the scope of this chapter, as to whether "chronic uveitis" in leprosy is actually a result of inflammation or whether it is the result of long-standing nerve damage to the ciliary nerves, which innervate the iris [5]. The

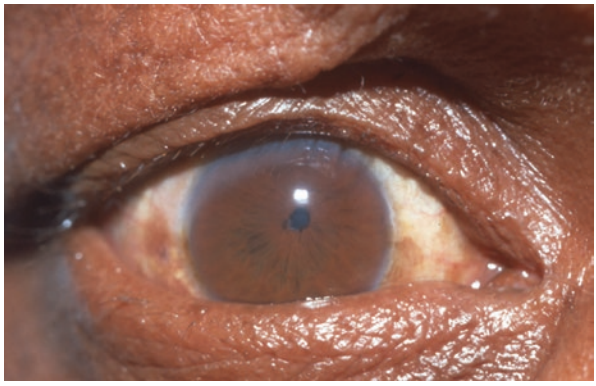

Fig. 23.3 In long-standing leprosy, the pupils may become miotic and poorly reactive, and the iris becomes atrophic. This is often referred to as chronic uveitis

process is insidious, and the affected eye is usually white and quiet, with occasional subacute episodes, which may go unnoticed. Gradually, the iris tissue atrophies, leading sometimes to irregular pupils or holes in the iris, sometimes to severe miosis, which does not respond to any mydriatic drugs (Fig. 23.3). In these eyes, posterior synechiae (adhesions of the iris to the lens behind it) are common, and large, apparently granulomatous keratic precipitates may appear on the endothelium of the cornea. Cataracts frequently accompany this; coupled with severe miosis, they are often the principal cause of loss of vision.

Acute uveitis in people with leprosy is quite a different issue, but is also less common; it occurs in MB patients during type 2 reaction. The acute inflammation of the iris and even surrounding sclera may be very severe and lead to irreversible vision loss. It is accompanied by painful red eye, possibly with decreased vision.

23.2.3.1 Treatment

The absence of any early clinical signs makes treatment and prevention of chronic uveitis extremely difficult. Up to 5% of MB patients will already have it at diagnosis, it can develop during MDT, and it has been shown to develop in up to 10% of patients after they have completed MDT [6–8]. Because of its insidious nature, the most practical recommendation is to perform routine visual screening on patients with leprosy. Those with visual impairment (VA < 6/18) require referral for evaluation by an eye specialist who can determine if the decreased vision may be improved by dilation of the pupil, anti-inflammatory therapy, or cataract surgery.

On the other hand, treatment of acute uveitis is a matter of urgency. Any patient who develops a red or painful eye during type 2 reaction needs urgent referral to an eye specialist. In the meantime, if the cornea is clear, steroid drops should be started four times a day.

23.2.4 Cataract

The majority of cataracts worldwide, as well as in people with leprosy, are age-related and not a complication of leprosy per se, with one exception noted below.

The treatment for cataract is good-quality surgery with an intraocular lens implant, and this applies to people with leprosy. It should not be assumed, however, that general improvements in cataract surgical services within a country or region are necessarily of benefit to leprosy patients. Firstly, people with leprosy usually live in rural areas, and many of the same barriers that prevent use of eye care services by all rural poor (cost of eye care, lack of awareness of its availability, distance to eye care facilities, fear of poor outcome, etc.) also apply to leprosy patients [9]. Secondly, leprosy patients, particularly those with physical deformities, often have stigma-related barriers to overcome. In some settings, they are actually barred from using general eye care services.

Diagnosis of cataract in leprosy patients is done in the same way as in anyone else. The combination of decreased visual acuity and lens opacity (visible when checking for a red reflex with a direct ophthalmoscope) indicates the presence of cataract and the need for referral to an ophthalmologist for evaluation for surgery. While not urgent, there is no reason to wait for surgery either, and patients who are experiencing difficulties with vision from cataract deserve an opportunity to have surgery.

The exception noted above is that there is probably a higher risk for and incidence of cataract among patients who have "chronic uveitis." Cataracts associated with inflammation are referred to as "complicated cataracts" and pose a slightly greater challenge than routine cataract surgery.

23.2.4.1 Treatment

High-quality modern cataract surgery, with intraocular lens implantation, results in excellent visual acuity. Leprosy, as long as there is no acute inflammation in the eye, is not a contraindication to cataract surgery with intraocular lens. The rare patients with iris atrophy (chronic uveitis) will be somewhat more difficult and should be operated by an experienced surgeon.

23.2.5 Other Ocular Manifestations of Leprosy

In addition to the potentially blinding complications mentioned above, there are a number of other manifestations of leprosy in the eye and ocular adnexa. Some of them are unique to leprosy but tend to be either rare or visually insignificant, and include such entities as avascular keratitis, beading of corneal nerves, formation of iris pearls, scleral nodules, and madarosis (loss of eyebrows). They generally occur in MB patients and reflect the high bacillary load.

23.3 Which Patients Are at Risk for Ocular Complications?

The distribution of the ocular complications of leprosy is determined by (1) the distribution of leprosy type (PB versus MB), (2) the age distribution of the patients, and (3) the implementation of MDT in the area [10].

As noted above, some complications are more common in MB compared with PB. Chronic uveitis, for example, is mostly confined to MB patients, and as a result, there may be slightly more cataracts (the complicated variety associated with inflammation) among MB patients. Acute iritis is a feature of MB disease during type 2 reaction. Patients with both types get lagophthalmos, but it is more likely to be treatable with steroid among PB patients.

Ocular pathology and blindness in leprosy develop as a result of chronic factors, so most ocular morbidity and blindness in leprosy occur in adulthood.

MDT reduces the incidence of ocular pathology in patients, but it will not eliminate it, for several reasons. Not all people with leprosy are on MDT, and to the extent that untreated leprosy exists, one should expect to see ocular complications. Furthermore, the time from onset of disease to diagnosis and treatment may be long, depending on the general healthcare infrastructure and the effectiveness of local case finding. The longer this period is, the greater the risk that ocular complications will have developed before treatment is started. Finally, even after treatment, around 12% of MB patients will develop incident ocular pathology [11–14].

Current recommendations are that all patients be screened for eye disease at time of leprosy diagnosis and at discharge from the antileprosy treatment program. Any patients with visual impairment or blindness, lagophthalmos, a facial patch, or persistent red eye need to have routine follow-up. Key to preventing visual loss in people with leprosy is to establish links between ophthalmologists and leprosy control programs.

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Otolaryngological Manifestations of Leprosy

24

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Ear, nose, and throat (ENT) examination is important in early diagnosis of leprosy, due to the frequent involvement of upper airways and external ear [1–3].

In lepromatous and borderline clinical forms, untreated or with insufficient treatment, ENT involvement is frequent and extensive, unlike the indeterminate and tuberculoid polar forms, where the lesions are rare and localized. An important characteristic in the evolution of leprosy in the upper respiratory and digestive tracts is its descending pattern. It initiates in the nasal fossa and moves on to the mouth, larynx, etc. [4].

24.1 Nose

The intranasal presentations can be divided into three groups:

24.1.1 Early Manifestations

The initial involvement occurs in the mucosae, which are infiltrated and become thickened and of pale or yellowish color. Next, slightly elevated nodular lesions appear. Unusual dryness of the nasal mucosa is relatively frequent, which can be due to compromised mucous glands that suffer alteration of the parasympathetic fibers responsible for their innervation [1, 2, 5, 6].

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24.1.2 Intermediate Manifestations

The lepromatous infiltration increases, with progressive thickening of the mucosa, which can result in nasal obstruction. This obstruction forces the patient to try to "clean" the nose, repetitively blowing or probing the nasal fossa with fingers to obtain relief; these attempts result in ulcers and favor an inflammatory reaction. When the patient speaks, it sounds like he or she has a cold. Another interesting fact is that the nasal congestion does not respond to vasoconstrictor medication applied in other obstructive rhinopathies [2, 5].

In this phase, an increase in nasal secretion can be observed, initially with a discreet, clear, fluid, evolving to a denser, yellow mucus with pus, and sometimes bloody discharge. Patients may manifest nasal symptoms of chronic rhinitis, including nasal congestion, rhinorrhea, hyposmia, and intermittent epistaxis [7, 8]. The secretion can form scabs because of the desiccation which aggravates the nasal obstruction (Fig. 24.1). The scabs become hardened and difficult to remove. The presence of scabs, maceration, and secondary infection leads to a fetid odor [5–7].

24.1.3 Late Manifestations

The ulceration, secondary infection of leprosy nodules, and infiltration associated with diminished blood flow of the perichondrium can generate perforation of the nasal septum cartilage (Fig. 24.2). The initially punctiform orifice resultant from the perforation can augment, eventually destroying the entire nasal septum bone and cartilage. Gradually, atrophy and fibrosis flourish in the mucosae; later, there can be destruction of the turbinates and the endonasal bone structure [2, 3, 5, 6].

The compromised mucosae suffer alteration in sensitivity. The nasolacrimal reflex is altered in many cases, and disorders of olfaction are common; in rare cases,

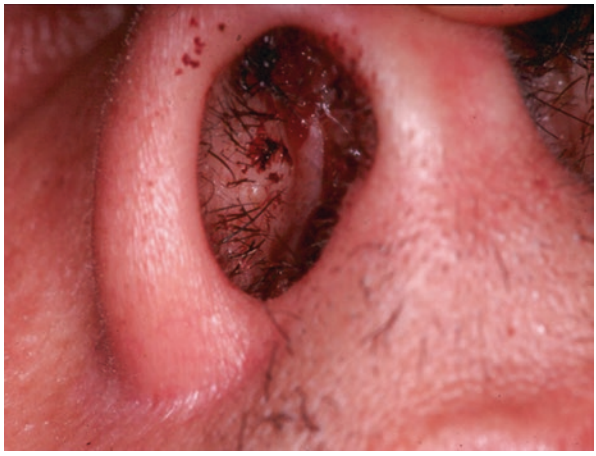

Fig. 24.1 Erosions and hematic scabs in the nasal fossa. Case with difficult differential diagnosis. Leishmaniasis was initially considered. Nasal septum biopsy and abdominal lesions established the diagnosis

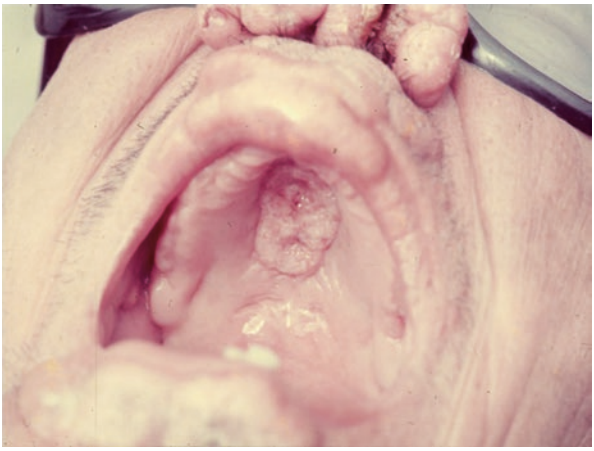

Fig. 24.2 Lepromatous leprosy. Ulcer in Hansen nodule in the palate along with superior lip and nasal wing infiltrations

anosmia can occur due to destruction of the olfactory bulb or to sequelae from adhesions, atresia, stenosis, etc. [5, 6].

It is accepted that lesions of the nasal mucosa are present in an average of 80% of lepromatous and borderline, bacilli-rich polar forms [7–10].

Nasal secretions of these patients are rich in bacilli. During speech, breathing, and clearing of the nose, there is elimination of a great number of bacilli. Currently, it is considered that this elimination is one of the main sources of transmission to close contact communicators [5–7].

In tuberculoid forms, lesions are seen in the external part of the nose and rarely can be found in the nasal vestibule [3, 5, 6].

It is important that patients be oriented not to force nasal deobstruction. Applying saline dressings and ointment is very useful for removal of scabs. In case of deformities, surgical correction will obtain good results [11].

The nasal manifestations diagnostics consists of cautions examination of nasal swabs, nasal secretion, and nasal tissue biopsies utilizing microscopic and molecular methods [12, 13].

24.2 Oropharynx

Lesions in the oropharynx are in most cases secondary to the nasal wounds and found in patients with long-term evolution of the disease. The lips, soft palate, hard palate, uvula, and gums can be affected. In the beginning, nonspecific inflammatory alterations can be verified, which gradually become infiltrated and present nodules of pale-pink or yellowish color. These wounds can extend to the posterior wall of the pharynx, nasopharynx, and soft palate tonsils [1, 2].

The uvula can be totally destroyed, and ulceration of the leprosy nodules in the palate can progress to perforation. Residual adherences and atresia of the pharynx

can cause difficulty in swallowing and nasal regurgitation. There have been specific descriptions of manifestations associated with bacilli-infested dental pulp [5, 11].

24.3 Larynx

Lesions of the larynx are observed in patients with long-term evolution and without treatment; these can be very severe. These lesions were very frequent in the period before modern therapeutic resources. They often lead to larynx obstruction and patient death [5, 11].

Compromise of the larynx initiates from the free extremity of the epiglottis, and then through the vocal chords. The lesions of the larynx are of two types: fibrotic and ulcerative. In fibrotic lesions—a tissue reaction due to *M. leprae*—there is immobilization of the vocal chords and, consequently, hoarseness. This involvement is progressive and can lead to complete stenosis of the larynx. The ulcerative forms are more severe; in these cases, granulomatous reactions develop more rapidly, forming a considerable amount of secretion, which can lead to hoarseness, pain, respiratory difficulty, and risk of death. These manifestations can be found in advanced lepromatous and borderline lepromatous leprosy cases without treatment or with irregular treatment [5, 11, 14].

24.4 Auricle

The auditory apparatus is divided into the outer, middle, and inner ear. In leprosy, it seems that only the auricle is compromised. The lesions prefer the free borders of the pinna; isolated and asymmetric lesions can be found in other polar clinical forms, and, in most cases, are symmetrical. The lesions tend to be symmetric in lepromatous and borderline lepromatous leprosy cases [11, 14].

The auricles are involved in approximately 80% of lepromatous and borderline lepromatous leprosy patients (Fig. 24.3) [14]. Presence of auricle lesions in indeterminate and tuberculoid leprosy is not frequent [11].

Leprosy nodules can develop ulcers, leading to development of secondary infections that facilitate perichondritis and deformation of the ears [11].

The rhinopharyngeal manifestations can collaborate to secondary otopathologies; these are not caused by *M. leprae* but rather by the lesions it produces in adjacent regions [5].

24.5 Differential Diagnosis

24.5.1 Common Cold and Chronic Rhinitis

The differential diagnosis should be considered in patients with nasal symptoms who do not respond adequately to standard therapies. Medical history and physical examination will allow for correct diagnosis [8, 12].

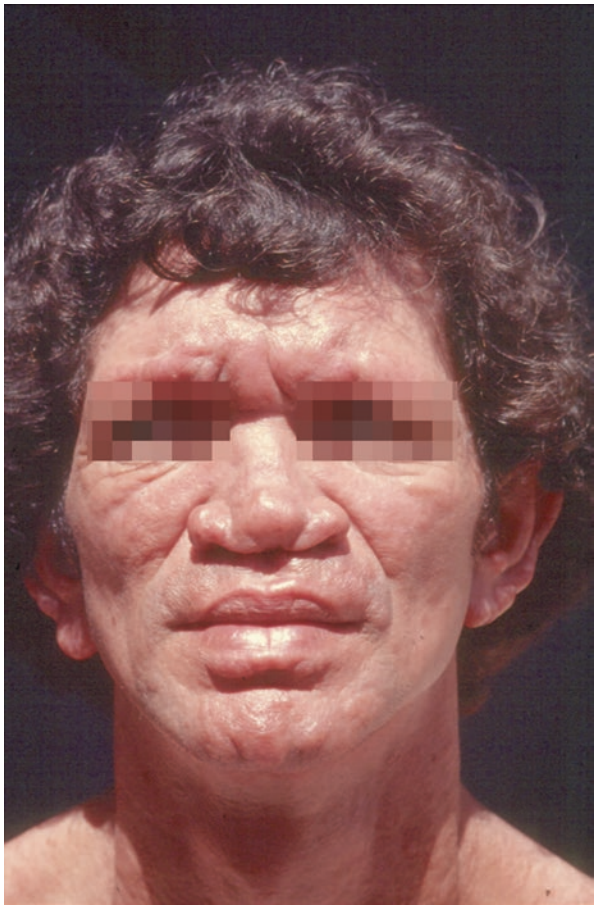

Fig. 24.3 Lepromatous leprosy. Delayed diagnosis. There is nasal involvement, madarosis, and bilateral infiltration of the auricles. Chronic nasal obstructions with bleeding episodes are common in these cases

24.5.2 Mucocutaneous Leishmaniasis

This initiates with ulcerated lesions, and if the patient is not treated correctly, lesions in the anterior part of the nasal septum can appear. The entire nasal septum can be destroyed; the resulting deformities are similar to the deformities from leprosy. Direct examination of bacilli and lack of abnormality in the sensitivity test will allow for definitive diagnosis. Other examinations that contribute to the diagnosis are immunological tests such as the Montenegro skin test [13]. With destruction of the nasal septum, the nasal tip drops, giving the nose an "elephant trunk" or "tapir nose" aspect. In more advanced cases, there can be partial or total destruction of the nasal wings and complete loss of the nasal dorsum [12, 13].

24.5.3 Congenital or Tertiary Syphilis, Yaws, or Rhinoscleroma

These should also be considered in the differential diagnosis of nasal manifestations of leprosy [12, 13].

24.5.4 Carcinoma, Sinus Lymphoma, and Paracoccidioidomycosis

These are important hypotheses in the differential diagnosis of larynx and oropharynx lesions [12, 13, 15].

24.5.5 Anergic Leishmaniasis and Lobomycosis

These are important differential diagnoses in cases with auricle involvement [12].

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Other Organs Involved in Leprosy

25

Enrico Nunzi and Salvatore Noto

Treatment regimen, follow-up of therapy, and release from treatment are based on clinical and microbiological cutaneous findings. Although *Mycobacterium leprae* (*M. leprae*) has a predilection for peripheral nerves and settles almost invariably on the skin, it is also found in other organs and systems. *Mycobacterium leprae* can be viable in these places, even after its disappearance from the skin, and this can lead to relapse.

Mycobacterium leprae spreads to internal organs via hematic and lymphatic systems. Histological changes typical of leprosy and bacilli have been identified in lymph nodes, liver, spleen, bones, striated muscles, testicles, adrenal glands, kidneys, and eyes [1].

Visceral localizations occur frequently in lepromatous patients. The bacteremia is not connected with clinical signs of hematic invasion; before treatment, it is present in 88% of cases in lepromatous patients and in 50% of borderline lepromatous patients.

Bacteremia disappears after 2 or 3 months of World Health Organization multidrug therapy (MDT) [2].

In patients affected by TT/BT leprosy, acid-fast bacilli (AFB) are mainly present in those organs rich in reticuloendothelial system, where they arrive via hematic and lymphatic vessels. This spread probably occurs before the complete setup of cell-mediated immunity (CMI) against *M. leprae*.

Visceral localization does not offer useful elements to formulate a leprosy diagnosis. In literature, a lepromatous leprosy case characterized by splenohepatomegaly is reported without any localization of the bacillus in peripheral nerves or in the skin [3].

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25.1 Lymph Nodes

Lymph nodes are the most frequent setting for *M. leprae* localization after peripheral nerves and the skin. In indeterminate leprosy, lymph nodes are not involved, while in the leprosy spectrum, adenopathy shows microbiological and histological features which are correlated to various forms.

(a) Tuberculoid leprosy
- Lymph nodes which drain infected cutaneous areas show small granulomas formed by epithelioid cells, Langhans cells, and lymphocytes in 60% of cases. In rare cases, scarce AFB can be identified. In the tuberculoid form characterized by high CMI, paracortical areas are infiltrated by lymphocytes.

(b) Lepromatous leprosy
- Involved lymph nodes become enlarged and hard in consistency. The cervical, axillary, inguinal, and abdominal areas and hepatosplenic lymph nodes are affected. Histopathology shows granulomas set up by macrophages where there is an AFB presence; AFB can be in globi or isolated. Foamy cells infiltrate cortical and medullar zones.

(c) Leprosy reaction type 2
- Lymph nodes involved in the inflammatory process increase in size and become painful. In severe leprosy reactions, they have a floating consistency. The axillary, cervical, inguinal, and epitrochlear regions are frequently involved. Microscope examination reveals neutrophils which infiltrate macrophagic granulomas; AFB are present.

25.2 Liver

The liver is the most frequent site for visceral *M. leprae* localization after lymph nodes.

(a) Tuberculoid leprosy
- Granulomas with epithelioid cells and giant cells are present in the liver. These can contain AFB surrounded by lymphocytes.

(b) Borderline leprosy
- In the liver, there are granulomas with variable histological features. From the BT to the BL form, an increase in the number of macrophages and bacteria can be seen, while epithelioid cells and lymphocytes decrease.

(c) Lepromatous leprosy
- About one-third of untreated lepromatous patients in advanced stages show hepatomegaly. In the hepatic tissue, miliary lepromas can be found. They involve Küpffer cells and are made up of foam cells which contain AFB, isolated or in globi. There is concordance between the cutaneous bacterial index (BI) and the hepatic bacterial load; AFB with solid staining can be identified

in the liver, even after disappearance of AFB from the skin. During episodes of type 2 reaction, granulomas are infiltrated by neutrophils.

In untreated late lepromatous leprosy, hepatic functional parameters are frequently modified. These alterations improve with therapy.

25.3 Spleen

Spleen involvement can be seen in lepromatous patients in active phase. Solid and granular staining bacilli are present in granulomas in red or white pulp. Splenomegaly improves during specific therapy. There is no spleen localization in the other forms of the spectrum.

25.4 Bone

25.4.1 Bone Marrow

(a) Tuberculoid leprosy
- Few AFB can be found.

(b) Lepromatous leprosy
- The bone marrow presents infiltration of macrophages and foam cells containing solid AFB. These bacilli can be present and alive even after slit-skin smear examination has become negative.
- In untreated lepromatous patients, in advanced phases, anemia proportional to the bone bacterial load can be observed.

25.4.2 Bone Structure

Specific or aspecific bone alterations can be observed in leprosy:

(a) Specific lesions
- These are characterized by presence of AFB; they are rare and can be observed in multibacillary patients, especially in advanced lepromatous leprosy. These lesions are localized in the hand, foot, and small bones of the nose, being made up of reactive inflammatory infiltrate. This invades the periosteum and destroys bone trabeculae, which are substituted by granulomatous tissue. AFB are present between the cells and inside macrophages and osteoblasts. Even without strong trauma, finger bones can break, so sudden edema can appear (Figs. 25.1 and 25.2). Adequate leprosy treatment leads to granulomatous tissue healing and bone recalcification. Tibial proliferative lepromatous periostitis can occur in lepromatous patients in advanced phases of disease. This localization can lead to the sabre tibia clinical aspect.

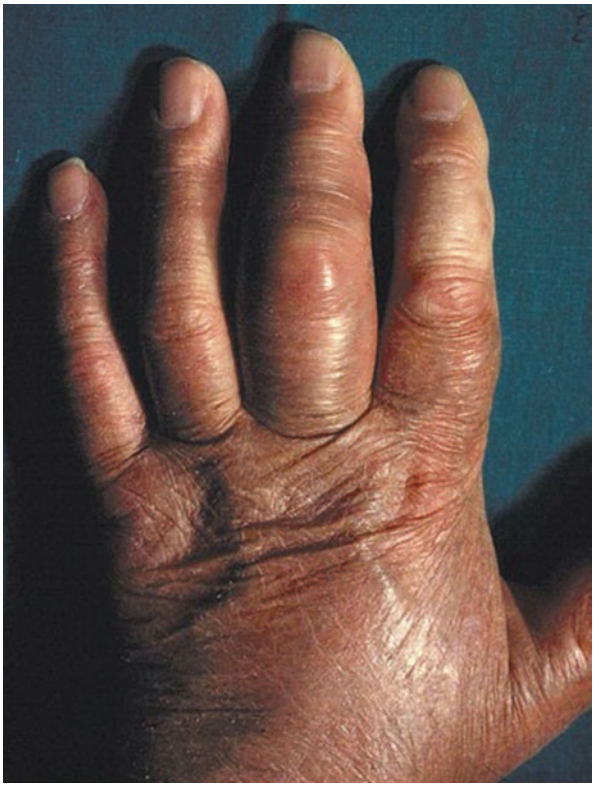

Fig. 25.1 Lepromatous leprosy. Patient treated with dapsone in monotherapy. Relapse appeared 10 years after end of therapy, with nodules, solid bacilli in slit-skin smears, and sudden edema of third finger of left hand

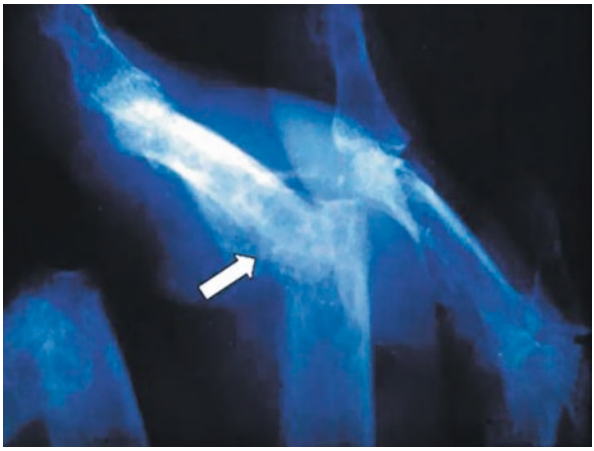

Fig. 25.2 Patient's hand X-ray from Fig. 25.1: fracture involved the second phalanx

(b) Aspecific lesions
- Most skeletal leprosy lesions found are not due to *M. leprae* bone localization. Xerotic, hypo-anesthetic skin of deformed hands and feet receives continuous traumas with cutaneous infection and ulceration. The continuous episodes may involve subcutaneous tissue and bone phalanx. These aspecific infections and consequent inflammatory episodes may activate osteoblasts and lead to reabsorption of distal phalanx.

25.5 Muscle

In superficial striated muscles of the lower limbs in lepromatous patients, nodular infiltrates with macrophages containing AFB can be seen. Inflammatory involvement of these granulomas during type 2 reaction leads to localized pain.

25.6 Testis

Low testicular temperature induces *M. leprae* testicular localizations; bacilli reach these organs through the bloodstream. Orchiepididymitis, often bilateral, can lead to testicular atrophy in advanced LL patients. In early phases, perivascular granulomas are made up of macrophages, plasma cells, and lymphocytes with interstitial tissue secondary involvement; AFB can be seen in macrophages and in endothelial cells. In advanced phases, there is fibrosis and hyalinization of tubula seminifera. The chronic evolution of orchiepididymitis is at times interrupted by acute inflammatory episodes of type 2 reaction: testicles present edema and pain. Other reactional symptoms such as fever, arthralgias, and erythema nodosum leprosum can be present. These episodes damage testicles more quickly and lead to atrophy. Acute orchiepididymitis symptoms can represent the only manifestation of type 2 reaction. In scrotum muscles, solid staining *M. leprae* has been found a long time after bacilli have disappeared from skin lesions [4, 5].

25.7 Breast

Gynecomastia can be seen in 8–9% of advanced untreated lepromatous patients. Its pathogenesis might be multifactorial and it is not only due to testicular involvement. In fact, this localization is very frequent; it can be diagnosed in 90% of lepromatous patients in the advanced phase of the disease. Low production of ketosteroid and increase in gonadotropins have been reported in patients with gynecomastia [5].

25.8 Adrenal

In the adrenal cortex of lepromatous patients, there can be the presence of miliary granulomas formed by macrophage and foamy cells containing AFB. Bacilli have been discovered in endothelial cells too. Chronic type 2 reaction can lead to hypoadrenalism [6].

25.9 Kidney

In advanced lepromatous patients, kidney lesions can be reported. Although *M. leprae* is also localized in glomerular endothelial cells, it does not cause kidney lesions, which are on the contrary due to immunocomplex deposition on kidney capillary walls. These immunocomplexes are formed during type 2 reaction; *M. leprae* antigens and autoantigens are present. Kidney damage leads to glomerulonephritis with hematuria, albuminuria, and cylindruria [7–9].

25.10 Central Nervous System

It is universally acknowledged that the central nervous system is not involved in leprosy. In differential diagnosis, it is dogmatically stated that leprosy must be excluded if patients show decrease or loss of deep reflexes and increase of pathological reflexes in neurological tests. In literature, presence of *M. leprae* in the medulla oblongata and spinal cord is reported [10]. In brain, meninges, and spinal cord of experimentally infected armadillo, lepromatous infiltrate with *M. leprae* was demonstrated. Bacilli were localized in endothelial and glial cells.

25.11 Amyloidosis

Secondary amyloidosis, with amyloid substance deposition in liver, spleen, and adrenal glands, can appear in patients with serious and repetitive episodes of leprosy type 2 reaction [11].

25.12 Causes of Death in Leprosy

Leprosy does not represent a direct cause of death. In endemic countries, life expectancy in lepromatous patients is lower than in other populations. Similar studies on tuberculoid patients did not show important differences.

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Part VII

Patient's Management in Leprosy

Diagnostic Work-Up of Leprosy

26

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The first step towards diagnosing leprosy is to think of the possibility of leprosy
D.L. Leiker

Leprosy is a serious disease, both for the patient because of the disabling sequelae with social margination and for the community because it is an infectious disease.

The diagnosis of leprosy must be established as early as possible and must not be generic but "accurate," specifying the form.

Early diagnosis has twofold importance: it defends the community, and it avoids the disabling physical consequences.

26.1 Integrated Leprosy

After the intensive leprosy control activities which took place in the 1990s, there was a drastic decrease in cases of leprosy. This situation led to a move from "heavy," vertical leprosy control programs to the adoption of "light" leprosy control activities integrated within general health services [1].

On the one hand, this situation produced social benefits such as better integration of patients into the community and reduced health system costs. On the other hand, integration reduces quality in health services, mainly due to lower diagnostic ability

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by multipurpose medical staff. This has been worsened by minimum interest in both teaching and scientific research in leprosy.

In the last decade, these facts have put leprosy in the World Health Organization (WHO) group of neglected tropical diseases.

To overcome this intellectual gap, in the Western world, physicians tried to develop expensive, ultraspecialized laboratory examinations (such as polymerase chain reaction, PCR). However, these "surrogates" make little or no contribution to diagnosis, which is made according to clinical knowledge and rational use of traditional laboratory systems. Nowadays, this modern analysis provides no useful information regarding therapy or prognosis.

Moreover, to get around the decreased knowledge on leprosy by physicians and paramedical staff, disease management has been divided into two levels: the peripheral level (integrated into general health services) and the referral level, where a range of specialists help in the diagnosis, therapy, and management of leprosy reactions and other complications.

In Western countries, these referral centers are usually associated with dermatological departments.

26.2 Diagnosis

Leprosy diagnosis is mostly clinical, helped by tests of skin lesions such as sensory tests and slit-skin smear examination.

Histopathology (the gold standard examination for leprosy) is necessary in those countries where leprosy is a rare and "imported" disease.

Suspicion of leprosy may arise in both physicians and pathologists in the course of routine observation. Histopathology must be matched with clinical signs in order to be consistent with the diagnosis of leprosy.

Leprosy is an infectious disease that mainly involves peripheral nerves and skin.

The diagnostic process of leprosy needs careful examination of:

- Whole skin, with special regard to morphological clinical aspects and search for AFB.
- Peripheral nerves usually damaged by leprosy. Physicians must palpate the sites of predilection. Moreover, anesthesia must be searched for in cutaneous lesions (macules or plaques) and in skin areas innervated by involved peripheral nerves.

Formulating an aspecific diagnosis of leprosy is the first step toward a diagnostic error. It is necessary to classify the disease using the Ridley–Jopling spectrum (5 forms + 1) [2] or using the simplest WHO classification (two forms: multibacillary and paucibacillary) [3]. This will lead to correct diagnosis, through obliged steps.

Moreover, classification helps in:

(1) Leading to the correct therapy
(2) Formulating a prognosis based on the "estimated" risk of leprosy reactions
(3) Underlining the importance of involvement of peripheral nerves

26.2.1 Basic Diagnostic Principle: Searching for Concordance Among Parameters

Each leprosy patient is characterized by a degree of cell-mediated immunity (CMI) which determines the form of the disease (see Chap. 6).

Each form of leprosy has a characteristic skin clinical aspect, a specific bacterial load, and a particular histopathology of skin lesions. These three parameters must be consistent because they depend on the same CMI (Table 26.1) [4].

The leprosy diagnosis is based on:

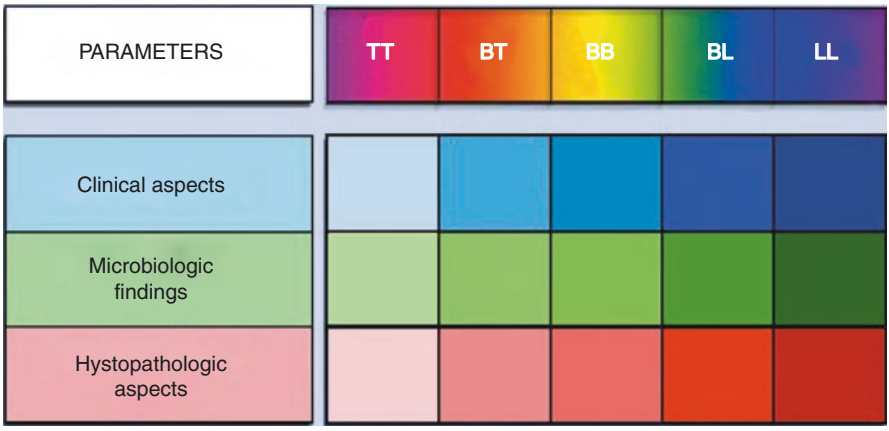
1. Searching for clinical, microbiological, and histopathological parameters
2. Establishing whether they are consistent among themselves in the same leprosy form

This diagnostic process is not valid in the indeterminate or pure neuritic forms of leprosy. The indeterminate form presents unclear CMI, and the pure neuritic form is characterized by nerve involvement without skin lesions. In these two forms, only histopathology may help in diagnosis.

26.2.2 Anamnesis

The first fundamental step is anamnesis, particularly in countries where leprosy is an "imported" disease. Physicians must investigate whether the patient comes from or has lived in tropical or subtropical areas in the last 10–15 years. It must be sought whether patients have relatives or cohabitants with leprosy or chronic nonspecified diseases which have led to disability or skin lesions. This information has less importance in areas with high incidence of leprosy.

Table 26.1 The basis of leprosy diagnosis: concordance among clinical, microbiological, and histopathological parameters in the various forms



26.2.3 Prodromal Stage

The disease may have its onset with a prodromal stage characterized by extracutaneous lesions that are hard to fit into a diagnosis because of their lack of specificity: nasal symptoms (repeated epistaxes, stuffiness, crust formation), paresthesia, neuralgia, arthralgia, and acral edemas. Interpretation of such symptoms is usually achieved during medical history-taking only after the appearance of typical signs of leprosy.

26.2.4 Onset

Nearly always, the clinical onset of leprosy is characterized by the appearance of cutaneous symptoms with slow onset and chronic evolution; multibacillary hypoanergic forms can be discovered by painless cutaneous traumas or burns.

Acute onset of the disease can take place when leprosy reactions occur in an apparently healthy subject. Pregnancy, delivery, vaccinations, or immunodepression caused by drugs can be precipitating factors.

Type 1 leprosy reaction appears with erythematous and edematous plaques. Acute onset of disease with type 1 leprosy reaction can represent an immune reconstitution inflammatory syndrome (IRIS) phenomenon in HIV/AIDS patients under treatment with highly active antiretroviral therapy (HAART), and only histology may lead to diagnosis.

Acute onset of disease can appear with type 2 leprosy reaction symptoms due to immune complexes: fever, erythema nodosum leprosum (ENL), neuralgias, polyarthralgias, and epididymo-orchitis. The etiology of this acute systemic pathology will be clarified by the anamnesis and the search for AFB in "cooler" skin areas.

26.3 Diagnosis at Peripheral Level

At this level, patients will be classified as pauci- or multibacillary according to the WHO classification for treatment purposes (see Chap. 6).

26.3.1 Physical Examination

The first step of the clinical skin examination identifies patterns of distribution of lesions on the body (see Table 10.2 in Chap. 10):

1. Asymmetric pattern (monolateral or bilateral)
2. Symmetric pattern

26.3.2 Pattern With Asymmetrical Arrangement (Monolateral or Bilateral)

Related Test: Test for Anesthesia in Skin Lesions

Asymmetrical arrangement of anesthetic lesions is typical of hyperergic and paucibacillary leprosy: TT, BT (with early damage of nerve bundles in skin lesions) (Figs. 26.1, 26.2, and 26.3).

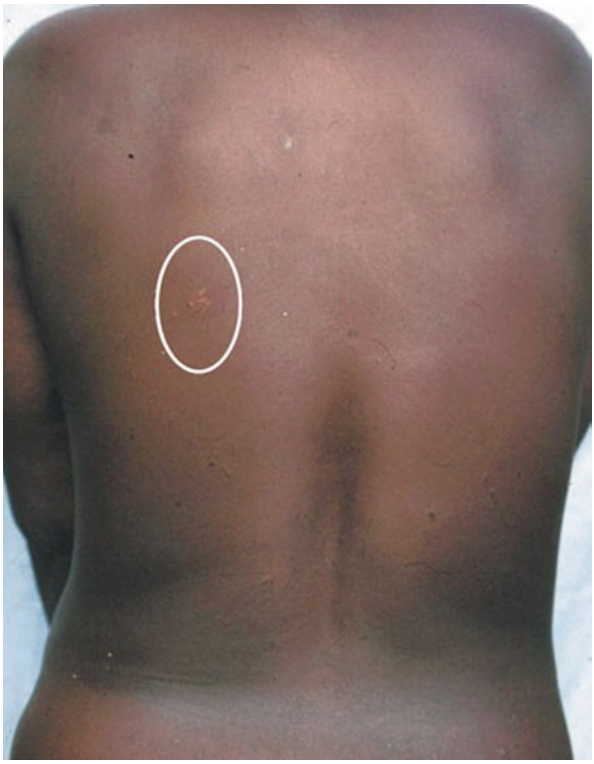


Fig. 26.1 Asymmetrical arrangement: monolateral

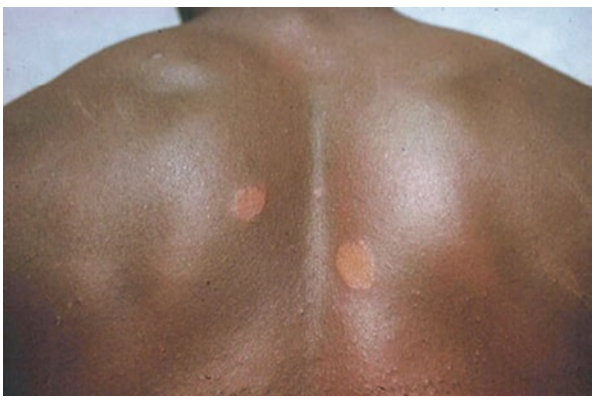


Fig. 26.2 Asymmetrical arrangement: bilateral (this figure is also presented in Chap. 10; see Fig. 10.12)

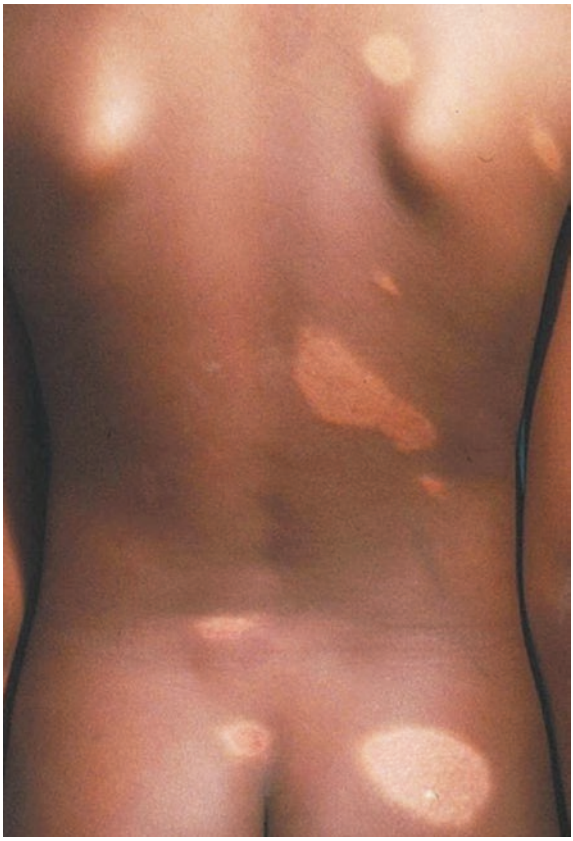


Fig. 26.3 Asymmetrical arrangement with more than five lesions

26.3.3 Pattern With Symmetrical Arrangement (Bilateral)

Related Test: Slit-Skin Smear Examination for AFB in Skin Lesions Symmetrical arrangement of lesions with AFB is typical of hypo-anergic multibacillary leprosy (Fig. 26.4).

Medical literature reports few exceptions to this rule; lepromatous patients with a single nodule or with asymmetric, monolateral distribution of nodules are anecdotal cases and do not invalidate the rule of consistency of the parameters.

26.4 Diagnosis at Referral Level

At this level, clinical examination should not be limited to observation of the distribution of skin lesions but should also identify their type and features. The clinical aspect must be compared with microbiological and histopathological findings to verify whether the three parameters are consistent with the same form of the spectrum (Table 26.1).

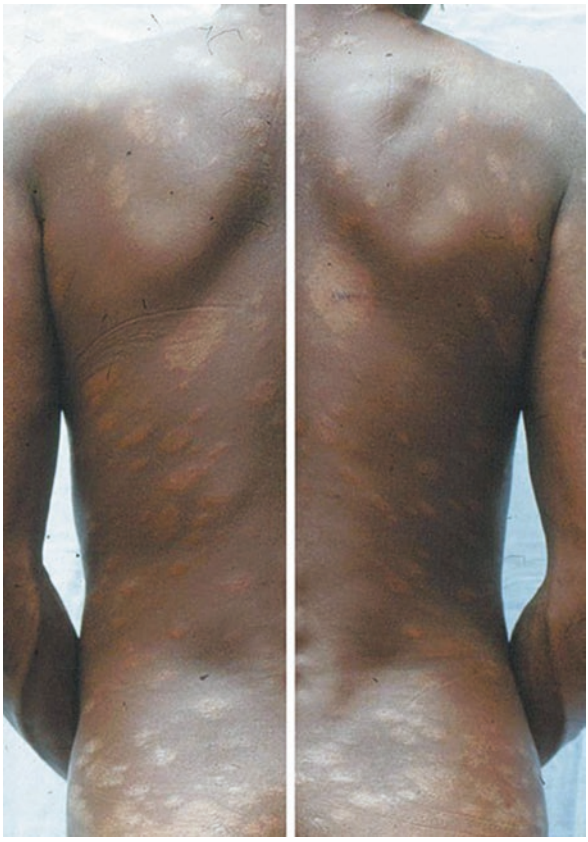


Fig. 26.4 Symmetrical arrangement

26.4.1 Steps in the Diagnostic/Classification Process

1. Collection of clinical, microbiological, and histopathological parameters
2. Consistency of the parameters for each clinical form

26.4.2 Clinical Parameters (Table 26.2)

- Arrangement of lesions (see Table 10.2 in Chap. 10)
- Type of lesions (Table 10.3 in Chap. 10)
- Clinical features (surface, edges, anesthesia, or esthesia)
- Shape (annular)

26.4.3 Microbiological Parameters (Slit-Skin Smear)

- AFB presence
- Bacterial index

Table 26.2 Clinical parameters

Clinical parameters	TT	BT	BB	BL	LL
Arrangement	Asymmetrical	Asymmetrical	Symmetrical	Symmetrical	Symmetrical
Annular aspect	Present	Present	Present	Absent	Absent
Surface	Rough	Rough	Smooth	Smooth	Smooth
Edge	Very sharp	Sharp / vague	Rather vague	Rather vague	Vague
Loss of sensation	Marked	Marked / moderate	Absent	Absent	Absent

26.4.4 Histopathological Parameters

- Type and localization of infiltrate
- AFB presence

26.5 Leprosy is a Peripheral Nerve Disease

The leprosy clinical picture is the result of skin lesions and peripheral nerve involvement.

To confirm diagnosis, after having made an assessment of the skin, it is necessary to search for clinical nerve involvement (anesthesia, anhidrosis, muscle hypoatrophy in innervated areas, and swelling and pain at site of predilection) (Table 26.3).

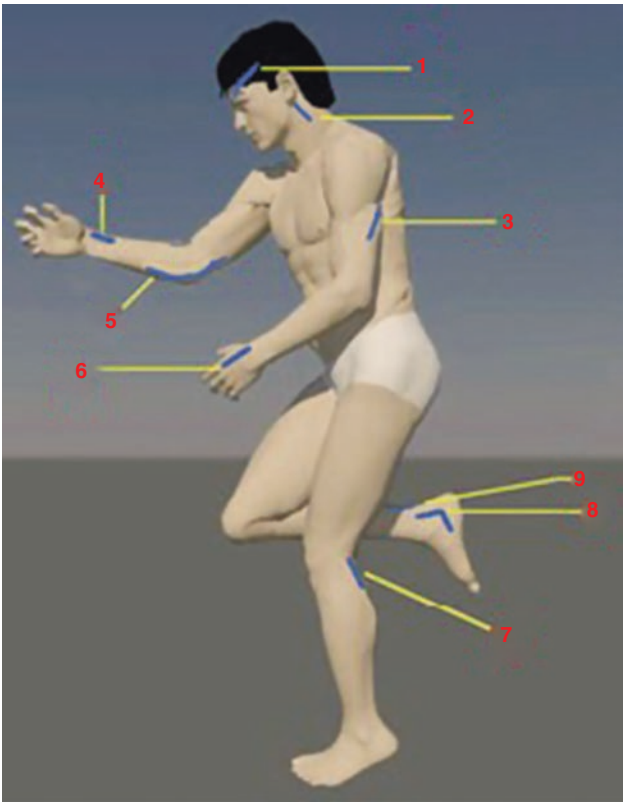
In rare case, leprosy presents with only nerve involvement (pure neuritic form). These cases must be sent to referral centers; only histopathology of biopsy performed on a sensory nerve (sural nerve or radial cutaneous nerve) can confirm the diagnosis.

26.6 Diagnosis: Main Points

Correlation of the following parameters:

1. Skin
   - Clinical aspects of lesions: arrangement, type, and features
   - Investigation of anesthesia
   - Presence of AFB
   - Histopathological aspects
2. Peripheral nerves
   - Swelling and pain at sites of predilection
   - Anesthesia in skin lesions or in cutaneous areas innervated by the involved nerve

Table 26.3 Peripheral nerves and sites of predilection in leprosy



1. upper orbital nerve
2. great auricular nerve
3. radial nerve
4. median nerve
5. ulnar nerve
6. radial cutaneous nerve
7. common peroneal nerve
8. posterior tibial nerve
9. sural nerve

Modified from Nunzi E, Leiker DL. Manuale di Leprologia, AIFO-Italia, Bologna; 1990

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Drugs in Leprosy

27

Sinésio Talhari and Mahreen Ameen

27.1 Introduction

In ancient times, before the advent of chemotherapy, leprosy was treated with the application of topical agents or injections to skin lesions. Vegetable oil preparations were used, such as hydnocarpus and chaulmoogra oil, studies demonstrating that the active principle chaulmoogric acid has some antileprosy actions [1]. The discovery of sulfonamides paved the way for use of antibiotic chemotherapy to treat leprosy. The goals of pharmacotherapy are to clear the infection, reduce morbidity, prevent complications, prevent transmission, and eradicate the disease. From the 1940s, for almost three decades, the only chemotherapeutic agent used for treating leprosy was dapsone. Dapsone demonstrated high efficacy, was relatively well tolerated, and was affordable. Given by mouth made on-the-spot administration easy, making it very suited to ambulatory treatment. It also has a long shelf life, which facilitated the logistics of drug distribution to remote areas. By 1951 the standard treatment for leprosy was oral dapsone and it was widely used as monotherapy in the 1950s and 1960s. This was an immense step forward in the treatment of leprosy. However, dapsone monotherapy eventually led to problems of drug resistance. This was particularly the case when it was given at inadequate doses, for prolonged periods, and when there was interruption of the treatment schedule. One of dapsone's drawbacks is that it is slow acting and as monotherapy it could take several years for lepromatous leprosy patients to become bacteriologically negative. Therefore, treatment often lasted for many years and sometimes a lifetime. This made compliance

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to treatment difficult, which contributed to the problem of drug resistance. Both primary and secondary resistance to dapsone became evident in the 1960s: primary resistance referring to resistance in leprosy patients who have never been exposed to dapsone and had acquired infection with drug-resistant *M. leprae* and secondary resistance referring to cases of relapse of leprosy in patients who had previously been treated with dapsone [2, 3]. Dapsone resistance against leprosy became widespread and was a public health problem as patients who developed it were a source of infection to their communities. Clofazimine was introduced in 1962 following reports of its antibacterial effects against *Mycobacterium leprae* and of its efficacy against leprosy reactions because of its anti-inflammatory effects [4]. Rifampicin was subsequently introduced in 1970 [5]. Rifampicin has strong bactericidal activity, and there are studies demonstrating that within days of commencing treatment with rifampicin, no viable bacteria are detected in biopsies of lepromatous cases [6, 7]. Furthermore, mouse footpad studies demonstrated that rifampicin was equally active against both dapsone-sensitive and dapsone-resistant *M. leprae* strains [7].

As rifampicin is the principal bactericidal drug used in MDT, the development of rifampicin resistance poses a serious threat to leprosy control programs. Brazil and India have reported the emergence of rifampicin-resistant mutants [8, 9]. In an effort to combat and monitor drug resistance, the WHO implemented a surveillance network in 19 endemic countries to monitor drug sensitivity patterns. It detected rifampicin resistance both in new leprosy cases and in relapses in 12 countries. Primary and secondary resistance to dapsone was also observed, and there were cases of resistance to both rifampicin and dapsone [10].

Multidrug therapy (MDT) came into practice after 1981 following recommendations from the World Health Organization (WHO). The rationale for MDT is that it would decrease the risk of development of drug-resistant mutants if two-drug or three-drug combinations were administered. Multidrug antimicrobial therapy is also associated with increased efficacy. Therefore, a two-drug regimen for paucibacillary (PB) leprosy and a three-drug regimen for multibacillary (MB) leprosy were advocated to effectively kill all live organisms. Since *M. leprae* characteristically persists in host tissues for many years, drug therapy would be required for prolonged periods. WHO initially recommended MDT regimens for 12 and 24 months of duration for PB and MB leprosy, respectively. These recommendations were reformulated in 1998, reducing the treatment duration to 6 and 12 months, respectively [11]. However, some leprosy centers continue to favor the longer MDT regimens. In 2018, the WHO developed the first guidelines for leprosy through evidence-based recommendations utilizing guideline development methods [12]. This was in contrast to previous guidance, which was developed though meeting reports and other technical documents. The 2018 guidelines addressed MDT regimens, the issue of drug resistance, and chemoprophylaxis. It advocated the use of three-drug regimen comprising rifampicin, dapsone, and clofazimine for all leprosy patients (both PB and MB leprosy) with duration of treatment remaining the same: 6 months for PB and 12 months for MB leprosy (Table 27.1). The WHO states that the use of the same three drugs to treat PB and MB leprosy simplifies the practicalities of treatment as the same blister packs can be used, and reduces the impact of

Table 27.1 MDT treatment regimen for children and adults: 6 months for PB and 12 months for MB leprosy

Drug	Pediatric dose (age <10 years or <40 kg)	Pediatric dose (age 10–14 years)	Age 15 years and above
Rifampicin	10 mg/kg monthly	450 mg monthly	600 mg monthly
Clofazimine	100 mg Monthly + 50 mg twice weekly	150 mg monthly + 50 mg Alternate days	300 mg Monthly + 50 mg daily
Dapsone	2 mg/kg daily	50 mg daily	100 mg daily

misclassification of MB leprosy as PB leprosy. For rifampicin-resistant leprosy, the guidelines recommend treatment with at least two second-line drugs (clarithromycin, minocycline, or a quinolone such as ofloxacin, levofloxacin, or moxifloxacin) plus clofazimine daily for 6 months followed by clofazimine plus one of the second-line drugs daily for an additional 18 months. For patients resistant to both rifampicin and ofloxacin, the guidelines state that a fluoroquinolone should not be used as part of second-line treatment. Instead, the recommended regimen is 6 months of clarithromycin, minocycline, and clofazimine followed by clarithromycin or minocycline plus clofazimine for an additional 18 months. The guidelines also recommend chemoprophylaxis for contacts of patients with leprosy: single-dose rifampicin for adults and children over the age of 2 years.

Since the late 1980s, three additional antileprosy drugs have been available: ofloxacin (a fluoroquinolone), clarithromycin (a macrolide), and minocycline (a tetracycline) [13]. There are ongoing clinical trials to investigate their efficacy. These drugs may potentially increase the effectiveness of standard MDT, shorten treatment duration, decrease the risk of the emergence of drug resistance, or be used as an alternative in cases of MDT resistance or in patients in whom one or more of the drugs constituting MDT are contraindicated.

For single skin lesion smear negative PB without nerve involvement, a single dose of rifampicin 600 mg, ofloxacin 400 mg, and minocycline 100 mg (ROM combination) has been trialed most extensively. Minocycline is contraindicated in early childhood and caution is advised with the use of ofloxacin in children and adolescents. Therefore, ROM regimens have been favored mainly for adults. Long-term ROM regimens have also been trialed from monthly regimens [14] to three times weekly regimens for both PB and MB leprosy. As yet there is still insufficient data to elucidate the optimum dosage and duration of ROM regimen as an alternative treatment for leprosy. A meta-analysis published in 2011 of ROM therapies for leprosy concluded that single-dose ROM therapy was less effective than MDT in PB patients and that there was insufficient data to determine whether multidose ROM therapy has a role for MB leprosy [15]. A further meta-analysis published in 2020 compared the standard MDT regimens against other regimens for the treatment of leprosy [16]. Diverse non-MDT treatment regimens including ROM were studied. However, the use of different outcome measurements and variations in follow-up periods made comparisons difficult.

27.2 Drugs Used to Treat Leprosy

27.2.1 Dapsone

Dapsone (4,4′-diaminodiphenyl sulfone) is a synthetic sulfone similar to the sulfonamides. It is bacteriostatic, acting by blocking folic acid synthesis by targeting dihydropteroate synthase, which is a key bacterial enzyme. Despite early reports of widespread resistance, it remains a very useful drug within MDT regimens. Its role in the PB MDT regimen is to prevent the emergence of rifampicin-resistant organisms. Dapsone in combination with clofazimine, as used in the MB regimen, has bactericidal efficacy, although this is not as powerful as the bactericidal activity of single-dose rifampicin. A daily 100 mg dose of dapsone is incorporated in both MDT regimens for leprosy.

27.2.1.1 Adverse Effects

Dapsone is generally well tolerated. Mild hemolytic anemias are common, and hemolysis is dose dependent. The doses used in treating leprosy (50–100 mg daily) are usually inconsequential. Severe hemolytic anemias are rare except in those with glucose-6-phosphate deficiency. Dapsone-associated agranulocytosis has also rarely been reported. Dapsone at doses of 100 mg daily may cause methemoglobinemia, but this is usually asymptomatic unless there is hypoxemia secondary to lung disease. Gastrointestinal disturbance with anorexia, nausea, and vomiting may occur. There have been occasional reports of delayed hypersensitivity reactions presenting as "dapsone syndrome" that has skin manifestations similar to Stevens–Johnson syndrome. This typically develops 4–6 weeks after treatment initiation, and patients should be advised to discontinue the drug and seek medical attention because it can be associated with significant morbidity and mortality. Dapsone syndrome is characterized by exfoliative dermatitis, generalized lymphadenopathy, fever, and hepatosplenomegaly. Dapsone is contraindicated in patients known to be sensitive to any of the sulfa drugs.

27.2.1.2 Pharmacology

Dapsone is well absorbed orally. It is distributed throughout body fluids. Its plasma half-life is 28 h, and there is some drug retention up to 3 weeks. Dapsone becomes acetylated, and 80% is excreted as metabolites in urine. Its dosage must therefore be reduced in renal failure.

27.2.1.3 Drug Interactions

Folic acid antagonists such as pyrimethamine may increase the risk of hematological reactions with dapsone, and therefore patients should be monitored for agranulocytosis during the second and third months of treatment. Concurrent administration of rifampicin may decrease levels of dapsone because of increased renal clearance.

27.2.2 Clofazimine

Clofazimine is an aminophenazone dye, which preferentially binds to mycobacterial DNA, both inhibiting mycobacterial growth and exerting a slow bactericidal effect on *M. leprae*. Clofazimine also has anti-inflammatory properties and is therefore used in type 2 reactions at higher (200–300 mg daily) and divided doses, acting as a steroid-sparing agent and allowing use of lower doses of steroids [17]. Reports of resistance to clofazimine are extremely rare.

27.2.2.1 Adverse Effects

Clofazimine is extremely well tolerated at the dosages used in leprosy MDT regimens, i.e., 300 mg monthly and 50 mg daily for adults. However, it does commonly cause darkening and dryness of the skin, particularly within leprosy lesions. The conjunctivae are also affected. Darker skin colors are more susceptible to the hyperpigmentary effects of the drug. Skin discoloration tends to develop from the third month of treatment but clears within 6–12 months of treatment discontinuation. Clofazimine is also excreted through breast milk and can cause mild discoloration of the skin of the infant. Prolonged use of high-dose clofazimine produces more significant skin pigmentation and ichthyosis and rarely severe gastrointestinal side effects due to crystal deposition in the intestinal tract.

27.2.2.2 Pharmacology

The pharmacokinetics of clofazimine is complex. Oral absorption is highly variable. It has a long half-life of approximately 70 days. Clofazimine is widely distributed throughout the reticuloendothelial tissues. It has a tendency to bind to lipids and is stored in the body and slowly released. Therefore, the loading dose of 300 mg, which is subsequently given monthly, ensures that an optimal amount of clofazimine is maintained in the body tissue even if patients occasionally miss their daily dose. Clofazimine is largely unmetabolized. Its major route of excretion is through bile, and less than 1% is excreted in urine [18].

27.2.2.3 Drug Interactions

Dapsone may inhibit the anti-inflammatory activity of clofazimine. Concurrent administration of clofazimine with aluminum- or magnesium-containing antacids must be avoided because this leads to decreased absorption of clofazimine. Clofazimine in combination with phenytoin will lead to a reduction in efficacy of phenytoin.

27.2.3 Rifampicin

Rifampicin, a DNA-dependent RNA polymerase inhibitor, is a powerful bactericidal drug against *M. leprae*. Because of its high bactericidal activity, it is only given once monthly. Monthly dosing produces equivalent efficacy to daily dosing

with the advantage of being associated with lower rates of adverse effects. A monthly dosing schedule also has the advantage of being very cost-effective for leprosy control programs. A single dose of 600 mg of rifampicin is capable of killing 99.9% or more of all viable organisms [7]. The rate of killing is not proportionately increased by subsequent doses. Reports of rifampicin-resistant leprosy came after dapsone-resistance cases, but as yet rates of rifampicin resistance in both clinical practice and reported studies remain low. Rifampicin resistance is believed to have developed as a result of its use in monotherapy or in combination with dapsone in dapsone-resistant patients. The molecular basis of rifampicin resistance in *M. leprae* involves mutations in the *rpoβ* gene, which encodes the beta subunit of RNA polymerase, leading to high levels of resistance to rifampicin [19].

27.2.3.1 Adverse Effects

Although the monthly dose protocol is associated with low toxicity, occasionally it does cause flu-like symptoms, thrombocytopenia, hepatitis, and renal failure. Rifampicin characteristically produces a reddish-brown color in urine, sweat, tears, and saliva.

27.2.3.2 Pharmacology

Rifampicin is rapidly and easily absorbed after oral administration. With a single dose of 600 mg, the peak serum concentration occurs 2 h after administration. Approximately 80% of rifampicin is transported in blood bound to plasma proteins, mainly albumin. Rifampicin is well distributed, although to a different degree, in the various tissues of the human body. Following deacetylation in the liver, rifampicin becomes more microbiologically active. Rifampicin is equally excreted in bile and urine, and therefore its excretion is slower in patients with impaired liver and kidney function.

27.2.3.3 Drug Interactions

Rifampicin induces cytochrome P450 microsomal enzymes and therefore may decrease the efficacy of a number of drugs including acetaminophen, barbiturates, benzodiazepines, beta-blockers, corticosteroids, cyclosporin oral anticoagulants, and oral contraceptives. Co-administration of rifampicin with isoniazid may increase the risk of hepatotoxicity.

27.2.4 Ofloxacin

Ofloxacin is a synthetic fluoroquinolone antibiotic with efficacy against *M. leprae*. It has bactericidal activity as it acts as a specific inhibitor of bacterial DNA gyrase.

27.2.4.1 Adverse Effects

These include gastrointestinal disturbance and central nervous system complaints such as headaches, dizziness, and insomnia. Since animal studies have demonstrated that high dose of quinolones may impair cartilage development, its use in children is restricted.

27.2.4.2 Pharmacology

Ofloxacin demonstrates excellent bioavailability. It has a long half-life and is given as a single daily dose.

27.2.4.3 Drug Interactions

Ofloxacin induces cytochrome P450 microsomal enzymes, which may decrease effects of anticonvulsants, sulfonylureas, and insulin. Absorption of fluoroquinolones is reduced by ferrous sulfate, antacids, or sucralfate.

27.2.5 Minocycline

Minocycline inhibits protein synthesis and is commonly used in management of acne vulgaris, where it is well tolerated even with long treatment courses of greater than 1 year. It is the only tetracycline that has significant bactericidal activity against *M. leprae* [20]. This is a result of its lipophilic properties, which allow it to penetrate cell walls. It has greater bactericidal activity against *M. leprae* than that of clarithromycin but much less than rifampicin.

27.2.5.1 Adverse Effects

These include gastrointestinal upset and photosensitivity and rarely a bluish discoloration of the skin. Rarely, it has been associated with an autoimmune hepatitis and systemic lupus erythematosus-like syndrome. Minocycline is not recommended in children under the age of 12 years and during pregnancy because of its effects on developing teeth.

27.2.5.2 Pharmacology

Oral minocycline is readily absorbed and distributed throughout body tissues because of its lipophilic properties. It is eliminated in both urine and feces. It is also excreted in breast milk.

27.2.5.3 Drug Interactions

The bioavailability of minocycline is decreased with concurrent administration of antacids containing aluminum, calcium, magnesium, or iron. Minocycline can decrease the efficacy of oral contraceptives.

27.2.6 Clarithromycin

Clarithromycin is a member of the macrolide group of antibiotics, which inhibit bacterial protein synthesis by linking to the 50S ribosomal subunit, preventing elongation of the protein chain. Although several macrolide drugs have been evaluated for treatment of leprosy, only clarithromycin has demonstrated significant efficacy. When clarithromycin is administered at a dosage of 500 mg daily to MB patients, it

kills 99% of bacilli within 28 days and 99.9% by 56 days. A single dose of clarithromycin in combination with minocycline has demonstrated high bactericidal activity against *M. leprae* in lepromatous patients [21].

27.2.6.1 Adverse Effects

Clarithromycin is generally very well tolerated. Gastrointestinal upset may occur with nausea, vomiting, and stomach upset, but this usually does not require therapy discontinuation.

27.2.6.2 Pharmacology

Clarithromycin is readily absorbed and is distributed to most tissues and phagocytes. During active phagocytosis, large concentrations of clarithromycin are released, and therefore its concentration in tissues can be considerably higher than in plasma.

27.2.6.3 Drug Interactions

Clarithromycin can potentially interact with many drugs. Importantly, it should never be used in HIV patients due to significant interaction with HIV drugs. It should not be given during pregnancy. It should not be administered concurrently with carbamazepine as it significantly increases its plasma level, predisposing to carbamazepine toxicity.

27.2.7 Global Provision of MDT

WHO has facilitated the provision of MDT medicines worldwide. It has been free of charge and was initially financed by the Nippon Foundation (from 1995 to 1999) and since 2000 by Novartis AG.

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Treatment and Prophylaxis of Leprosy

28

Ousmane Faye and Pierre Bobin

Management of leprosy was for centuries based on traditional medicine, particularly in Asia where chaulmoogra oil was widely used [1]. The modern era of leprosy therapy began with the use of sulfone by Faget in 1941 in the United States [2]. The introduction of multidrug therapy (MDT) four decades later by members of a WHO Expert Committee [3] revolutionized leprosy control. Over the years following the implementation of MDT, the prevalence of leprosy has decreased dramatically to a point where the WHO envisaged, for the first time, a possible elimination of the disease by the year 2000. Although MDT is very effective, 30–40% of patients suffering from leprosy may experience reactions during the course of disease which, if not properly managed, can lead to irreversible neurological damage and the related disability that might require further interventions such as surgery. In practice, the treatment of leprosy should comply with the following objectives:

- Stopping disease transmission.
- Preventing and managing complications.

Achievement of these two objectives will help and facilitate the rehabilitation and social reintegration of the patient into the community.

This chapter will cover the basic and component elements of MDT recommended by WHO, management of complications (leprosy reactions, plantar ulcers), surgical treatment, and chemoprophylaxis.

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28.1 Multidrug Therapy (MDT)

28.1.1 Basics and Aims of MDT

Standard multidrug therapy (MDT) currently recommended by the WHO is a combination of antibiotics with various bactericidal effects aimed at killing all viable bacilli in a short period of time without selecting a single mutant resistant strain.

Dapsone was the first effective antibiotic for the treatment of leprosy. Its use as monotherapy for all types of leprosy including the lepromatous form resulted, in the 1970s, in the emergence of resistant mutants of *Mycobacterium leprae* (*M. leprae*). Similarly and a few years later, resistance also appeared with newer powerful antibiotics such as rifampicin as the result of long-life monotherapy in lepromatous patients. Therefore, it became necessary to adjust therapeutic strategies in order to preserve the effectiveness of the antimicrobials used for the control of leprosy. Meanwhile, awareness has grown that in leprosy, as with other mycobacterial diseases with high bacterial loads, the use of combination therapy is mandatory in order to prevent the emergence of resistant bacterial mutants. In the untreated lepromatous patient, the number of viable *M. leprae* is estimated to be 10^9 to 10^{10} and the chance of developing drug-resistant mutants is never greater than 1 per million bacteria [4]. By analogy with tuberculosis, the likelihood of finding resistant mutants in a wild-type strain is 1 in 10^7 for rifampicin and 1 in 10^5 for both clofazimine and dapsone [5], in the range of 1 in 10^{12} for a strain resistant to rifampicin and/or dapsone and clofazimine and 10^{17} for all three drugs. This suggests that the likelihood of finding a mutant resistant to a combination therapy of two or more drugs requires the patient to carry a large number of viable bacteria, which is extremely rare if not impossible. In 1981, this prompted the WHO Expert Committee for leprosy to recommend multidrug for all types of leprosy for the reasons abovementioned.

28.1.2 WHO MDT Anti-leprosy Drugs

The MDT regimen recommended by the World Health Organization [3] in 1981 consisted of rifampicin and dapsone for paucibacillary leprosy (PB leprosy) and of rifampicin, dapsone, and clofazimine for multibacillary leprosy (MB leprosy). The treatment duration was 6 months for PB, and it was reduced from an initial duration of 24–12 months for MB leprosy on the recommendation of the WHO Expert Committee [6].

28.1.2.1 Dapsone

Dapsone or 4-4-diamino-diphenyl-sulfone (DDS, Disulone) is a chemical analogue of sulfapyridine first synthesized in 1908. It acts as a bacteriostatic agent by inhibition of folic acid synthesis in *M. leprae*. There is also evidence that dapsone has an anti-inflammatory effect; this justifies its use in other dermatological conditions. Following oral administration of 100 mg, the peak serum concentration attained within 2–8 h exceeds by 500 times the minimum inhibitory concentration (MIC) of *M. leprae* (3 ng/

mL), and 10 days later, a serum concentration higher than the MIC of *M. leprae* can be detected in blood and urine [7, 8]. Dapsone is almost completely absorbed from the gut and penetrates well to all compartments of the body. It crosses the blood-brain barrier and placenta and the breast milk, as well. It is metabolized by the liver but also by activated polymorphonuclear leukocytes and is mainly eliminated in the urine.

Dapsone is available in 50 and 100 mg tablets (white in color) and administered orally at the dose of 1–2 mg per kg, 100 mg in adults and 50 mg in those under 14 years of age.

It should be noted that dapsone has been attributed with aphrodisiac properties. This might explain why, in endemic areas, some patients, even those who have been cured, continue to take the medication.

Dapsone is contraindicated in patients who are allergic to the drug or have severe anemia, liver dysfunction, and renal failure. As a sulfonamide analogue, it should be used with caution in patients with glucose-6-phosphate dehydrogenase deficiency.

28.1.2.2 Clofazimine

Also known under the name of B663, clofazimine is a riminophenazine dye with weak bactericidal activity against *M. leprae*. It has been used for the treatment of leprosy since the early 1960s. Although its exact mechanism of action remains unclear, the bacterial outer membrane appears to be the primary site of action for clofazimine, but other putative targets include the bacterial respiratory chain and ion transporters. In addition, it possesses some anti-inflammatory effects at a dose of 200–300 mg per day [8]. In vitro, it causes significant suppression of the mitogen- and antigen-driven proliferative responses of isolated T lymphocytes [9]. In humans, the absorption of clofazimine varies considerably (45–62%) depending on whether the drug is taken with or without food [10]. Oral ingestion of 100 mg results in an average plasma level of 0.7 mg/L. Because of its lipophilicity, clofazimine distributes into fatty tissues as well as the mononuclear phagocyte system. The major part of the absorbed drug is eliminated via feces, less with urine.

The drug is available in both 50 and 100 mg brown-colored capsules.

Except for reddish discoloration of the skin, clofazimine is generally well tolerated at the usual therapeutic doses, and no teratogenic effects have been reported so far.

28.1.2.3 Rifampicin

Used for the first time in the treatment of leprosy in 1968 [11], rifampicin (RMP) is a powerful bactericidal agent that acts by inhibiting the DNA-dependent RNA polymerase of microorganisms and thereby interferes with the synthesis of microbial RNA. Administered orally, it is rapidly absorbed in the digestive tract and has a wide tissue distribution [8]. Peak plasma levels are reached in 2–4 h. The minimal inhibiting concentration (MIC) for *M. leprae* is 0.3 mg/L. At a dosage of 600 mg, the maximum serum concentration is 30 times higher than the MIC. In addition, a single dose of 600–1500 mg kills 99% of viable bacilli within 3–4 days [12]. No difference in bactericidal activity has been demonstrated between RMP administrated daily and monthly [8].

Rifampicin is available in both 150 and 300 mg capsules and is given at a dosage of 10 mg/kg per day. The drug is partially eliminated in the urine, but almost two-thirds is eliminated via the gastrointestinal tract.

28.1.3 Other Anti-leprosy Drugs

Therapeutic research on *M. leprae* has greatly benefited from progress on treatments for tuberculosis. Many new active molecules currently available as second-line drugs for the treatment of leprosy are the result of the research on tuberculosis. They essentially belong to the fluoroquinolone (pefloxacin, ofloxacin, bedaquiline) [13], cycline (minocycline), macrolide (clarithromycin) groups [14], or derivates of rifampicin (rifapentine, rifabutin). Except in therapeutic trials, these molecules have rarely been used in practice, and trials to shorten the duration of treatment have sometimes been disappointing due to a high number of relapses, as in the rifampicin-ofloxacin combination trial in lepromatous patients in Bamako (Marchoux Institute) [15].

28.1.4 Therapeutic Scheme Recommended by WHO

The MDT regimen recommended by the World Health Organization [3] in 1981 consisted of rifampicin and dapsone for PB leprosy (Fig. 28.1) and of rifampicin, dapsone, and clofazimine for MB leprosy (Fig. 28.2). These regimens have now been superseded by the revised recommendations (uniform multidrug therapy, U-MDT) of all three drugs 6 months for PB and 12 months for MB [17]. The earlier dose regimen is currently in transition and will be subsumed by the new one once current stocks of blister packs are exhausted.

Potential advantages of the new recommendation are simplification of the treatment regimen, reduced impact of misclassification of leprosy cases (persons with MB leprosy incorrectly classified as PB leprosy), and simplified logistics since only two types of blister packs of drugs (adult and child) would be required (Fig. 28.3).

These regimens still require all patients with leprosy to be classified as either PB or MB according to the number of skin lesions that is easy to perform in the field, as compared with the bacteriological index that needs both skill and equipment that are not available in all settings (Tables 28.1 and 28.2).

MDT is provided free of cost and distributed in blister packs to national leprosy control programs by WHO in most endemic countries.

It should be noted that in children, dosages should be adjusted according to body weight. Also, in industrialized countries, daily multidrug therapy is used, which differs from that recommended by the WHO: RMP is taken daily and clofazimine is given at a dose of 100 mg per day. The duration of treatment in PB does not change, but MB patients are treated until bacteriological negativity is reached (Table 28.3).

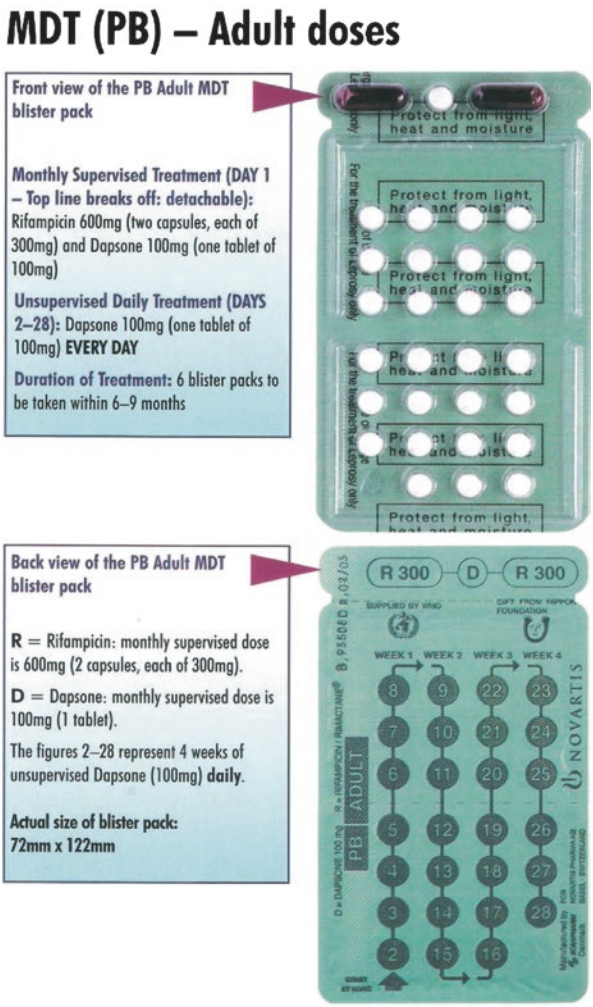

Fig. 28.1 One-month multidrug therapy (MDT) for adult paucibacillary (PB) leprosy; from McDougall and Yuasa [16] (with permission)

Studies comparing patients treated with uniform MDT (U-MDT) and regular MDT couldn't find statistical difference between the two groups regarding the risk of relapse, decrease in bacteriological index, the risk of developing leprosy reactions, and disability progression [18].

28.1.5 Other Therapeutic Schemes

The single dose of rifampicin (600 mg) plus ofloxacin (400 mg) and minocycline (100 mg) could be used to treat PB patients with a single lesion.

In addition, new treatment guidelines for drug-resistant *M. leprae* are available.

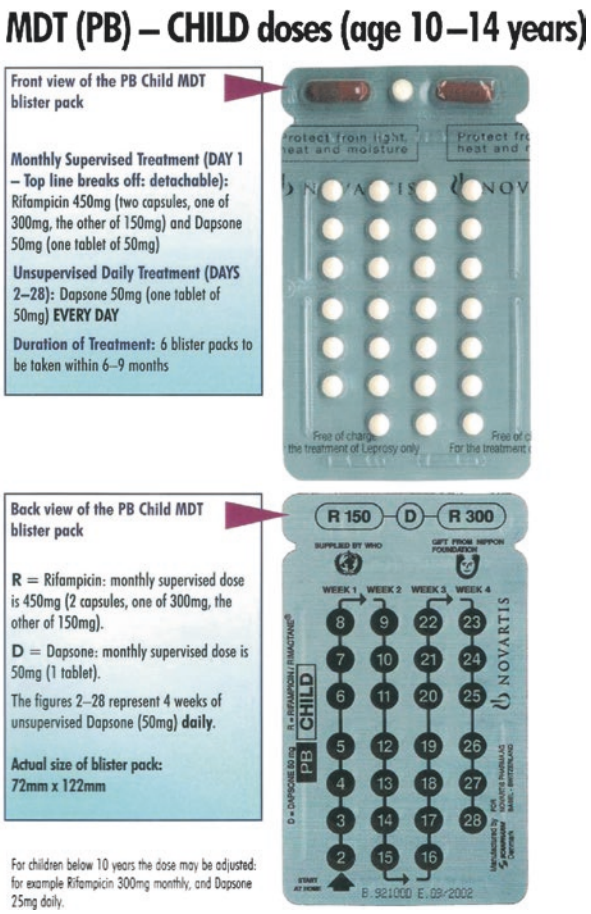

Fig. 28.2 One-month multidrug therapy (MDT) for child paucibacillary (PB) leprosy; from McDougall and Yuasa [16] (with permission)

The WHO **recommends** for leprosy patients with rifampicin resistance to be treated with at least two of the following second-line drugs: clarithromycin, minocycline, or a quinolone (ofloxacin, levofloxacin, or moxifloxacin), plus clofazimine daily for 6 months, followed by clofazimine plus one of the second-line drugs daily for an additional 18 months. In cases of rifampicin plus ofloxacin resistance, a quinolone should not be chosen; therefore, the recommended regimen is clarithromycin, minocycline, and clofazimine for 6 months followed by clarithromycin or minocycline plus clofazimine for an additional 18 months.

28.1.6 Surveillance

All patients during MDT should be monitored to detect drug-related side effects, nerve function impairments, and reactions. When available, a minimum biological assessment should be carried out before starting MDT: this includes a full blood

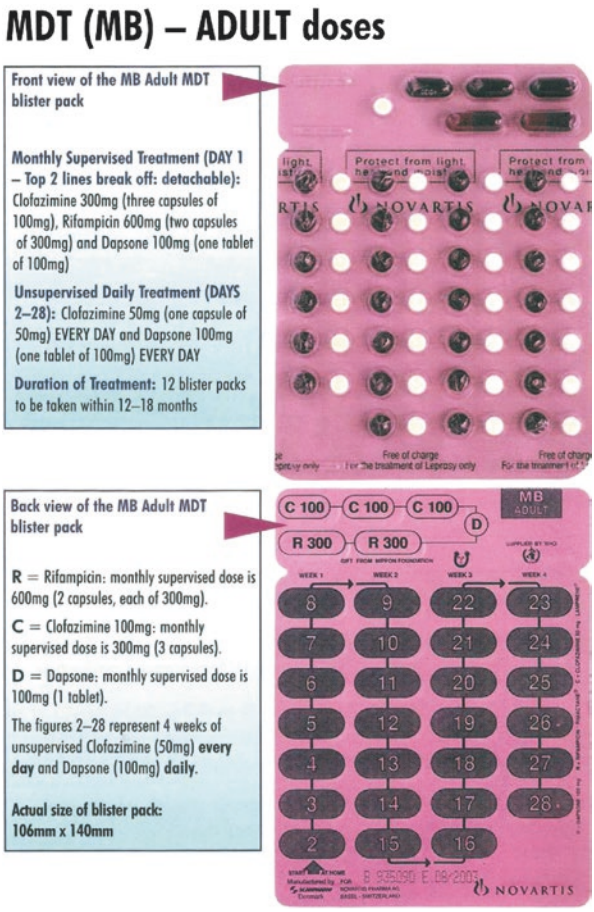

Fig. 28.3 One-month multidrug therapy (MDT) for adult multibacillary (MB) leprosy; from McDougall and Yuasa [16] (with permission)

Table 28.1 MDT regimen for paucibacillary leprosy as recommended by WHO in 1981

Drugs	Adults	Child (10–14 years)
Rifampicin	600 mg once a month	450 mg once a month
Dapsone	100 mg daily	50 mg daily
Duration	6 months (6 blister packs of 28 days each)	6 months (6 blister packs of 28 days each)

Table 28.2 MDT regimen for multibacillary leprosy as recommended by WHO in 1981

Drugs	Adults	Child (10–14 years)
Rifampicin	600 mg once a month	450 mg once a month
Clofazimine	300 mg once a month, and 50 mg daily	150 mg once a month, and 50 mg every other day
Dapsone	100 mg daily	50 mg daily
Duration	12 months (12 blister packs of 28 days each)	12 months (12 blister packs of 28 days each)

Table 28.3 WHO MDT scheme currently recommended for the treatment of leprosy [6]

Age group	Drug	Dosage and frequency	Duration	
			MB	PB
Adult	Rifampicin	600 mg once a month	12 months	6 months
	Clofazimine	300 mg once a month and 50 mg daily		
	Dapsone	100 mg daily		
Children (10–14 years)	Rifampicin	450 mg once a month (day 1)	12 months	6 months
	Clofazimine	150 mg once a month, 50 mg on alternate days		
	Dapsone	50 mg daily		
Children < 10 years or < 40 kg	Rifampicin	10 mg/kg once a month	12 months	6 months
	Clofazimine	100 mg once a month, 50 mg twice weekly		
	Dapsone	2 mg/kg/daily		

count, liver and renal function tests, blood sugar (in case steroid treatment is given), and G6PD activity.

Adverse effects of dapsone include skin manifestations (rashes, fixed drug reaction, urticaria, exfoliative dermatitis, erythema multiforme, toxic epidermal necrolysis) [19], hematological disorders (hemolytic anemia, methemoglobinemia, agranulocytosis), and gastrointestinal effects (abdominal pain, nausea, vomiting). Although rarely observed, a hypersensitivity syndrome is a severe adverse event which is associated with hematological changes, hepatitis, and mucosal involvement. Fatal outcome occurs in 10% of patients, but early withdrawal of the drug is associated with a better prognosis.

As a rule, dapsone is most often the drug implicated, and the most frequent side effects reported are fixed drug reactions and exfoliative dermatitis. It should be kept in mind that skin adverse events due to dapsone mostly occur from approximately the end of the first blister pack to the beginning of the second blister pack (i.e., from the last week of the first month to the first week of the second month of treatment).

To reduce the risk of dapsone-related hemolysis, some manufacturers combine this molecule with iron oxalate and folic acid.

Clofazimine is responsible for brown discoloration of the skin and ichthyosis, symptoms more visible in fair skin than in colored skin. The most serious toxicity of the drug affects the gastrointestinal tract and presents mild cramps or epigastric pain occasionally associated with nausea and vomiting. This usually occurs when higher doses than those used in MDT are given, for instance, in the treatment of reactions.

Beyond gastrointestinal disorders and acute hemolytic anemia, rifampicin can cause a “Flu syndrome” (flu-like syndrome) and generalized pruritus. Rifampicin is an enzyme inducer that may cause liver dysfunction, especially when it is associated with hepatotoxic drugs such as isoniazid or ethionamide. Hepatic insufficiency is a major contraindication to its use.

Post-treatment follow-up is needed to detect relapses that occur mostly after the fifth year and in particular in patients with high bacterial loads [20].

To ensure that treatment is conducted properly, the regularity and adherence to the treatment regimen must be checked. To achieve this, all patients at the outset of treatment must receive all the information necessary for understanding in order to demystify the disease and encourage patients to follow the instructions.

28.1.7 Results

MDT is, to date, the most effective treatment for leprosy. Several weeks to a few months following the initiation of treatment, the skin lesions will become less infiltrated, the plaques and nodules tend to sag into wrinkles, while skin folds are more visible especially on the face in the lepromatous patient. In PB patients, pale patches may persist for months or years after the treatment is completed. The decrease in bacillary index is correlated with the bacterial load of the patient. It is less rapid in MB where there is a high initial bacillary index when compared to those with low BI. The decrease is about 0.6–1 log per year, and negativity is obtained within 4–5 years in patients with BIs of 5+ on the Ridley scale. After 2 years of MDT, skin smears are still positive in a significant proportion of MB patients when the treatment is stopped. As a result, the demonstration of acid-fast bacilli in skin smears alone is no longer sufficient for the diagnosis of relapse [20]. The risk of relapse is usually very low in field conditions and less than 1% according to WHO. However, in a study conducted at Marchoux in 35 mb patients treated with MDT, 7 patients relapsed after a follow period of 5–7 years. The seven relapses occurred in the group of patients with an average BI >4.0 before MDT (18 cases) as compared to the group with an average BI <4.0 where no relapse was found [20].

This study suggested that the relapse rate is closely correlated with the bacterial load, and relapse is significantly more frequent among patients with a BI of 4.0 before MDT or a BI of 3.0 at the end of MDT.

This clearly shows the need to follow up all patients after MDT is completed for a period of time ranging from 2 for PB to more than 5 years for MB especially those with high bacillary index before and after MDT.

28.2 Treatment of Complications

28.2.1 Treatment of Reversal Reaction

Editor note: The treatment of reactions is dealt herewith and in Chap. 21 "Reactions in Leprosy". The chapters represent two approaches, both largely used, to the management of these complications.

The treatment of leprosy reaction has been recently updated with the release of new guidelines by WHO [21]. An algorithm for the detection and management of reversal reactions (RR) and neuritis was also developed. Oral steroids remain the main choice for the treatment of RR and neuritis. The precise steroid regimen used to treat reactions and neuritis continues to be debated, both in terms of dose and duration. It is best to start as soon as possible after detection of the reaction, at a dose of 1 mg/kg body weight, and gradually decrease as symptoms improve. The WHO guidelines recommend a steroid

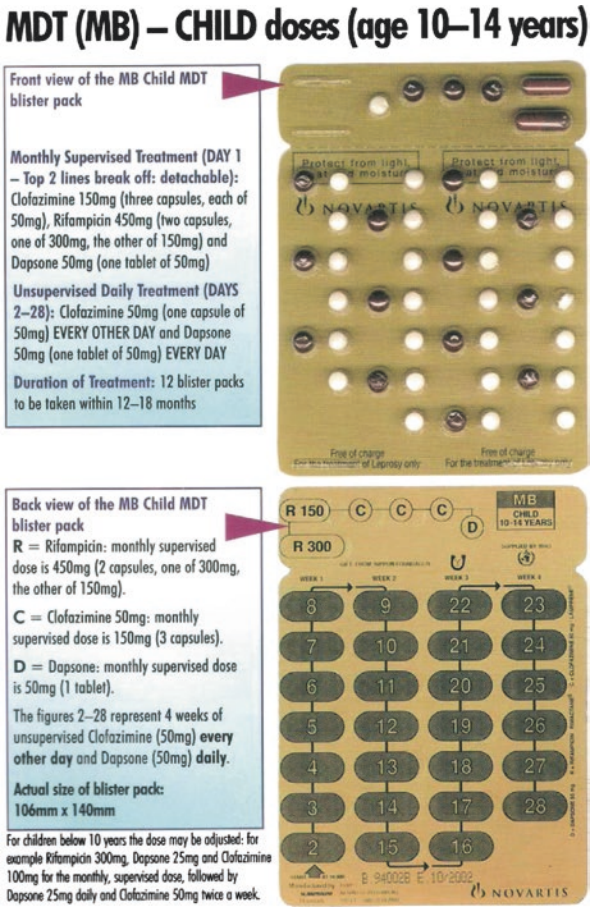

Fig. 28.4 One-month multidrug therapy (MDT) for child multibacillary (MB) leprosy; from McDougall and Yuasa [16] (with permission)

Table 28.4 Course of steroids recommended for the treatment of reversal reaction [21]

Drug	Dosage	Duration
Prednisone	30 mg/day	2 weeks
	25 mg/day	2 weeks
	20 mg/day	8 weeks
	10 mg/day	4 weeks
	5 mg/day	4 weeks
Total		20 weeks

course of 20 weeks with a starting dose of 30 mg/day (Fig. 28.4). Most reactions subside over a period of weeks or months, but they sometimes recur and become more chronic. In all cases, rest is essential as well as detection and correction of any triggering factors (stress, infection, pregnancy, immunization, etc.).

The treatment of mild reactions should be limited to the prescription of analgesics: aspirin or paracetamol or nonsteroid anti-inflammatory drugs (Table 28.4).

In cases of treatment failure or in order to spare cortisone, especially in obese and cortico-dependent subjects, other medications can be used: azathioprine (3 mg/kg/day), cyclosporine (5 mg/kg/day), methotrexate, or tacrolimus (in topical form). Clofazimine seems to have some effect.

The risk of side effects due to the long-term use of steroid should be kept in mind. The basic principles of treatment and accompanying measures should be respected: these include albendazole use or stool examination for parasites, cysts, and eggs, blood sugar, and detection of latent infection or comorbidity.

28.2.2 Treatment of Erythema Nodosum Leprosum

Treatment depends on the severity of the episode and should control the inflammation and pain. The ENLIST group (global consortium to improve understanding and treatment of ENL) has contributed to developing studies of ENL. According to the severity score, ENL is classified into three types: mild (score > 8), moderate, and severe (score > 8).

Mild ENL cases are managed with analgesics (aspirin, indomethacin, ibuprofen, diclofenac, acetaminophen, tramadol) [21] and pentoxifylline (slow-acting drug, needing 30–60 days). If there is worsening and an increase in the score to >8, ENL should be treated as "severe" and managed accordingly.

Thalidomide remains the treatment of choice for severe ENL. Unfortunately, this chemical is no longer available, except in few specialized centers. Moreover, its teratogenicity and the risk of highly dose-dependent neuropathy limit its use [22]. Thalidomide also passes into the seminal fluid. The following treatment scheme has been used at the Marchoux Institute for more than 50 years: four tablets (100 mg each) in two doses morning and evening for 3–5 days and then decreased by 100 mg/week for a total duration of approximately 4–6 weeks.

To date, steroids are the first-line treatment for moderate to severe ENL. The recommended starting dose is 30–40 mg per day. In patients who do not respond or in cases where there is a poor response to steroids, the dose may need to be increased up to 40–60 mg per day depending on the body weight and then gradually reduced if symptoms improve. The treatment duration is 20 weeks. Given the risk of mortality secondary to the use of steroids, patients should be monitored every 2 weeks and principles of use of steroid therapy strictly followed.

Of interest, clofazimine is an alternative for the treatment of chronic ENL. The treatment regimen starts with 200–300 mg per day for 1–2 months followed by 200 mg per day for 2 months and 100 mg per day for additional 2 months, and the overall duration is 4–6 months. Patients should be monitored for clofazimine-related side effects (abdominal pain, diarrhea).

Iridocyclitis isolated or associated with ENL is managed with topical ophthalmic steroids.

Other drugs have been used as second-line treatments for ENL in a limited number of patients. These are pentoxifylline, methotrexate, cyclosporine, and azathioprine. TNF alpha inhibitors (e.g., infliximab, etanercept) and apremilast have also been used (Table 28.5).

Table 28.5 Course of steroids recommended for the treatment of ENL [21]

Drug	Dosage	Duration
Prednisolone	40 mg/day	2 weeks
	30 mg/day	2 weeks
	20 mg/day	4 weeks
	15 mg/day	4 weeks
	10 mg/day	4 weeks
	5 mg/day	4 weeks
Total		20 weeks

28.2.3 Treatment of Plantar Leprosy Ulcers

Management of plantar ulcers is hospital-based and requires long in-patient stays. Achieving and maintaining the healing of a plantar ulcer is an objective which is difficult to obtain, hence the importance of prevention. The treatment consists of:

- Rest and suspension of weight bearing by the installation of a device (plated insole that can support the weight of the body) to avoid pressure when walking.
- Daily local care with antiseptics such as Dakin and dilute potassium permanganate.
- Regular removal of the callus at the edges where epidermal proliferation occurs.

An X-ray is necessary to detect osteitis which can delay the healing process.

28.2.4 Surgical Treatment

The role of surgery in leprosy has been considerably reduced in recent years as the result of improvements in care, better surveillance, and intensification of Prevention of Disabilities and Physical Rehabilitation through national programs for the control of leprosy.

The surgery covers the neurological complications of leprosy, either by direct intervention on the nerve itself or on the complications of neuritis (paralysis, amyotrophy, trophic change) [23].

The intervention on the nerve called neurolysis involves freeing the compressed nerve in the bone canal by making a longitudinal incision through a thickened nerve sheath, which results in decompression and therefore pain relief.

Palliative surgery is aimed at restoring most of the movement that has been impaired or lost. In the hands, it involves correction of claw deformities by tendon transfer, capsular shortening, and arthrodesis. On the feet, interventions include correction of drop foot by anterior transposition of the posterior muscles not affected by neuropathy (Technique de Carayon). On the face, the correction may concern the anterior nose, and in the case of lagophthalmos, partial blepharoplasty or palpebral reconstruction by temporal musculoaponeurotic transfer can be performed.

Rehabilitation is an essential complement to surgery; it allows the patient to recover. It involves wearing orthopedic shoes with microcell rubber, daily care of hands and feet, and physiotherapy.

28.3 Prophylaxis

Segregation of patients affected by leprosy, as in the Middle Ages, is today regarded as both unnecessary and inhuman. Initiation of MDT as early as possible, contact tracing, and examinations for leprosy are the mainstay of disease prevention [24]. After a single dose of rifampicin, some contacts of patients are considered noncontagious because 99.99% of viable bacilli are killed.

28.3.1 Prevention of Leprosy Through Chemoprophylaxis

The WHO GDG **recommends** the use of single-dose rifampicin (SDR) as preventive treatment for contacts of leprosy patients (adults and children 2 years of age and above), after excluding leprosy and tuberculosis, and in the absence of other contraindications.

Studies have shown that SDR in leprosy contacts is associated with a reduction in risk of leprosy of 57% over 2 years and of 30% over 5–6 years [25]. For every 1000 contacts treated with SDR, there were four leprosy cases prevented after 1–2 years and three cases prevented after 5–6 years. The protective effect of SDR occurred in the first 2 years, with no additional effect after 4 and 6 years [26]. However, the total impact of the intervention remained statistically significant after 6 years, but it is not effective in contacts of patients with multibacillary forms of leprosy. Administration of SDR is questionable. In a study performed at Marchoux Institute (Bamako, Mali), administration of SDR of 1500 mg could neither prevent relapse nor reduce its rate among multibacillary patients who had already become clinically and skin-smear negative after dapsone monotherapy: 15 relapses occurred in 136 patients and the risk of relapse was 2.1 per 100 patients-years [27].

Finally, the question remains as to whether post-exposure prophylaxis as recommended by the WHO is really feasible in all settings and above all effective over time.

28.3.2 Prevention of Leprosy Through Immunoprophylaxis (Vaccines)

There is evidence that BCG vaccination can prevent leprosy. Surprisingly, it is widely given at birth in most developing countries where leprosy is still endemic. In Malawi, a randomized vaccine trial with BCG alone or in combination with strains of *M. leprae* showed protection of around 50%. The combination of *M. leprae* with BCG does not increase protection. On the other hand, this protection is maximal if the vaccination takes place before the age of 15 years [28]. To date,

there has been no WHO recommendation for the use of BCG as a preventive tool, but immunization should be maintained, at least, in all leprosy high-burden countries or settings.

28.4 Conclusion

MDT has reduced the number of cases on treatment but has had less impact on the number of new cases that have remained stable over the past decade. Early detection of new cases remains the major concern of most national programs for leprosy; it requires frontline healthcare workers being trained in the management and recognition of neglected tropical skin diseases or skin NTDs as well as common skin diseases including early signs of leprosy, contact tracing, and treatment with MDT. Achievement of these targets is needed to meet the goal of Global Leprosy Strategy for the period 2021–2030 [29]. Engagement of dermatologists and persons affected by leprosy will be helpful.

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Prognosis of Leprosy

29

Enrico Nunzi

World Health Organization (WHO) multidrug therapy (MDT) [1] is strongly effective against *Mycobacterium leprae* (*M. leprae*) and can easily reduce and eliminate bacterial load. However, global leprosy management goes further than just killing bacilli.

In this disease with bacterial etiology, immunity plays an important role, not only in the passage from infection to disease and its expression in different forms but also in the development of leprosy reactions, which cause peripheral neuropathies and disabilities.

Today, drugs with strong bactericidal and bacteriostatic action against *M. leprae* are available, but it is still hard to manage leprosy reactions, which affect prognosis and even today remain the major problem of leprosy.

Prognosis can only be made after having exactly determined the patient's disease form; borderline leprosy patients risk developing leprosy type 1 reaction and therefore neuropathies/disabilities, while lepromatous leprosy patients (BL, LL) can develop immunocomplex pathologies (leprosy type 2 reaction) which can harm nerves, joints, eyes, kidneys, and testicles.

29.1 Indeterminate Form

In indeterminate leprosy there are neither leprosy reaction episodes nor peripheral nerve involvement. For this reason, prognosis is clear. If diagnosis is made at an early stage, therapy prevents disease evolution towards the borderline part of the spectrum.

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29.2 Tuberculoid Form

The tuberculoid leprosy form is hyperergic and stable, can heal spontaneously, and responds very well to therapy. In some cases, it can damage a peripheral nerve and also create abscesses.

29.3 Borderline Group

Patients with most damage to peripheral nerves are in the borderline group. In the BT form, there is interaction between CMI and bacillary load, which creates the worst damage to peripheral nerves, requiring careful quoad valetudinem prognosis. The BT form of leprosy is usually classified into the paucibacillary forms.

BT patients with numerous lesions (according to the WHO, more than five cutaneous lesions) with tendency for symmetric distribution must be examined from a bacteriological point of view. Presence of bacilli in lesions requires the multibacillary regimen to avoid relapses.

Borderline patients, especially those located in the central part of the borderline spectrum (BB), can easily experience leprosy type 1 reactions, with heavy damage to peripheral nerves. In absence of treatment, these patients move towards the lepromatous pole.

These patients must be frequently checked, monitoring the function of peripheral nerves to identify the first signs of nerve damage, whose appearance requires rapid therapy revision.

The BL form, if untreated, may move to the lepromatous pole, adopting the clinical features of lepromatous subpolar leprosy (LLs).

29.4 Lepromatous Form

The lepromatous leprosy form has high bacillary load. MDT together with early diagnosis has resolved the "bacterial problem."

Advanced LL is characterized by infiltration of body areas with anesthesia and by bacillary infiltration of upper respiratory tract, nasal pyramid collapse, palate perforation, and larynx obstruction causing hoarseness. These pre-MDT clinical aspects should belong to the past.

Leprosy type 2 reaction (erythema nodosum leprosum) is still difficult to keep under control even today. These inflammatory episodes are caused by immunocomplexes which can lead to glomerulonephritis that determines prognosis. If early diagnosis is made and correct therapy applied to stop leprosy type 2 reaction episodes, prognosis should be good.

In women affected by leprosy, physiological episodes (delivery, breast-feeding) must be carefully monitored [2]. Events such as vaccination and viral disease can worsen leprosy, especially in peripheral nerves, even in an asymptomatic way (silent neuritis).

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Neurolysis in Leprosy

30

Alberto Balestrino and Sergio Gennaro

30.1 Neuropathies Caused by Compression or Entrapment

A key feature of leprosy is a selective involvement of the peripheral nervous system. This is due to the particular neurotropism of *Mycobacterium leprae* [1], the causative agent of the disease, and represents a unique peculiarity in the microbial world. *M. leprae* penetrates and develops in peripheral nerves, causing nerve damage, the main cause of disability in leprosy. The motor-sensory branches and autonomic section of the peripheral nervous system can both be involved. The clinical manifestations of nerve damage may have early onset in the hyperergic forms, where damage is caused by the immune response of the host, or tardive in the anergic forms, where damage is due to multiplication of the bacillus in the nerve.

In general, the origin of the compressive or entrapment neuropathy can be extrinsic or intrinsic.

Extrinsic compressive neuropathy is caused by tissues contiguous to the nerve such as large callus, thickened sclerous synovial capsule, hypertrophic muscular mass, anomalous muscular-tendinous insertions, persistence of fibrous shoots, and presence of contiguous neoformations. Intrinsic compressive neuropathy is caused by presence of a localized, primitive or secondary, neoformation of the nerve or by an excessive hypertrophic reaction which involves all the structural parts of the nerve with a pronounced increase in its volume. The latter is typical of infectious forms and therefore also of leprosy.

In the entrapment neuropathy seen in leprosy, the physiologic and anatomic relationship between the container (the anatomic tunnel structure) and its content (the nerve) is modified. The nerve, increased in volume due to the disease, suffers at the

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level of one or more anatomical narrowings as anatomic tunnels, with consequent functional damage. The severity of the nerve damage depends on the speed of onset, on duration of symptoms, and on degree of intra- and extraneural anatomic involvement. In order to better understand nerve damage in leprosy, it has to be noted that peripheral nerve fibers are more vulnerable than central nerve fibers.

Nerve inflammation leads to development of edema with subsequent increased pressure within the fibro-osseous tunnels and obstruction of the vasa nervorum that worsens the nerve damage with hypoxic and ischemic mechanisms. Damage can be selective and limited to some fascicles in relation to the anatomic integrity of the intraneural fascicle structure. Large nerve fascicles surrounded by thin epineurium are more vulnerable compared to smaller nerve fascicles, which are surrounded by plentiful perineurium.

30.2 Neurolysis

Neurolysis is a surgical procedure performed to free the nerve from compression, either extrinsic or intrinsic, that hinders or blocks its functions without physically interrupting the fascicles.

Extrinsic compression is created by hypertrophic tissue surrounding the nerve that may derive from traumas that did not directly damage the nerve but led to formation of hypertrophic cicatricial tissue. Intrinsic compression is usually a consequence of repeated nerve friction against rigid structures (e.g., tendons, aponeurotic edges, bone prominences) to which a nerve can be subjected during movements of the limb within which it runs. More rarely, injection of irritant drugs directly into the nerve may cause interstitial fibrosis.

Neurolysis can be distinguished into external or internal. External neurolysis consists in freeing the nerve trunk from external reactive-inflammatory-cicatricial tissue. This is done following a precise surgical technique. At first the healthy nerve is identified proximal and distal to the lesion. Then the functional integrity of the nerve is evaluated by direct electrical stimulation proximal to the scar in order to determine whether the relevant muscles contract or not. A lack of contraction indicates that there is also intrinsic damage, and therefore neurolysis would be useless. In this case resection of the damaged tract and restoration of nerve continuity may be indicated. On the contrary, signs of muscle contraction indicate that immature axons have reached the periphery and that the nerve should be preserved. In the (far from rare) case where the scar is crossed by undamaged collateral nerve branches, emerging proximally, direct stimulation is useful to identify and respect them.

At this point the nerve is freed from compression, and an anatomic bed made of soft tissue (e.g., adipose tissue or interstice between two unhurt muscle bellies) has to be created. Particular care should be taken during preparation of the anatomic bed with blunt dissection and the least possible damage to the tissues in order to prevent the formation of a harmful scar. It has been proven that covering the freed nerve with muffs of various materials not only does not provide benefits but may also be

harmful. Whichever material is used, it tends to stimulate formation of fibrous tissue, thus creating the opposite to the desired effect [2].

Internal neurolysis (also called interstitial neurolysis) is a nerve decompression technique that consists in microsurgical removal of the fibrous tissue within the nerve. Considering the significant risks of nerve damage and the risks of postoperative fibrosis that prevents nerve healing, this technique is nowadays mostly a historic procedure being rarely performed only in very selected cases [3]. In any case it is nowadays mandatory to perform such procedure under microscopic view, avoiding performance with naked eye.

Alternative procedures like attempting to free the nerve fascicles by forcefully injecting a physiologic solution into the nerve and cutting the epineurium are useless or even harmful.

30.3 External Neurolysis in Leprosy

External neurolysis in leprosy can be performed with either therapeutic or preventive purposes [4–7]. Therapeutic neurolysis is performed to decompress a nerve which lies in a narrowing; this can be required during the acute edematous phenomenon of type 1 leprosy reaction (T1R or reversal reaction) or for chronic cicatricial phenomena.

It is important to remember that decompressive surgery for leprosy compression neuropathy should always be performed concomitantly to corticosteroid therapy in order to reach the maximal therapeutic effect.

Preventive neurolysis is suggested in immunologically unstable leprosy patients as those affected by borderline forms, who frequently suffer from T1R. Preventive opening up of the tunnels of nerves at higher risk of involvement prevents future acute inflammatory reactions from causing irreversible damage. Additionally, a comprehensive evaluation of patients by means of clinical examination and radiological and neurophysiological studies allows to determine subclinical or mildly symptomatic nerve compressions that will benefit from decompression surgery.

External neurolysis opens the anatomic bottleneck in which the nerve is compressed and also directly treats the cause of the compression by removing the inflammatory reactive tissue covering the nerve trunk. The sleeve compression caused by inflammatory reactive tissue determines a hypoxic-ischemic condition that worsens nerve's edema and increases its volume, thus forming a vicious circle.

Detailed informations for performance of the correct surgical procedure are provided by echography and nuclear magnetic resonance (NMR) studies. These diagnostic tests provide detailed imaging of peripheral nerve trunks highlighting the respective fascicles and interstice, thus allowing a precise determination of the extent and location of compression and fibrosis.

Therapeutic and preventive neurolysis prevent irreversible nerve damage (motor, sensory, and vegetative impairment according to the nerve and nerve fibers involved) and allow prompt regression of symptoms [8].

Clinical assessment of the patient including sensory and motor testing and electrophysiological [9], echographic, and NMR studies of the nerves most frequently involved in leprosy (ulnar, median, common peroneal, and posterior tibial nerves) provide necessary parameters for identification of patients and nerves at risk of neuropathy, thus being candidates for early surgery. At present in leprosy, decompression surgery by means of external neurolysis is usually performed at the cubital (Fig. 30.1), carpal (Fig. 30.2), and tarsal (Fig. 30.3) tunnels.

Nerve decompression associated with external neurolysis is a simple surgery performed under local anesthesia with low morbidity and complication rates [10]. It

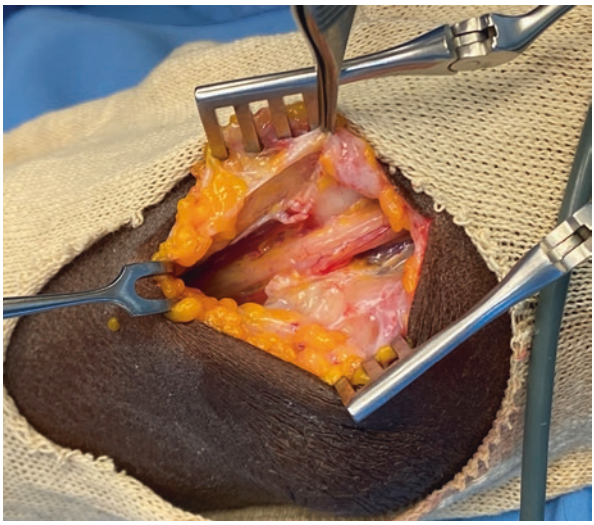

Fig. 30.1 Ulnar nerve: external neurolysis at the cubital tunnel

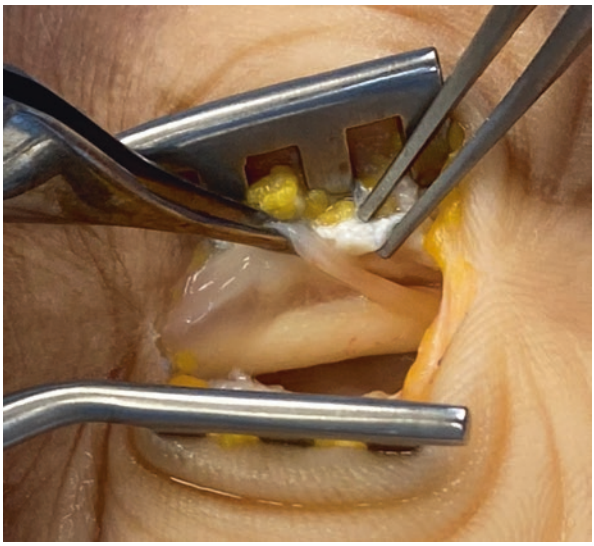

Fig. 30.2 Median nerve: external neurolysis at the carpal tunnel

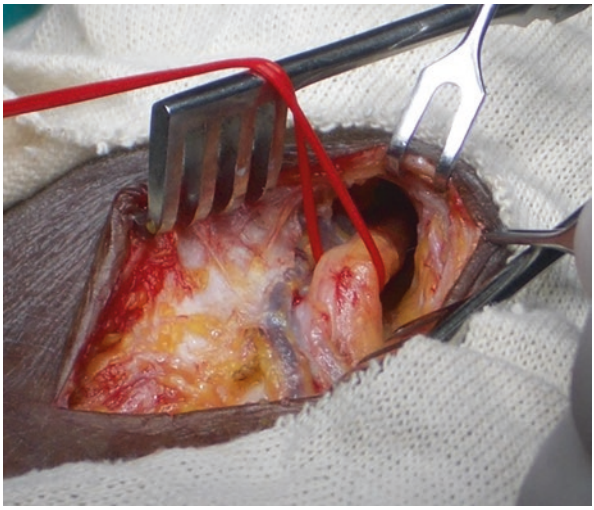

Fig. 30.3 Posterior tibial nerve: external neurolysis at the tarsal tunnel

both treats and prevents nerve compression and leads to significant improvement of the symptoms of the neuropathy and does not impact on patient's normal life activities.

30.4 Nerve Abscesses in Leprosy

In cases in which the nerve compression derives from an endoneural lepromatous abscess, a variation of the aforementioned internal neurolysis technique is indicated. Endoneural abscesses in leprosy are rare, but their recognition is critical for correct planning of the procedure [11]. A misdiagnosis may lead to an incorrect treatment that will not result in optimal outcomes.

Treatment of choice is surgical excision of the abscess.

The level of the nerve involved by the abscess is pre- and intraoperatively determined with the aid of NMR and echography imaging. Once the level of abscess is correctly located, the nerve is exposed and epineurotomy is performed. Gentle dissection of fascicles is needed until the abscess is found (Fig. 30.4). Lepromatous abscesses usually present as roundish caseous masses. At this point the abscess is gently dissected from the surrounding fibers and then excised.

Decompression of the nerve by surgical excision of the abscess allows relief of symptoms and improvement of patients' neurological function [11].

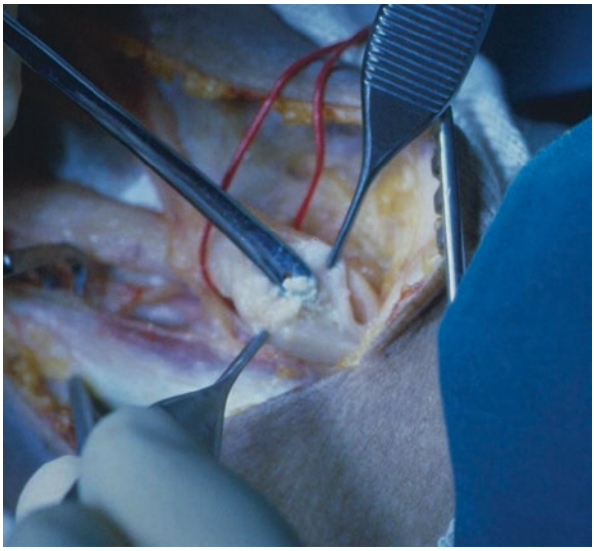

Fig. 30.4 Common peroneal nerve: surgical excision of an endoneural abscess

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Prevention of Disability and Ulcer Care in Leprosy

31

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In the first section of this chapter, a theoretical framework of disability is presented, with definition of terms, etiology, and dimensions of disability.

The second part of this chapter discusses in more detail the pathogenesis and management (prevention and care) of the most common secondary impairment in leprosy-affected people: ulcers.

Before discussing the various known health problems that may result from leprosy, we want to emphasize that the key to prevention of physical and mental well-being consequences of the disease is in an early diagnosis of the disease and adequate treatment of its complications (Chaps.10, 13, 15, 16).

31.1 Definitions and Introduction to ICF

In the International Classification of Functioning, Disability and Health (ref. ICF-WHO. [1]), disability is considered the umbrella term for impairments, activity limitations, and participation restrictions caused by a health condition (Fig. 31.1).

Impairments are changes, deviations, or losses in body function or structure. Some examples in leprosy are areas of skin with loss of sensation, eyes with vision loss, hands and feet with muscle weakness or paralysis and sensory loss, joint contractures, ulcers, and decrease in mental well-being and depression.

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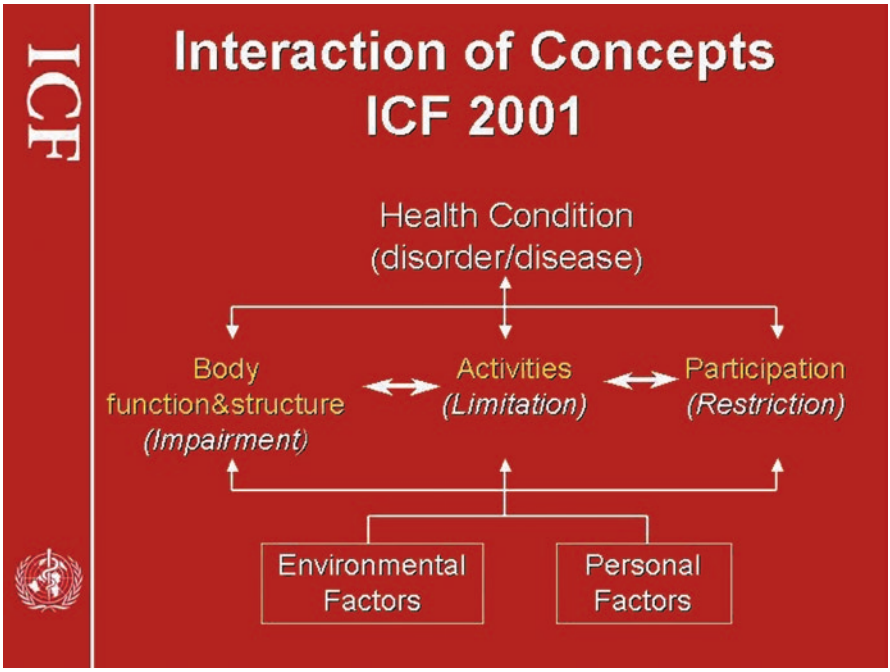

Fig. 31.1 Interaction of concepts. (From "ICF—International classification of Functioning, Disability and Health" [1])

Activity limitations are difficulties an individual may have in executing activities. Examples in leprosy are difficulty reading, writing, doing personal care, walking, or preparing meals as expected.

Participation restrictions[1] are problems an individual may experience being involved in life situations due to societal beliefs, values, laws, or others. Persons affected by leprosy may not be allowed to get married, to attend school, to work, or to participate in family and community activities.[2]

Functioning and disability can be influenced negatively or positively by environmental and personal factors. The physical, cultural, and attitudinal environment can impede or facilitate inclusion and participation of people affected by leprosy.

Environmental factors may include social beliefs and attitudes about leprosy and accessibility of the physical (geographical) environment for people with mobility or visual problems.

Personal factors include factors such as age, gender, self-esteem, poverty, level of education, and level of coping skills.

[1] Some prefer the term participation "problem" rather than "restriction" because problem indicates that intervention(s) might be helpful rather than restriction which sounds more permanent situation.

[2] See for more information on the ICF, the ICF itself, educational tools, PowerPoint presentations, etc.; see the WHO website: www.who.int/classifications/icf/en.

The aim of a comprehensive assessment is to prevent the furthering of disability and restore people affected by leprosy to their fullest potential. It is essential to have a complete picture of the presence, the extent, and the severity of the effects of the disease on the person within their environmental and personal context.

31.2 Prevention of Disability

Four important factors contribute to disability as defined above:

1. Direct "leprous" infiltration of skin (nodules) and of the face/eyes/nose/ears. These impairments are visible in advanced stages of lepromatous leprosy. Early diagnosis and adequate treatment should prevent or limit the extent of these eye, nose, and skin complications (see Chap. 6).
2. Nerve function impairment (NFI) with or without leprosy (immune system) reactions. The main cause for "disability" is nerve function impairment. The key to prevention of NFI is early diagnosis and treatment of the disease (MDT) and an early recognition of leprosy reaction and NFI accompanied by adequate treatment of the leprosy reaction and NFI with prednisolone when it is detected (see Chap. 21). Leprosy reactions, especially ENL, can be very chronic and debilitating, affecting activities and participation.
3. Societal beliefs and attitudes (stigma and discrimination). The adverse effects of societal beliefs and attitudes primarily result in participation problems and exclusion (Chaps. 7 and 32).
4. Emotional. People affected by leprosy and their families can experience anxiety, depression, and mental distress. Timely interventions with peer support groups, informal counselling, or referral to a specialist can improve mental well-being and resilience.

Chronic nerve pain can be very debilitating in people having (had) reactions and can in some people persist for years post-treatment. Neuropathic chronic pain usually responds better to drugs such as tricyclic antidepressants and anticonvulsants vs prednisolone.

An overview of primary and secondary impairments that may result from nerve function impairment is presented in Fig. 31.2.

31.2.1 Prevention of Nerve Impairments

Most of the impairments, activity limitations, and participation restrictions that occur in leprosy-affected people can be attributed to immune system reactions and nerve function impairment (NFI). Regular nerve function assessment (NFA) to detect early loss is essential at time of diagnosis and routinely assessed after diagnosis (ref. Nerve workshop).

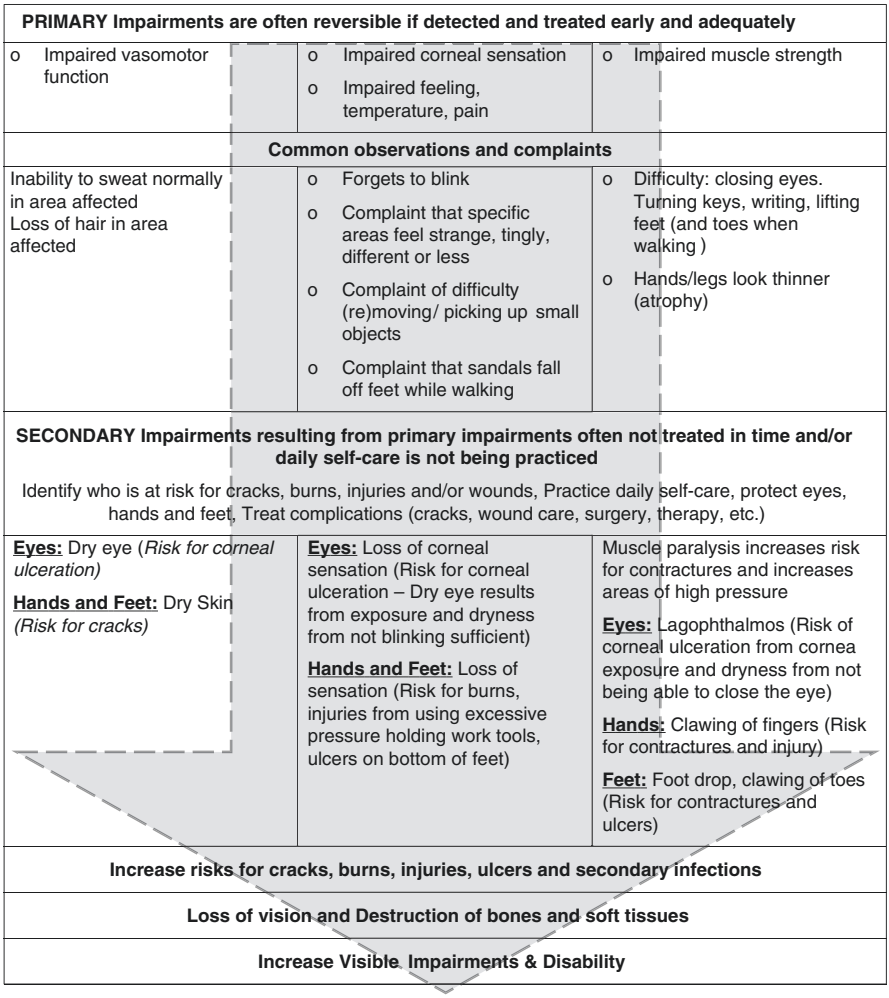

Fig. 31.2 Peripheral nerve impairments in Hansen's disease/leprosy

Timely diagnosis of NFI and appropriate treatment can reverse or prevent permanent nerve function loss. Nerves that are at risk are summarized in Fig. 31.3 (of Nerves 3 × 3).

The primary impairments are motor, sensory, and autonomic. This results in muscle weakness (or paralysis); loss of tactile, temperature, and protective sensation; loss of sweating and skin dryness. The tools used to assess and evaluate peripheral nerve function are manual muscle strength testing and sensory testing.

- Muscle strength testing:

  Four out of nine nerves that are most affected in leprosy have an important motor function. These are the facial, ulnar, median, and common peroneal nerves. Each nerve is evaluated by asking the person to do a specific movement. When

Nerve	*When impaired…..*		*VMT*	*ST*	*Palpation*
Facial	Lagophthalmos		Y	-	-
Trigeminal		Impaired corneal sensation	-	Y	-
Great Auricular	No significant loss		-	-	Y
Ulnar	Claw-fingers	Impaired sensation	Y	Y	Y
Median	Loss of thumb opposition	Impaired sensation			
Radial	Drop wrist		Y	1	Y
Comm. Peroneal	Drop foot	Impaired sensation	Y	2	Y
Posterior Tibial	Claw toes	Impaired sensation	3	Y	Y
Sural	No significant loss		-	4	(Y)

1. Radial nerve supplies sensation to the dorsal side of the thumb and the thumb web space. Not routinely tested.
2. Common peroneal nerve supplies sensation to the dorsum of the foot. Not routinely tested
3. Posterior tibial nerve supplies all intrinsic muscles of the foot. Not routinely tested.
4. Sural nerve is a sensory nerve supplying the lateral border of the foot. Not routinely tested.

VMT: Voluntary Muscle Testing; ST: Sensory Testing;
Y: yes; (Y) Sural nerves can be palpated but in actual clinical parctice usually not done

Fig. 31.3 Nerves in leprosy (3 × 3)

this movement is completed, the examiner will apply pressure against the movement and grade the resistance. The following nerves are tested by doing the specific movements below.

- *Facial nerve*: eye closure.
- *Ulnar nerve*: abduction of the little finger.
- *Median nerve*: abduction of the thumb.
- *Common peroneal nerve*: dorsi-flexion of the foot. (Sometimes there may be weakness in great toe extension without weakness in dorsiflexion of the foot.)
- The *radial nerve* is rarely affected, and when involved, it is usually obvious (drop wrist).
- The *posterior tibial nerve* is very often involved, but, in most programs, only the sensory function is assessed. A reliable test for motor function of this nerve is available [2].

• Sensory testing:

The trigeminal, ulnar, median, and posterior tibial nerves have important sensory functions. The loss of protective sensation is an important risk factor for unrecognized injury, wounds, and ulcers that are secondary impairments.

Sensation is preferably tested by monofilaments (ref. NF workshop and Textbook).

The WHO does not recommend testing the sensation of the cornea of the eye.

However, if corneal sensation is absent (trigeminal nerve), the person will not feel the need to blink. A careful observer may be able to conclude that there is not sufficient protective sensation of the eye, if there is no regular blink, even though closure may be partial resulting from muscle paralysis (facial nerve). The ability for the eye to move sufficiently upward helps protect and clean the cornea when blinking (Bell's phenomenon).

Persons with red and/or painful eyes with or without recent loss of vision should immediately be referred for a detailed eye assessment.

Preservation of eyesight is critical for persons with loss of protective sensation of hands and feet. They must use their vision to help maintain and improve hand and foot function and protect them from injury during daily activities.

31.3 Prevention of Secondary Impairments: Contractures and Wounds/Ulcers

Difficulties closing the eyes due to muscle paralysis (facial nerve) result in greater cornea exposure and dryness during daily activities and when sleeping at night. This increases risks for corneal injuries and ulcerations.

Forgetting to blink also causes corneal dryness and increased risk for secondary impairments of corneal ulcers which can lead to loss of vision.

Muscle paralysis may result in imbalance in specific joints in leprosy-affected persons.

The common paralytic impairments in the upper extremity are claw fingers (ulnar nerve) and loss of opposition (median nerve).

In the lower extremity, a foot drop (common peroneal nerve) and claw toes (posterior tibial nerve) may happen. This imbalance causes increased pressures on specific areas of the hand and foot during work and walking, and when combined with the loss of protective sensation, it can lead to a higher risk for injury and ulcers.

31.3.1 Skin Care

The combination of sensory loss and dry cornea or skin increases risks for cracks and ulceration.

A daily routine of soaking hands and feet with loss of sensation is needed to hydrate the skin and keep the skin soft and flexible. Soaking and oiling the skin should precede the exercises or could be done as part of some exercises.

Maintaining the skin in good condition and maintaining joint mobility will reduce the risk of developing cracks and ulcers. If reconstructive surgery is available, the patients with paralyzed but mobile joints could be referred for specialized surgery and rehabilitation (Chap. 32).

31.3.2 Protection

When there is sensory loss and paralysis, extra care is needed to protect the eye, hand, and feet.

It requires daily self-care practices that start with inspection of the eyes, hands, and feet.

If a crack or wound is noticed, immediate action is needed to rest and protect the affected area.

In addition, some people may need to use protective glasses during the day and night to prevent corneal dryness and prevent debris from entering the eye causing injury.

Injuries in hands with loss of protective sensation can be prevented or reduced by cushioning work tool handles, using gloves when cooking.

Feet with sensory loss require adequate protective footwear which are frequently checked for foreign objects and checked for need of repair or replacement. It is important to learn to choose acceptable protective footwear for use inside and outside the home. Adapting work tools, keys, pencils, zippers, etc., can also reduce pressure and make doing the activity easier with less risk for high pressure.

31.3.3 Exercises

The aim of exercises for the paralyzed hand is to maintain or improve joint mobility.

It is important to distinguish between the mobile hand (no contractures) and the hand with contractures.

a. *Mobile hand*: Place the hand to be exercised with palm up on your thigh or tabletop. Press with the other hand (or forearm) the palm/knuckle joints firmly down and hold when actively straightening the interphalangeal joints. Hold straight for a few seconds, and repeat 10–15 times.
b. *Contractures*: with the hand supported on the thigh/palm (as above), passively straighten the finger joints with care with the other hand (or forearm), and hold for few seconds. Remember to hydrate the skin well so that skin is not cracked when gently straightening the fingers.

If the hand is mobile, exercise "a" is enough. If contractures are present, then first do exercise "b" followed by exercise "a."

Preventing contractures will maintain joint range of motion enabling a better grip with a larger contact bearing surface which reduces high pressures on the skin that can cause injury, a secondary impairment.

31.3.4 Exercises for the Paralyzed Foot

Persons with foot drop position can develop a tight heel cord. In some countries where leprosy is endemic, persons regularly squat to sit down and chat with people or to defecate. Cycling, walking stairs, or walking uphill would also maintain functional dorsiflexion. These cultural habits maintain the range of motion in the ankle joint. Passive stretching exercises may be needed for people in countries that do not have these cultural habits or for persons that are bedridden for a prolonged period. When persons have (tendency for) clawed toes position, they can routinely passively straighten out the toes with their hands when soaking their feet.

31.3.5 Exercises for the Paralyzed Eye

The function of the eyelids can in some way be seen as similar to the function of the windscreen wipers on the windscreen of a car. When the eyelids open and close, they moisten the eyeball, lubricating and removing dangerous particles (dust) from the eyeball.

Persons with loss of effective blink should be advised to close their eyes regularly even though there may not be full eye closure.

The attempted closure will result in an upward movement of the eyes which will wash and lubricate the eyes, and the movement of the eyeball under the upper eyelid will remove foreign particles from the eyeball. If the eye cannot close completely, extra protection to reduce the drying effects of the air and wind and protect the eye against foreign debris falling into the eye is needed during the day and night.

Besides exercises, care may also include daily auto-vision checks, artificial tears, and protective eye shields during the day and night.

- *"The mechanical and more obvious mechanism for the protection of the eye is a ready motion of the eyelids (blink) and the shedding of tears which coming as it were from a little fountain, play over the surface of the eye, and wash away whatever is offensive (Facial nerve)…*
- *…for the action of this hydraulic and mechanical apparatus there is required an exquisite sensibility to direct it … a property resembling the tenderness of the skin, yet happily adapted, by its fineness, to the condition of the organ (Trigeminal nerve)*
- *…Now it sometimes happens that this nerve is injured. Smoke, offensive particles, restupon the eye, without producing sensation and exxciting the mechanical apparatus to act. They do stimulate (irritate) and will produce inflammation… this causes opacity in the fine transparent membranes of the eye… although the proper nerve of function remains entire (Optic nerve)* [3].

So far we have discussed the primary and the secondary impairments due to muscle imbalance from paralysis. Again, primary impairments can in large extent be prevented by early disease diagnosis in addition to timely diagnosis and treatment of NFI (Chap. 23). Exercises prevent soft tissue contractures and can prevent joint stiffness.

In the next section, the secondary impairments that can be attributed to the loss of protective sensation in hands and feet will be discussed.

31.4 Wound Prevention and Care (Written by Hugh Cross)

Perhaps the most distressing secondary complication for people affected by leprosy is neuropathic ulceration. Compared with a sudden wound, ulceration is a process of slow, sometimes hidden, breakdown of tissue. The body's response to ulceration

is the same as it is to any cause of tissue breakdown; ulcers will heal by secondary union, but the process of union will only begin when the destruction stops.

The composition of soft tissue varies from one part of the body to another. It develops in such a way that it can adapt to different stresses without being damaged during normal activities. Collagen and elastin are two types of fiber that give soft tissue strength and elasticity, respectively. In certain body parts (e.g., the foot), globules of specialized fat also help by acting as shock absorbers. If these fibers are put under too much stress, they will become weak through fatigue. Fatigue can be caused either by vertical forces that create pressure or by horizontal forces that cause shearing stress.

The ulcers commonly found in the feet of people affected by leprosy are usually the result of moderate pressure or shearing stress that is applied repeatedly over a long period of time. This is different from pressure ulceration (also known as ischemic ulceration) which results from low but continuous pressure being applied to tissue for several hours, thereby blocking the supply of oxygen and nutrients. Under normal circumstances, when tissues are placed under unremitting stress, they release mediators of inflammation (including interleukin and prostaglandin) which stimulate nerve endings and excite sensory feedback to the central nervous system. The greater the stimulus, the more likely it is that the impulses will be registered as pain. Pain demands that the damaged part be rested. During a period of rest, the tissue manages to recover.

If there is no nerve function, the tissue may be put under stress, and although mediators are active, sensory feedback does not function, the central nervous system cannot respond, and no action is taken to protect the threatened part. Eventually the tissue that is under stress reaches a point of fatigue and breaks down.

As tissue fatigues and breaks down, it releases other chemotactic elements. In response, the area around the damaged tissue becomes packed with fibrin, neutrophils, platelets, and plasma, the body's self-repair mechanism. However, the massing of repair chemicals and blood causes edema. Body tissue is usually confined in very tight compartments (particularly in the feet), so the buildup of edema puts pressure on the small blood vessels that serve the tissue and eventually blocks off the blood supply. As the tissue becomes starved of nutrients, more tissue dies. As more tissue dies, more chemical messengers are released, and again the body responds by activating an even stronger tissue repair response. Unless immediate action is taken to stop this cycle, the area under stress breaks down to such an extent that there is no more space for further accumulation of plasma and broken down tissue. The skin gives way to internal pressure and eventually breaks to allow an escape of fluid, and an open ulcer appears.

31.4.1 How Ulcers Heal

Provided that nothing interferes with the repair process, the body will heal itself effectively and efficiently. Leukocytes are activated to break down dead tissue (the

first debriding agent), and monocytes release enzymes that break down collagen and other proteins. These destructive activities spread inward until good, viable tissue is reached.

During these activities, transudate assists the repair process by bringing nutrients and carrying away the breakdown products. The breakdown products change the clear, straw-colored transudate into a creamy, discolored fluid with a distinctive odor. This thick and sticky fluid is exudate. After about 1 week, the cells and plasma particles of the exudate begin to bond together into a necrotic coagulum termed slough. Slough may remain relatively fluid or dehydrate to become a hardened eschar.

If good conditions are maintained, the process of tissue repair moves onto a phase in which cells multiply very quickly and new tissue is built up. An important sign that an ulcer is healing is the appearance of granulation tissue at the base of an ulcer. Granulation tissue is a mass of vascular and lymph vessels that form in a gel-like substance known as granulation matrix. The entire mass is held together in a fibrous collagen network. The granulation matrix is made up of necessary ingredients to build tissue and also of various chemicals that act as defenses against infection. The blood vessels carry nutrients to macrophages and fibroblasts, while the lymphatic vessels prevent edema by constantly draining the area. Granulation tissue is produced until it fills the wound cavity, almost to the level of the surrounding skin. As granulation builds up to the level of the skin, it stimulates the release of further chemotactic elements that cause epithelium to start spreading over the wound surface.

When a large area of tissue has been damaged, the problem of scar tissue becomes an issue. Scar tissue is not as elastic as normal skin tissue and has a maximum strength of 20% less than that of undamaged skin. As it forms it also contracts and can leave the body part seriously deformed and with very much reduced ability to function. During the final phase of ulcer healing, complete skin cover can give the impression that the repair process is complete. However, the process is not complete, and much can be done during this phase to prevent the effects of scarring.

31.4.2 What Can Be Done to Prevent Ulceration?

Knowing that there is a risk is the first action in preventing ulceration, but if immediate action is taken during the fatigue phase, it is also possible to prevent frank ulceration.

Most foot ulcers are formed because some people have feet on which small areas of high pressure build up and unremitting application of pressure will cause tissue fatigue and breakdown. If people are required to walk with feet that have lost sensory feedback, they should feel their feet frequently to try and find whether there are any places on the foot that feel hot (assuming that their hands can still feel). If localized hot spots are found, the foot should be rested before continuing to walk. Other signs of inflammation are redness and swelling; consequently, people at risk should also look at their feet frequently (especially if they cannot feel with their hands). If

there are any localized areas of puffy redness, this may indicate early tissue breakdown, in which case immediate rest is also required to prevent breakdown.

Soft, cushioned insoles in footwear are useful because cushioning reduces force. If force is reduced, there will also be a reduction of pressure. Correct foot orthoses can also offer relief from high pressure.

If the body's ability to control sweating has been lost, the skin dries and loses its elasticity. When elasticity is lost, the skin is much more vulnerable to damage. The skin of feet can be kept elastic either by daily soaking in water or by use of emollient creams (e.g., soft paraffin or lanolin-based creams). If soaking is the method of choice, the feet should be immersed in water for 30 min, after which they should be mopped dry. When the excess water has been mopped away, a barrier ointment (e.g., Vaseline) should be applied. Application of an emollient is an alternative to soaking. Emollients penetrate the skin, taking water into the skin as they do so. Either method will help to keep the skin elastic.

31.4.3 Treatment for Simple Ulcers

Very often people do not notice that there is a problem until frank ulceration is found. The earliest signs of breakdown should be considered an emergency. If the ulcer is not infected and does not involve any tissue deeper than the dermis, it is termed a simple ulcer. The most essential actions to be taken to address simple ulceration are rest and elevation. Further consideration should be given to maintaining the best possible wound environment; this will require optimal dressing materials without any medication.

31.4.4 The Qualities of the Ideal Dressing

- Removes exudate.
- Maintains a humid environment at the wound surface.
- Allows exchange of gases.
- Prevents entry of microorganisms.
- Maintains a suitable and stable temperature.
- Does not stick to the wound surface or introduce toxic substances into the wound.

31.4.5 Treatment for Complicated Ulcers

Ulcers that extend to tissues deeper than the dermis (fascia, ligaments, tendons, bones, etc.) are termed complicated ulcers. The first priority when starting treatment of a complicated ulcer is to decide whether the wound is infected (it usually is).

31.4.6 Infection

If a wound is infected, there are likely to be signs of cellulitis and lymphadenitis:

- The area around the wound will be hot, red, and swollen.
- The lymph nodes will be swollen and tender.
- There will be much foul-smelling exudate from the ulcer.

If the wound is infected, it is essential to make sure that any detached necrotic tissue is removed and that drainage for outward flow of pus is secured.

Once an open drainage system is made, careful hand pressure, in a movement that forces the pus in a direction away from the person's heart, can help to clear pus from a wound. Having forced as much pus out of the wound as possible, a 10-ml syringe filled with saline can be used to flush out the wound. This should be done repeatedly until the wound is clear of pus.

Although loose tissue (including bone) should be removed immediately, no action should be taken to further debride the wound. A ribbon of gauze can be drawn through the wound if it has associated fistulae, or the wound can be lightly packed with gauze to make sure that it does not close. Gauze dressings, held in place with a bandage, should be applied over the wound, and the part should be elevated for rest.

Systemic antibiotic treatment should be started immediately, and dressings need to be changed daily. If there has been a buildup of pus, it must be removed as before, but if there is no buildup of pus, the wound should be cleaned with saline and dressed as before. After 3 days, if there is no pus and the inflammation has reduced, the wound will be ready for surgical debridement.

Debridement is necessary to ensure that all dead tissue is removed. Toxic substances which are the products of dead tissue continuously leak into the wound and can seriously delay healing. Until all irritants are removed from the wound, the body will not be able to begin the repair phase of healing. Sequestra and osteomyelitis can be particularly damaging, so all fragments of bone must be removed from the wound, and all pieces of infected bone must be excised.

All overhanging skin with any other weak or avascular tissue should be excised, and all callus that may have built up around the ulcer should also be reduced. Following debridement, the wound should be packed with gauze soaked in Betadine® solution and secured with bandage. Postoperatively, the limb should ideally be immobilized and rested in a position that is higher than the level of the heart.

Dressings should be removed the following day, at which time the wound should be examined. If there is no pus and the inflammation in the wound has reduced, the wound should be cleaned gently with saline and packed with gauze dressings made moist with saline. If it is certain that the infection has been adequately addressed and there is no further evidence of pus, the wound can be left for 2 days before examining it again. The wound should continue to be inspected on alternate days. The wound will improve with careful cleaning and replacement of saline-moist

dressings. If the patient can be given crutches or the use of a wheelchair, this will help the healing process significantly.

The wound will heal over time, but the duration will depend on the following factors:

- The depth and area of the wound.
- The behavior of the patient (if the patient does not rest the part, it will take longer to heal).
- Patient self-esteem and morale.
- The general health of the patient (nutrition, cardiovascular status, nicotine intake, etc.)
- Levels of personal and environmental hygiene.

Fourteen days after debridement, a former complicated ulcer should show signs that it has stabilized and is progressing through tissue repair (inflammation will be controlled, swelling will be reduced, and there will only be slight amounts of serous discharge). At this stage, total contact plaster casting may be considered.

31.4.7 Avoidance of Medication

Widespread use of antiseptics and topical antibiotics is problematic for the following reasons:

- The toxic agents used in many antiseptics destroy body tissues as well as microbes and can significantly delay healing. Some antiseptics also affect the bactericidal ability of the blood because they destroy leukocytes.
- Patients are lulled into believing that the medicine will cure the ulcer, and as a result they do not take responsibility to change or modify their activities.
- There is evidence that resistant strains of bacteria are developing partly as an effect of careless use of antiseptics.
- Valuable resources are used up unnecessarily (wounds heal without costly antiseptics).

If antiseptics are to be used, they should be chosen for a specific action against an identified organism.

31.4.8 Other Important Factors that Influence Wound Healing

- Vitamin C and oxygen are essential for manufacture of collagen.
- Vitamin A deficiency has been recorded as delaying re-epithelialization.
- Protein deficiency results in a reduction of amino acid. Poor supply of amino acid will affect production of granulation tissue. (Protein deficiency also reduces defenses against infection.)

- Deficiency of trace elements, particularly zinc and copper, is also responsible for delay in healing. Zinc is essential for the process of re-epithelialization and collagen synthesis. Copper is essential for production of the enzyme that links collagen molecules together.
- Psychological well-being. Where people are excluded from participating in normal social activities, they are likely to suffer from low self-esteem, which can lead to poor motivation. Not only do self-esteem and confidence result in better levels of care, but low stress levels result in better immune responses.

Almost all wounds will heal with rest, while almost no wounds will heal without rest.

31.5 Addendum

In recent years a new dressing technique has been introduced in the treatment of (chronic) plantar ulcers and lower limb (stasis) ulcers: leukocyte platelet-rich fibrin dressings. The first reports seem promising and resulting in faster and better tissue healing.

However, an important warning seems in place. Most ulcers are caused by underlying biomechanical factors, too much walking, tight shoes, foot deformity and individual foot mechanics, etc. If these are not addressed, then ulcers might recur.

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International textbook of leprosy (Open Access): https://internationaltextbookofleprosy.org/

Chap. 4.3: Physical rehabilitation. https://internationaltextbookofleprosy.org/chapter/physical-rehabilitation

Chap. 4.5: Stigma related to Leprosy. https://internationaltextbookofleprosy.org/chapter/stigma-quantitative

ILEP: www.ilep.uk

AIFO: http://www.aifoeng.it/category/leprosy/learning-materials/

Leprosy Mailing List. leprosymailinglist@googlegroups.com

Surgical and Social Rehabilitation in Leprosy

32

J. Wim Brandsma and Sunil Deepak

In Chap. 31 general disability issues were discussed, namely, the pathogenesis of disability and prevention of disability with an emphasis on ulcer care and management. This chapter is devoted to physical-surgical and social aspects of rehabilitation.

In the physical rehabilitation section, the focus will be on correction of paralytic deformities. The social rehabilitation section will discuss issues related to stigma prevention, reduction and socioeconomic reintegration.

Even though these two aspects of rehabilitation are discussed in separate sections, it should be clear that in many instances, the two are interdependent.

For a review of important aspects of paralyzed limbs in leprosy, the reader is referred to the consensus documents of international workshops [1, 2].

32.1 Surgical Rehabilitation

This section will limit itself to the more common paralytic conditions that result from leprosy neuropathy [3]. Neurolysis as a surgical procedure to restore or prevent nerve function impairment (NFI) will not be discussed. This remains a controversial issue in leprosy surgery, whose possible benefits have not been established [4]. For information about aesthetic-cosmetic surgical procedures, e.g., nose correction, the reader is also referred to the leprosy E-textbook [5].

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Some important principles related to reconstructive limb surgery are listed below:

1. Reconstructive surgery should only be performed by surgeons with sufficient training in leprosy reconstructive surgery. The surgeon should ideally have spent time with an experienced leprosy reconstructive surgeon.
2. Surgeons and therapists should be confident that patients understand that movements will be restored, not sensation. Patients should have shown good understanding and practice of living with a limb, or limbs, with loss of protective sensation.
3. No tendon transfer surgery should be done without the assurance that patients will be followed with postoperative therapy.
4. Assess corrected hands and feet after 6–12 months to determine occurrence of possible secondary impairments (wounds, contractures) and to assess functional social benefits.

32.2 Tendon Surgery for the Paralyzed Hand

32.2.1 Ulnar Palsy

The ulnar nerve is one of the most effected nerves in leprosy. Loss of ulnar function results in claw finger deformity, overt or latent, of all four fingers. The thumb will also show weakness because of paralysis of the ulnar innervated muscles that are important in pinch: adductor pollicis, flexor pollicis brevis, and first dorsal interosseous.

However, decreased thumb strength is not routinely addressed but may be for the accurately assessed and selected patient a very much needed requirement which may then need correction.

The ideal hand for tendon transfer is the hand without secondary defects, i.e., the hand with full assisted extension, i.e., when metacarpophalangeal (MCP) extension is “blocked,” the fingers can be fully actively extended. This indicates full integrity of the extensor mechanism in the absence of contractures. The patient may need a period of preoperative therapy including splinting to prepare the hand for the best possible outcome of tendon surgery. Tendon surgery will not correct the pre-existing secondary impairments.

32.2.2 Surgery for Ulnar Nerve Paralysis

The operation of choice nowadays is the so-called Zancolli lasso operation. In this operation the flexor digitorum superficialis (FDS) of either ring (R) or middle (M) finger is harvested, split into four tails, and looped around the A2 pulley. Activation of this muscle now results in primary MCP flexion allowing the extensor digitorum communis to extend the interphalangeal joints. Whether FDS-R or FDS-M finger is used depends on activity of FDP-R and/or concomitant median palsy.

Many other techniques are available for which the surgeon is referred to the appropriate textbooks.

Postoperative therapy is essential and can start 2–3 days post-surgery. Rath et al. [6] recently published some randomized clinical studies which showed that it is safe to start with early active (protected) motion.

It is recommended that patients are called back for review 6–12 months after discharge to assess the hand. This is the time to see if possible secondary defects may have occurred and find out from the leprosy-affected people to what extent surgery has been of functional and social benefit using the appropriate questionnaires and scales.

32.2.3 Median Palsy

Median palsy is usually associated with ulnar palsy but can occur in isolation. Some people with isolated median palsy may still have adequate opposition especially when flexor pollicis brevis is entirely ulnar innervated. In such cases tendon transfer surgery might not be needed.

When combined ulnar and median palsy are present, some surgeons prefer to correct the hand in two sessions. However, depending on surgeon's and therapist's experience with tendon transfers, the procedures to correct fingers and thumb can be combined in one session. Combining the two would naturally be of great economic benefit to the patient, i.e., no loss of work (hospitalization).

The most common procedure is use of an FDS, usually R finger, which is rerouted to the thumb and can be inserted in different ways depending on the individual mechanics of the thumb. Palmaris longus and extensor indicis have also been used for opposition reconstruction.

Strength of the donor muscle is not required for positioning of the thumb in opposition (pulp to pulp pinch). Strength is needed in a key pinch. When strength is required, a second tendon transfer may be needed to strengthen adduction.

Postoperative re-education is relatively easy and should preferably be done through functional and purposeful activities.

32.2.4 Combined Ulnar and Median Palsy

Combined ulnar and median palsy is not unusual in leprosy and may even be present bilaterally, representing a true challenge for surgeon and therapist.

It is advisable not to correct both hands while patients are hospitalized. This would require long hospitalization and could "dislodge" the patient from his social environment. It is better to do one hand first, send the patient home, and have the patient come back after 6–12 months. This also allows for a timely review of the first surgery.

32.2.5 Radial Palsy

Radial palsy is relatively rare. When present, it is usually associated with ulnar and median palsy. This pattern of the so-called triple palsy can be variable, with muscle weakness or paralysis present to different degrees.

Drop wrist can be easily corrected with a transfer of the pronator teres muscle. For finger and thumb extension, different procedures are available.

32.3 Lower Extremity Reconstructive Surgery

32.3.1 Foot Drop Surgery

Common peroneal nerve involvement may lead to foot drop. Because the nerve bifurcates at the site of predilection, the head of the fibula, the foot drop may be partial. The muscle of choice for transfer is the tibialis posterior. Routing of the transfer may differ, interosseous or circumtibial, and insertion depends on the pattern of the foot drop.

32.3.2 Claw Toe Correction

Various procedures exist for correction of claw toe deformity depending on severity. Indications for surgery depend on severity and associated foot deformities.

32.3.3 Ulcer Surgery

For ulcer surgery the reader is referred to the textbook by Brandsma and Schwartz which can be downloaded from the web (https://www.infolep.org/resource/surgical-reconstruction-and-rehabilitation-leprosy-and-other-neuropathies).

Deformities (impairments) can be prevented and/or corrected. This may help the leprosy-affected person to function better and not be recognized physically as a leprosy-affected person. However, having (had) leprosy be medically cured may however still have significant consequences at the societal level.

32.4 Social Rehabilitation

32.4.1 Disabilities Due to Leprosy and Evolution of Rehabilitation Strategies

Leprosy can result in neuropathy leading to disabilities. The presence of disabilities is associated with greater social stigma. Early diagnosis and treatment of leprosy is important for preventing disabilities and stigma [7]. The simplified leprosy

disability grading system proposed by the World Health Organization (WHO) in 1988 [8] is not geared towards rehabilitation of individuals with leprosy-related disabilities, but is an epidemiological tool to assess the delay in diagnosis of leprosy.

The Global Leprosy Strategy 2016–2020 had laid a greater emphasis on a reduction in disability among new cases, especially children, and supporting community-based rehabilitation for people with leprosy-related disabilities. It had also proposed the objective of having zero countries with laws or legislation that allow discrimination on the basis of leprosy [9].

During 2019, around 6.3% of newly diagnosed leprosy cases had a grade 2 disability and included many children [10]. Thus, the objectives decided for 2016–2020 remain distant. At the same time, our knowledge about prevalence of leprosy-related disabilities remains still limited. Finding out this information is important for organizing rehabilitation services. For example, knowing the number of persons having loss of sensation due to leprosy in hands or feet in a community is useful for promoting prevention of disabilities by regular self-care and use of protective footwear.

32.4.2 Community-Based Rehabilitation (CBR) and Leprosy

Traditionally different rehabilitation activities of socioeconomic rehabilitation have been followed by nongovernmental organizations. Significant decreases in leprosy prevalence over the past decade and the change from vertical leprosy programs to integrated leprosy services as a part of primary health care have prompted changes in rehabilitation strategies, by reaching out to persons affected with leprosy in existing CBR programs.

According to the CBR Guidelines, in 2010 there were CBR programs in more than 90 countries [11]. They are an opportunity for leprosy programs to create networking with local CBR programs to ensure the inclusion of persons with leprosy-related disabilities in these programs.

The CBR Guidelines include a separate chapter on inclusion of persons affected with leprosy in the CBR programs. The basic principles of CBR stress on active participation by affected persons, human rights approach, and mainstreaming (access to existing services rather than creating separate or specific services), across five domains of activities of CBR Matrix—health, education, occupation, social, and empowerment. (insert CBR matrix, enclosed).

Inclusion of persons affected with leprosy in CBR programs requires preparation and support from different stakeholders including learning to work together with other disabled persons; tackling issues of stigma, discrimination, and self-stigma; and training of CBR personnel about leprosy-related disabilities.

Self-care groups for prevention of new disabilities and care of existing disabilities and self-help groups for working together with other disabled persons in promoting activities of empowerment, income generation, loans, skills training, etc., are key elements of CBR programs. Over a period of time, persons with disabilities

can organize themselves in formal organizations and get involved in advocacy for change of laws and access to local and national resources. They can also play an active role in planning, implementation, and monitoring of CBR programs.

32.4.3 Associations of Persons Affected with Leprosy

Over the past decade, a number of organizations of persons affected with leprosy have been formed in endemic countries, such as Morhan (Brazil), Handa (China), IDEA (India), ARPAL (Angola), AMPAL (Mozambique), IDEA (Nepal), IDEA (Ghana), etc. Increasingly these organizations are playing an active role in changing perceptions, policies, programs, priorities, and procedures related to leprosy. They are involved in a wide number of initiatives such as leading self-care groups and promoting socioeconomic rehabilitation.

Along with social stigma, many countries continue to have discriminatory laws regarding persons affected with leprosy [10]. The organizations of affected persons are also advocating for repeal of those discriminatory national laws.

Together with WHO, representatives of these organizations are asking to stop the use of derogatory terms such as "leper" and have prepared guidelines on the involvement of leprosy-affected persons in related programs and activities [12], including the following:

- *Stigma and discrimination*: work with affected persons to evaluate whether words or images promote dignity or perpetuate stigma.
- *Equality, social justice, human rights*: work with affected persons and their organizations to educate other affected persons, program staff, and community.
- *Information, education and communication*: encourage affected persons to serve as a role model for others.
- *Advocacy*. Work with affected persons to promote equal access to services.
- *Counselling*: provide affected persons with the opportunity to be trained as counsellors.
- *Gender issues*: work with affected persons to advocate for equal opportunities for men and women.
- *Monitoring and evaluation*: use experience of individuals who have disabilities to help identify gaps in the public health system—before, during, and after treatment.
- *Research*: encourage affected persons to work with researchers to ensure research methodology does not deprive people of their identity and that they are informed of their rights.

32.5 Conclusions

Needs of persons with leprosy-related disabilities are huge. Some countries still have old villages and homes where elderly persons, often with severe disabilities, live. More than 16 million persons have been treated with MDT in past decades.

Many of them, even though cured of leprosy infection, need regular support for complications like ulcers and disabilities, as well as socioeconomic rehabilitation measures. Working with existing rehabilitation programs such as CBR is important. Promoting participation, empowerment and advocacy for access to existing services are important to answer these needs.

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Part VIII

Leprosy and Community

Leprosy in Pregnancy

33

Elizabeth Duncan

33.1 Biological Immunosuppression in Women

All women of reproductive age are immunosuppressed for 6 months every year and for 10 months during pregnancy and lactation, the immunosuppression "coming off" abruptly, literally overnight, about 40 days after birth of the baby. Women with leprosy therefore need to be understood and treated differently from men.

Women have cyclic immunosuppression during the menstrual cycle. Immunosuppression starts with ovulation and comes off at onset of menstruation. Eight women of reproductive age associated with the Armauer Hansen Research Institute in Addis Ababa had lymphocyte transformation tests with phytohemagglutinin thrice weekly for 2 months. All showed a biphasic pattern with immunosuppression starting abruptly with ovulation and continuing through the second half of the menstrual cycle, coming off at onset of menstruation with one exception; she had continuing immunosuppression and pregnancy confirmed 1 month later [L Reitan and E Duncan, unpublished data].

Immunosuppression during pregnancy is physiological, so that the fertilized ovum, later to become the fetus, is not rejected because its genetic make-up (50% from each parent) is incompatible with the mother. Without our own mothers' being immunosuppressed, not one of us would be here today.

Many of the damaging complications of leprosy are caused by an unstable host–parasite relationship related to changes in the host's immune response potential.

To Elizabeth Duncan
Elisabeth, who dedicated her life to African women affected by leprosy, passed away.
These pages, fruit of her intellect, continue her work in the years to come as an expression of her love for the most forgotten people.

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Immunosuppression results in new cases of leprosy becoming overt and apparently cured women showing relapse and reactivation. Others may have emergence of drug-resistant leprosy as drug-resistant *Mycobacterium leprae* multiply freely during the 10 months of immunosuppression during pregnancy and the puerperium. Type 2 leprosy reaction (T-2-R) or erythema nodosum leprosum (ENL), frequently with nerve function impairment (NFI), occurs during pregnancy, initially in the first trimester when it may be the first indicator of pregnancy, and especially during the third trimester, and continues postpartum. With recovery of cell-mediated immunity 6 weeks postpartum, type 1 leprosy reaction (T-1-R) or reversal reaction in skin and nerve with NFI is a major problem, with persistent NFI for up to 2 years postpartum (Fig. 33.1) [1]. In severe cases this can result in crippling paralysis and extensive foot and hand ulceration.

The recommendations made in this chapter are based on observations made on women of reproductive age followed for almost 30 years and amounting to 3000 "reproductive life years." The "A9" study was a collaborative study with the Medical Research Council (MRC) Leprosy Project, the Armauer Hansen Research Institute, and the Addis Ababa Leprosy Hospital (ALERT). For review of leprosy in women, see [2].

For the majority of people with leprosy, it is a disease of poverty. With no state security, pension, or sickness benefit, mothers with leprosy need enough living healthy children to care for them in sickness and old age as carers and breadwinners. These women run the risk of further pregnancies, although they know and have stated that the maximum number of pregnancies that they should risk is two. For women with leprosy who have to undergo several pregnancies to achieve the desired family size, repeated immunosuppression can result in progressive shift across the Ridley–Jopling scale from borderline tuberculoid (BT), to borderline (BB), to borderline lepromatous (BL), and even to subpolar lepromatous (LLs) in successive pregnancies.

With urbanization and rising cost of food, traditional nourishing diets of pulses and vegetables are unaffordable, and a cheaper Western diet of pasta now supplies calories without nutritional factors essential for cell-mediated immunity. Immunosuppression is further aggravated by ethnic unrest and war, flood and drought, crop failure and famine, and poverty, all causing massive displacement of people. HIV/AIDS and tuberculosis also compound the problem. Intestinal parasitosis such as amebiasis and giardiasis, especially the latter, is per se immunosuppressive.

33.2 Obstetric Care

33.2.1 Pregnancy

Ideally, every leprosy hospital should have its own antenatal clinic (ANC) and family planning clinic (FPC). This is where pregnant women with leprosy can have their leprosy monitored and receive health education. Pregnancy in women with leprosy is remarkably uncomplicated. Routine antenatal care should concentrate on

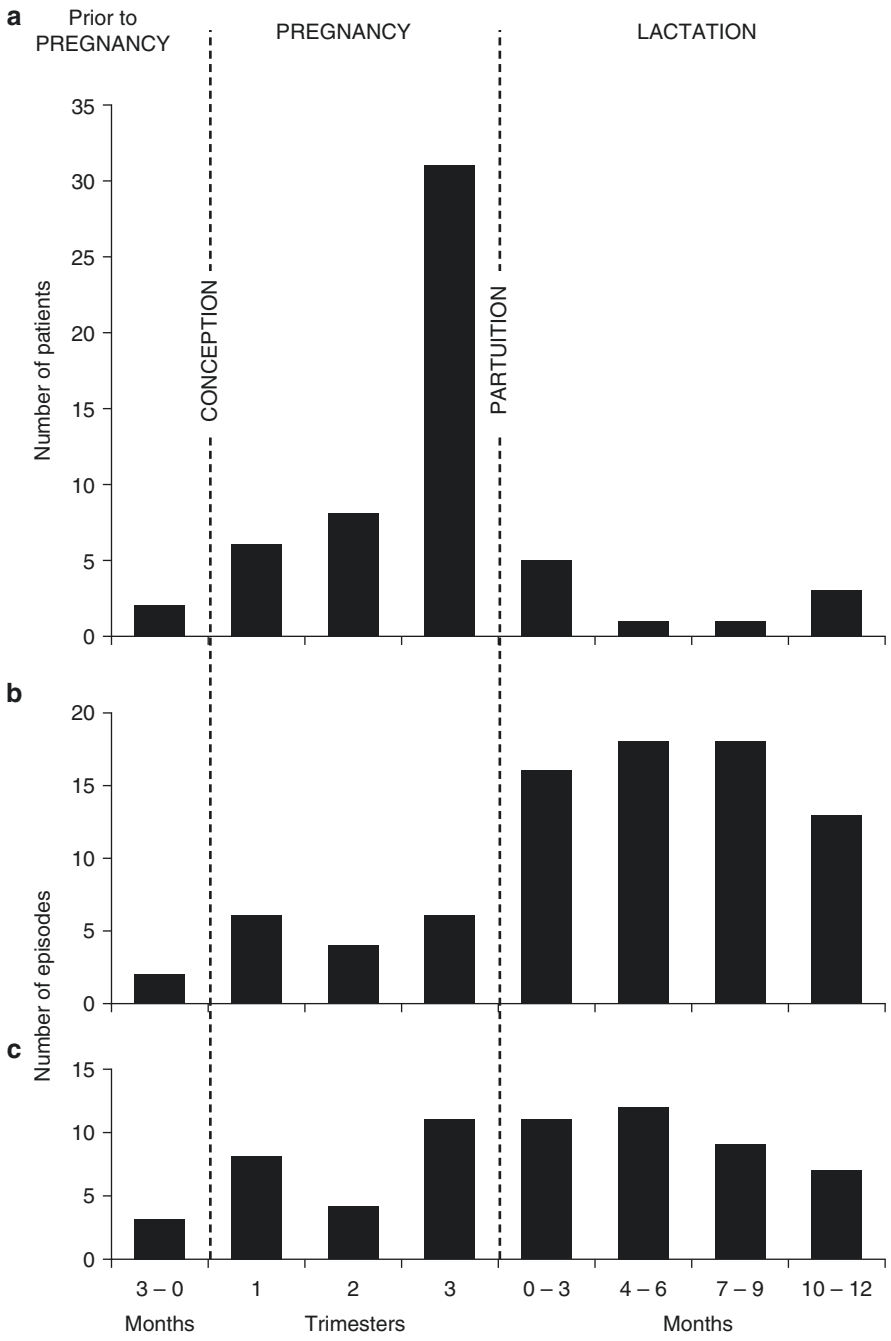

Fig. 33.1 (**a**) Relapse, reactivation, and appearance of new cases of leprosy; (**b**) reversal reaction; and (**c**) erythema nodosum leprosum (ENL) are for first episode only. (Data from Mother and Child Leprosy Study Ethiopia 1975–1978. Results are shown by 3-month intervals: 3 months prior to pregnancy as "control"; first, second, and third trimester of pregnancy; and 3-month intervals for the first year after birth of the baby)

treatment of anemia and detection of intrauterine growth retardation (IUGR). Most pregnant women with leprosy live in areas where routine ultrasound scanning is not available. Traditionally, midwives have measured the uterine fundal height, an indicator of fetal growth, in finger breadths. A better method is to record fundal height in centimeters (cm) above the pubic symphysis; this should rise by 1 cm weekly. The gravid uterus is a pelvic organ until approximately 14-week gestation. By 16-week gestation, the gravid uterus can be palpated above the pubic symphysis. IUGR can be suspected as early as 16-week gestation and is observed in BL and LL women, especially LL women, irrespective of leprosy activity or treatment. These women should be seen more frequently and may require admission to hospital for rest prior to onset of labor to ensure safe delivery of the baby. Where these recommendations are not followed, babies of BL and LL mothers die unnecessarily.

33.2.2 Labor

Normal vaginal delivery should be expected. There should be careful monitoring, especially when IUGR is suspected, to detect fetal distress in case emergency operative intervention is required. If possible, a pediatrician should be alerted for active resuscitation after delivery and, if necessary, management of any respiratory problems. Where an incubator is not available, a small baby wearing only a diaper can be snuggled between its mother's breasts and kept warm under a blanket by her body temperature. Colostrum and expressed breast milk are safer than formula. Even small babies can be fed expressed breast milk using a cup and teaspoon, which for most is more easily sterilized than a bottle. A mother with acute ulnar or median neuritis may require help in feeding her baby. Mothers should not be sent home before breastfeeding is well established and the baby has regained its birth weight.

In a prospective study of 147 Ethiopian women during 156 pregnancies, there were 114 women with leprosy (120 pregnancies) and 32 women without leprosy (36 pregnancies). Baby weight, trimmed placental weight, and placental coefficient (placenta weight/baby weight) from healthy control (HC) women and women with TT and BT, BL, and LL leprosy, with full-term, singleton pregnancies without fetal abnormality, are presented in Table 33.1. Fetal distress or Apgar scores of less than 4 at 1 min after birth were recorded in 20% of babies of BL or LL mothers, and respiratory problems were a significant cause of neonatal mortality in babies of LL mothers [3]. Histological examination of placentae showed no abnormalities [4]. Acid-fast bacilli were not seen on routine microscopy but were found by concentration methods in two out of seven placentae from women with very active leprosy [5]. Small placental size was due to reduced cytoplasmic mass [4].

33.2.3 Follow-Up of Babies

Babies should be seen for weighing and measuring, monthly for the first 3 months, then 3-monthly for 2 years, and more frequently if any problems arise. In the A9 study, babies were seen according to this schedule. The baby weights are shown in Fig. 33.2. After the first 3 months, babies of BL and LL mothers showed failure

Table 33.1 Baby birth weight, placental weight, and placental coefficient (placental weight/baby weight) according to the clinical classification of the mother

	Birth weight (g)	Placental weight (g)	Placental coefficient
Healthy controls	3280.6 ± 87.6 (18)	595.0 ± 34.5 (13)	0.184 ± 0.01 (13)
Tuberculoid or borderline tuberculoid leprosy	3075.0 ± 61.1 (30)	569.4 ± 19.4 (25)	0.181 ± 0.05 (25)
Borderline lepromatous leprosy	2985.6 ± 69.9 (33)	521.0 ± 26.4 (26)	0.173 ± 0.01 (26)
Lepromatous leprosy	2558.1 ± 60.5 (21)	362.0 ± 19.1 (15)	0.144 ± 0.01 (15)

All observations are for singleton, full-term pregnancies without fetal abnormality. Results are given as mean ± standard error of mean with number of observations in parenthesis

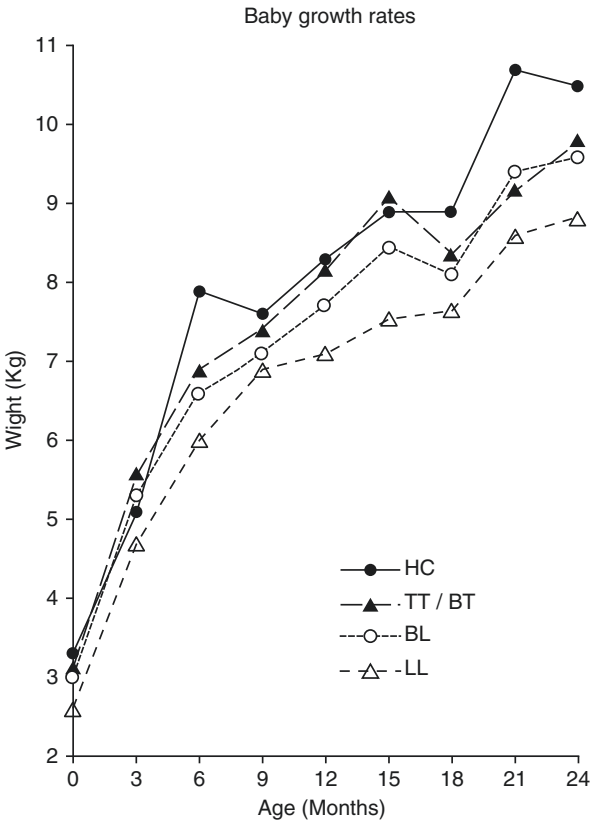

Fig. 33.2 Baby growth rates showing average baby weight at 3-monthly intervals from birth to age 2 years according to the classification of the mother. The pattern of baby birth weight is HC [TT and BT [BL [LL. The same relationship holds at age 2 years. HC healthy controls, TT tuberculoid leprosy, BT borderline tuberculoid leprosy, BL borderline lepromatous leprosy, LL lepromatous leprosy

to thrive and were increasingly subject to minor ailments which, if untreated, became serious problems requiring admission for treatment and supervised feeding. The growth chart for weights of two low-birth-weight babies is shown in Fig. 33.3. Child mortality for the first 2 years was 22%, 12%, 10%, and 10% for babies of LL, BL, TT and BT, and HC mothers, respectively. Breastfeeding should be continued for 2 years, with supplementary feeding introduced at 6 months. Prolonged lactation is for many a natural contraceptive and thus acceptable to both partners.

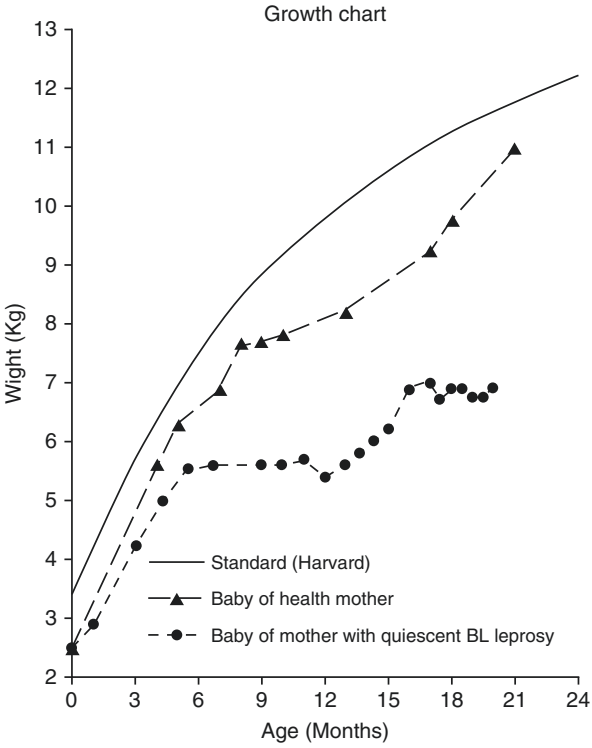

Fig. 33.3 Comparison of growth of two babies, both weighing 2.5 kg at birth. Both babies were breastfed until 1 year with supplementary feeding thereafter. Baby 1, born to a healthy control mother, attended the clinic less frequently and latterly only if the baby was sick. Baby 2 was born to a BL mother with quiescent leprosy (BI = 0). This baby's growth curve flattened out at 6 months, and the baby was being seen for recurrent infections, feeding problems, and failure to thrive

33.2.4 Transmission of Leprosy from Mother to Baby

Mycobacterium leprae has been demonstrated on the fetal side of the placenta [6]. Five percent of babies born to mothers with active lepromatous leprosy had self-healing indeterminate leprosy under the age of 2 years and also anti-*M. leprae* antibodies of class IgA, IgG, and IgM [7]. Separation of mother and baby at birth is not advised, nor is it necessary for the lactating mother to wear gown and mask when handling and feeding her baby. Prophylactic administration of antileprotic drugs to the baby is probably unnecessary (as the drugs commonly used pass the placenta and milk barrier), clofazimine causing typical reddening of the baby's skin. Prophylactic administration of antileprotic drugs is also less valuable than adequately treating and watching the mother during pregnancy and lactation and observing the baby regularly during the first few years of life.

33.2.5 Puerperium

Routine care is given, with attention to breasts, involution of the uterus, color of lochia, and perineal sutures. Puerperal infections are uncommon, especially for those already on dapsone. Dapsone has been called the "fertility drug" where gonorrhea is widespread, as it is very effective against gonorrhea, and we believe also against chlamydial pelvic infections. Early ambulation and care of the baby is the norm.

33.3 Management of Leprosy

Examination of skin and nerves for evidence of new leprosy, reactivation, or exacerbation of leprosy should be made on first attendance at the ANC, 3-monthly during pregnancy, and for 2 years postpartum. A standardized body chart showing front and back views is useful. Old leprosy lesions can be recorded in black and new lesions or ENL in red. Slit-skin smear examination for bacteriological index (BI) and morphological index (MI) should be carried out from an active lesion(s) if possible, and the site(s) marked on the body chart, and from standard sites (both earlobes, elbows, and knees). Punch biopsy and slit-skin smears should be taken every 3 months from the second trimester until 6 months postpartum from active skin lesions, or from the buttocks. Positive BI and/or MI or a punch biopsy from the buttocks may be the first indication of worsening or reactivation of leprosy.

33.3.1 Nerve Function and Neuritis

Nerve function should be monitored with voluntary muscle test (VMT) using the MRC scale; graded sensory skin test (STG) using Semmes–Weinstein filaments (0.05, 0.2, 2, 4, 10, 20, 50, and 300 g) for sensation of hands and feet; corneal reflex (CR); and two-point discrimination, moving and static (2-PDM and 2-PDS), for 2, 3, 4, up to 15 mm on hands and feet. For assessment of STG, 2-PDM, and 2-PDS, we followed routine ALERT practice of testing ten sites on each hand and foot. Facial STG (FaSTG) for assessment of the face used 12 sites on each side of the face [8], and stocking and glove anesthesia (SGA) was tested with a piece of cotton wool brushed lightly over the skin of feet and legs and hands and arms. FaSTG and SGA are particularly important for women aged 35 years and over.

Van Brakel's definition of NFI is recommended for STG, in hands and feet, i.e., sensory increase at one site by two levels of STG or at three sites by one level of STG in any one hand or foot [9]. Similar levels are used for 2-PDM and 2-PDS.

Silent neuritis with insidious loss of sensory and motor function during lactation is a particularly dangerous risk of pregnancy [1]. Recurrent ENL and reversal reaction cause continuing nerve damage for 2 years postpartum. Each episode of NFI or overt or silent neuritis should be treated promptly as outlined below. The World Health Organization recommendation of testing for "protective sensation" using a 10 g filament or ballpoint pen to cause skin indentation is not appropriate for women.

33.4 Treatment

Leprosy treatment should be continued throughout pregnancy and for 1 year after delivery. If acute reaction with neuritis or plantar ulceration occurs during pregnancy and lactation, the mother may require rest and prolonged hospital treatment. Home circumstances should be assessed carefully to ensure that, during her absence, her husband does not take another wife to care for her home and children. Severe ENL may be a sign of undiagnosed tuberculosis, which should be investigated. For treatment of ENL or leprosy neuritis during pregnancy, use clofazimine 100 mg

three times daily; it is safe and effective, crossing the placenta and into breast milk (which is colored pink) harmlessly. Prednisolone should not be given for leprosy neuritis during pregnancy because of suppression of fetal adrenal function. Thalidomide should not be given to women of childbearing age because of its teratotoxicity. Mothers should be advised that regurgitated milk may be colored pink, that this shows that the mother's clofazimine is reaching the baby via the breast milk, and that while it may discolor the baby's skin temporarily, it is good for the baby. When a woman with active LL develops ENL involving skin of the face and neck, careful inpatient monitoring is required with facilities for emergency tracheotomy if breathing is obstructed.Reversal reaction with neuritis and silent neuritis after delivery may require prolonged treatment with prednisolone starting with 40 mg daily. Response to treatment is monitored by VMT and STG twice weekly, increasing prednisolone by 5 mg twice weekly until VMT and STG respond. Prednisolone should be then continued at that dosage, which may be as high as 60 mg or even 80 mg, daily for 4 weeks. When VMT and STG are improving and nerves are no longer tender, prednisolone can be reduced by 5 mg every 2 weeks.

33.5 Surgical Treatment

Surgical treatment of plantar ulcers is with debridement and saucerization with dressings twice weekly. Use of a mixture of honey with either castor oil or zinc ointment spread thinly on a dressing is cheap and effective in clearing infection. Reconstructive hand surgery for claw hand is better postponed until the baby is 2 years old and a sibling can care for it for the 3 weeks that the mother's hand is immobilized in a gypsum cast and up to 6 months until active physiotherapy has enabled the woman to use her hand again. If the baby dies within a few months of birth, hand reconstruction can be done earlier, provided that the woman is given effective contraceptive to prevent pregnancy within 6 months after the gypsum cast is removed.

33.6 Follow-Up of Women

Even in absence of pregnancy, because women of childbearing age are immunosuppressed for 6 months of the year, they run the risk of reactivation of leprosy. In countries where sterilization by tubal ligation is done after one or more pregnancies, these women will still be immunosuppressed for 6 months per year. Ideally, annual clinical assessment should be done with BI and MI from standard sites, and VMT and STG, and for older women FaSTG and testing for SGA. Perimenopausal women with leprosy or leprosy contacts who complain of facial burning require urgent investigation. FaSTG may give the first indication of loss of facial sensation, which when untreated or with delayed treatment may lead to blindness. For the individual with insensitive extremities, blindness is disastrous and is true blindness, needing constant home care.

33.7 Recommendation for Further Pregnancies

Women should be advised to attend the ANC if at any time they suspect that they may be pregnant. In the event of pregnancy, antenatal care and supervision of leprosy should be carried out in every pregnancy as outlined. Termination of pregnancy, surgically or medically, is not effective for prevention of pregnancy- associated neuritis. In the event of reactivation of leprosy or development of new nerve damage, a minimum of 1 year's course of multibacillary (MB) multidrug treatment (MDT) should be given. Women with MB leprosy may relapse several years after the initial treatment, even presenting with MB neural leprosy. Biopsy of a fascicle of a sensory nerve is advised. If MB neural leprosy is strongly suspected or clinically confirmed, 2 years of MB MDT is recommended.

33.8 Health Education

Every clinical assessment is an opportunity for health education by the leprosy worker with the recommendation to pass it on. In most cultures, women meet socially whether for tea or coffee and a chat, or at the well or while washing clothes at the river. These are ideal opportunities for dissemination of health education including how to carry out daily inspection of insensitive hands and feet; daily soaking of hands and feet in cold water to which a few drops of cooking oil are added to ensure skin is kept moist; exercising of stiff clawed fingers to prepare for reconstructive hand surgery; use of protective gloves for lifting cooking pots off a fire or cooking stove; prompt attendance at hospital in the event of injury; non-weight-bearing rest for those with ulcerated feet; and baby care, feeding problems, common ailments, and immunization. Women with leprosy may have severe disfigurement and deformities of hands and feet, but the majority are industrious and intelligent, and many have been able to see their children through school and college or university. They all should be treated with respect and encouragement and respond positively to kindly enquiries after their children, especially if referred to by name.

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Leprosy in Childhood

34

Paolo Fiallo

34.1 Epidemiology, Sources, and Way of Infection

Although leprosy was universally "eliminated as a public health problem" (defined as a registered prevalence of less than 1 case per 10000 population) in 2000, new cases of leprosy continue to be seen in people from endemic areas, including children (less than 14 years of age) (see Chap. 36 for more information).

Annual statistics on leprosy for 2018 report that among the total of 208,619 new leprosy cases, 16,013 involved children, corresponding to 7.6% of all new cases [1]. The number, although modestly decreasing over the past 5 years, is still significant and used as a strong indicator of continuing transmission of the disease and a useful marker of undiagnosed cases in the community.

Incidence of childhood leprosy in endemic areas is mainly due to overcrowded housing favoring increased aerial exposure to *M. leprae* and poor hygienic conditions favoring skin diseases, mostly itching (ectoparasitosis, pyoderma, mycoses) that facilitates transdermal penetration of *M. leprae*.

A strong factor in childhood leprosy is the presence at home, among family members or close neighbors, of an adult suffering from the disease. Fifty percent of leprosy cases in children occur in household contacts. Among familial contacts, there is a fourfold risk of developing leprosy if the index case is suffering from multibacillary (MB) leprosy as compared to paucibacillary (PB) leprosy.

Among children with leprosy, the age group with the higher incidence is between 5 and 14 years of age (with only 5–6% less than 5 years of age) [2]. This is explained by the disease's long incubation period of approximately 3–5 years.

Although leprosy should not be expected in infant, because of its long incubation period, cases of leprosy in children less than 1 year of age, although very uncommon, were reported [3]. In this regard it should be considered that *M. leprae* can

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enter the body, other than respiratory tract and skin, through other routes, such as the transplacental and/or by breast milk in women affected with untreated multibacillary leprosy. For instance, the transplacental pathway is demonstrated by the finding of viable *M. leprae* in the placenta of untreated multibacillary women and in the umbilical cord of their newborns [4].

Even if it is universally accepted that an intimate and prolonged contact with an untreated multibacillary patient is necessary to contract leprosy and that the disease has a long incubation period, occurrence of leprosy in infants of a few months of life suggests additional hypotheses on the pathogenesis. The short incubation period of only a few weeks in the case of infants could be explained by the fact that the immune system not fully developed in infant would allow the *M. leprae*, penetrated transplacentally, to rapidly develop the disease. Moreover, laboratory observations show that the generation time of *M. leprae* can be much faster than commonly reported in literature (26 h instead of 12–13 days).

Nevertheless, from an epidemiological point of view, the passage of bacilli by transplacental route, although possible, is less important than direct contact as demonstrated by the fact that when babies are removed from their lepromatous mother until the age of 6 months, childhood leprosy significantly decreases [5].

Sex ratio in child leprosy is uncertain and differs among studies. Thus, there is no evidence that allows us to establish any significant difference in prevalence between the sexes.

The genetics of leprosy has been investigated in children affected with leprosy by conducting studies on monozygotic and dizygotic twins. The findings confirm the existence of a genetic mechanism controlling susceptibility to both leprosy infection and clinical manifestation (for a thorough exposure on this topic, see Chap. 3).

34.2 Natural History After Contact With *M. leprae*

Upon penetration of *M. leprae* in the human body, varying outcomes may occur:

(1) No overt evidence of infection appears, and the child never develops leprosy.
(2) Early lesions of leprosy may appear. They may remain stationary for variable time and disappear entirely in a few months as a result of self-healing due to an effective immunological response leaving no trace or in a minimal form. In these cases the temporary clinical manifestations are more frequently hypopigmented macules difficult to be detected.
(3) The lesion/s may progress, and the child (or in adulthood) develops advanced leprosy.

34.3 Clinical Presentation of Advanced Leprosy

34.3.1 Clinical History

In case of advanced leprosy, skin and nerves may be variably affected in children, and all clinical forms may develop.

Indeterminate leprosy (I) is an early and transitory stage of leprosy characterized by one of a few hypopigmented macules with indefinite margins lasting from 2 to 5 years (Fig. 34.1). Greater attention must be dedicated to detect them by using proper light when visiting and accurately examining all the skin surface. Most of the lesions are bacteriologically negative and disappear in a few months leaving no trace or in a minimal form. When spontaneous healing does not occur, this form evolves into one of the subtypes in the spectrum of the disease (Fig. 34.2).

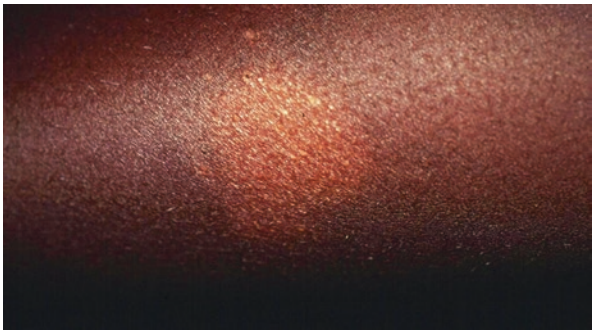

Fig. 34.1 I leprosy. Coppery macule, normal sensitivity, smooth surface and ill-defined edge

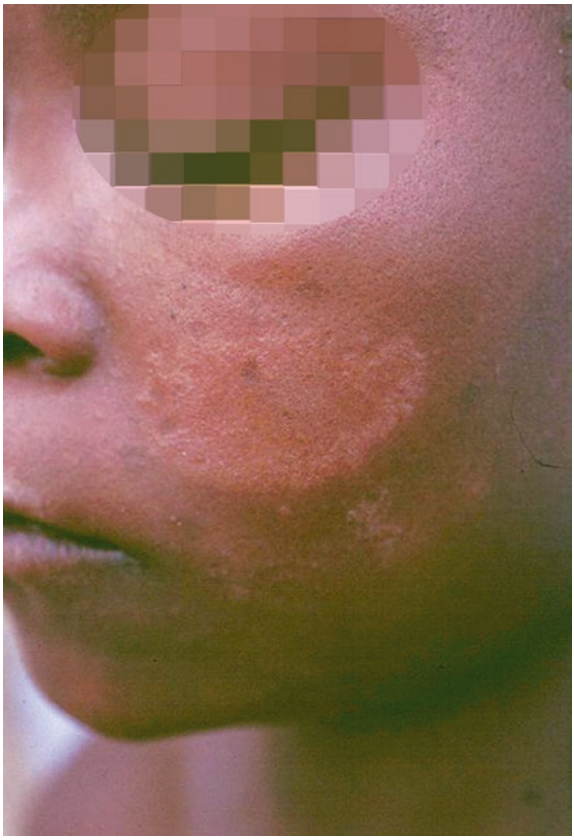

Fig. 34.2 I leprosy evolving into a hyperergic form

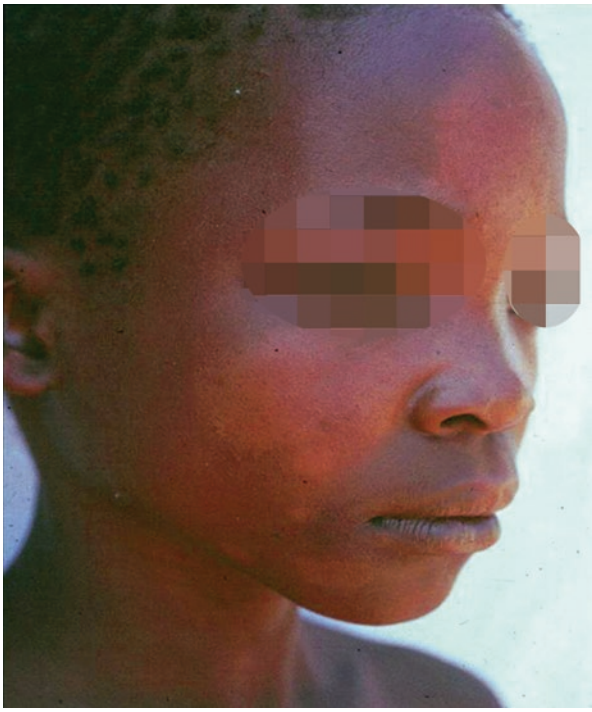

Fig. 34.3 BT leprosy. Large hypopigmented macule with well-defined margins and satellite lesions

Paucibacillary forms (tuberculoid leprosy or TT and borderline tuberculoid leprosy or BT) are the commonest clinical type recorded. This is due to the fact that infection contracted in childhood and evolving into multibacillary leprosy would become apparent after puberty due to the long period of incubation. Furthermore cell-mediate response in children is more likely hyperergic as a consequence of the penetration of *M. leprae* through the skin (as described in Sect. 34.1).

The most frequent skin lesion of childhood paucibacillary leprosy is the hypopigmented macule with definite margins (Fig. 34.3). Other skin lesions are those of hyperergic leprosy: papules and plaques. Plaques may appear annular because of central healing. In most cases (90%), the lesion is single and located on the upper and lower limbs, followed by the trunk and face. Lesions are usually few in number, distributed monolaterally or bilateral (in the BT type) but asymmetrically.

When multibacillary leprosy develops (mid-borderline leprosy or BB, borderline lepromatous leprosy or BL, lepromatous leprosy or LL), it initially appears as multiple, small, nearly symmetrically distributed hypopigmented to coppery macules with indistinct borders. They may appear erythematous or reddish-brown and later on may develop into nodules (Fig. 34.4). With the time, the lesions diffuse symmetrically involving the entire skin.

Anesthesia of the skin lesions, as well in the areas of the affected nerves, occurs in TT and BT leprosy. It is easier to be diagnosed confidently in older children (12–14 years of age) especially if the lesion/s are on the limbs and trunk (more difficult on the face). By contrast, eliciting sensory loss to fine touch in young children

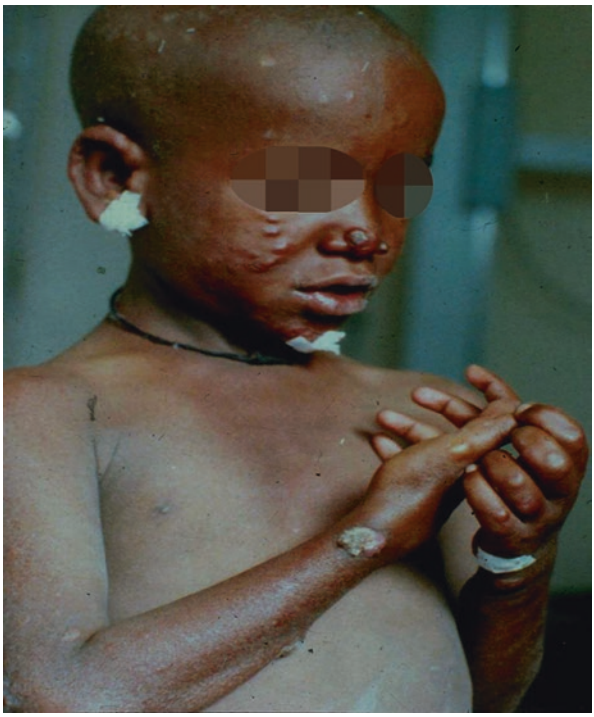

Fig. 34.4 BL leprosy (Courtesy of C. Travaglino)

is often inaccurate or even impossible. A loss of sensitivity can be evidenced in children of 3–4 years old or more, if they trust the examiner and understand what he wants. In these cases it is necessary to spend a few minutes with the little patient before the medical examination in order to gain his trust and friendship, for instance, by tickling and asking him where he was touched and then by repeating the game with his eyes closed. If you want to assess the tenderness of nerve trunks, it is better to slowly palpate and look at his eyes even before waiting for his reaction to the pain. The most affected peripheral nerve is the ulnar nerve, followed by the external sciatic-popliteal and large auricular.

In MB spectrum sensory loss is symmetrical and diffuse; the absence of pain precedes loss of touch sensitivity.

34.3.2 Reactions

Leprosy reactions are relatively rare in children under 15 years of age with varying frequencies in different studies [6]. In all of them, the Type 1 reaction is most commonly reported given that the most frequent clinical form is BT (Fig. 34.5). Older children, and those with borderline forms, are at higher risk for reactions (Fig. 34.6) [7]. Severe reactions are accompanied by fever, malaise, anorexia, swelling of the face and extremities, and neuritis. Rarely, the only manifestation of a reaction is an isolated neuritis.

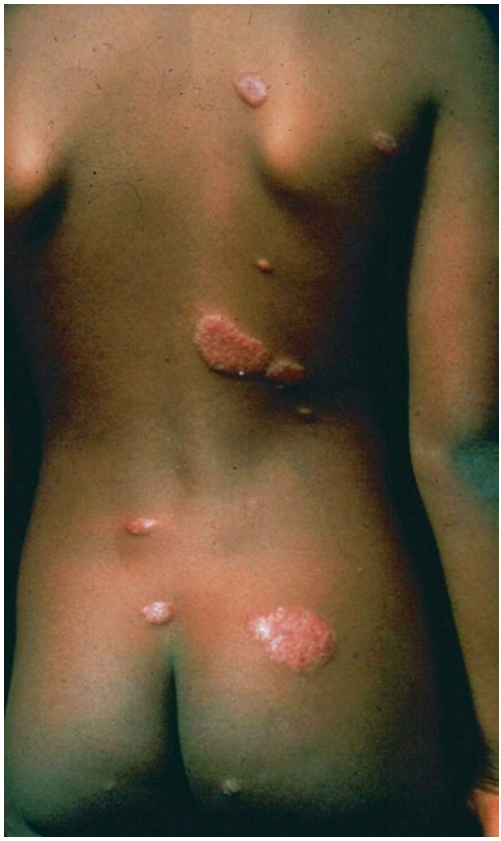

Fig. 34.5 Type 1 leprosy reaction in BT patient (before treatment). The same patient, under treatment, is presented in Fig. 26.3

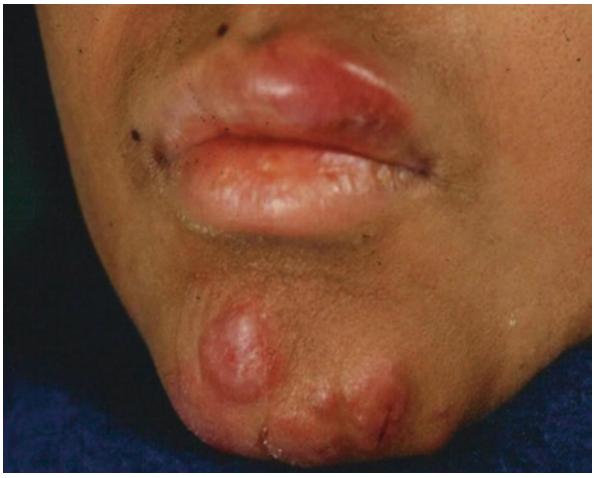

Fig. 34.6 Type 2 leprosy reaction (erythema nodosum leprosum). (From Massone C, Nunzi E (2009) Note di Leprologia. AIFO-Italia, Bologna)

34.4 Diagnosis

For a thorough explanation of the clinical diagnosis of leprosy, see Chap. 26.

Furthermore, when diagnosing leprosy in children, we should take into account some peculiarities.

In a large proportion of early cases of childhood leprosy, search for acid-fast bacilli (AFB) is negative because most of them are indeterminate, TT, or BT. However, in the presence of multiple erythematous macules with indefinite borders due to multibacillary leprosy, skin smears are positive with rates increasing with age [8].

Histopathology examination, other than being an essential cardinal step for leprosy diagnosis, particularly in non-endemic areas, is also a useful tool for accurate proper classification of leprosy and detection of any shift in the patient's position in the spectrum [9]. Due to the incompletely developed immune system of children, non-specific histological features may be encountered, particularly in indeterminate leprosy or in early phase of tuberculoid leprosy.

None of the serological markers identified in leprosy can be used as confirmatory tests for diagnosing leprosy in children. However, titration of anti-PGL-1 has been suggested to identify, among school children and household contacts, those at high risk of developing multibacillary leprosy [10].

34.4.1 Differential Diagnosis

The clinical suspicion of leprosy is facilitated in children with a familial case, more reasonably if multibacillary.

Diagnosis of leprosy in children should be based on the cardinal signs of leprosy (clinic, bacteriology, histopathology) (see Chap. 26).

Other skin diseases resulting in similar hypopigmented lesions such as pityriasis alba (Fig. 34.7), early vitiligo, birthmarks, and pityriasis versicolor should be considered in differential diagnosis of leprosy.

With regard to nerve involvement, inherited peripheral nerve disorders, e.g., hereditary sensory and sensory-motor neuropathies of various types, neurofibromatosis, neuropathies associated with developmental defects, and acquired neurological conditions, including traumatic neuropathies, should also be taken into consideration in differential diagnosis.

34.5 Treatment

The introduction of multidrug therapy (MDT) has been largely successful. Standard blister packs for children aged 10–14 years are available for both paucibacillary leprosy (dapsone 50 mg given daily; rifampicin 450 mg given once a month under supervision) and multibacillary leprosy (dapsone 50 mg given daily; rifampicin 450 mg given once a month under supervision; clofazimine 50 mg given every other

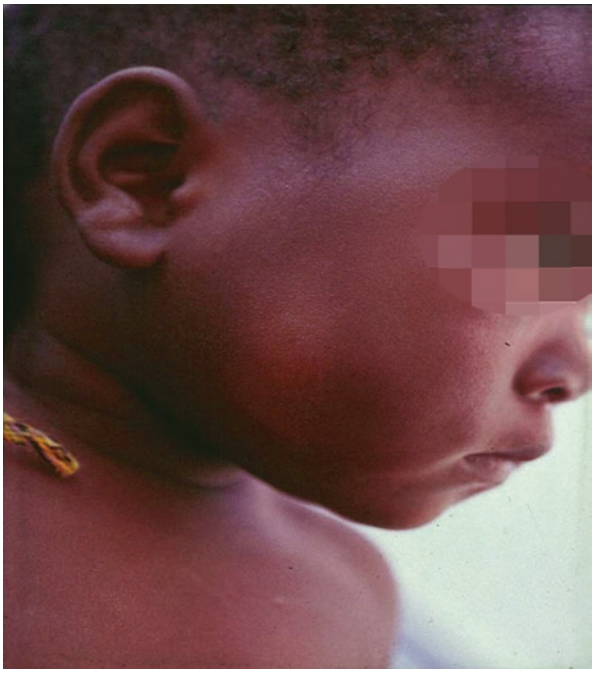

Fig. 34.7 Pityriasis alba. Coppery-colored macule with ill-defined margin

day and 150 mg given once a month under supervision). The paucibacillary regimen is administered for 6 months and the multibacillary for 12 months.

For children less than 10 years, the dose must be adjusted to body weight: (A) rifampicin, 10 mg/kg body weight monthly; (B) clofazimine, 1 mg/kg body weight daily and 6 mg/kg body weight monthly; and (C) dapsone, 2 mg/kg body weight daily.

In case of hypersensitivity to dapsone, clofazimine and rifampicin can be used in combination as an alternative to conventional MDT.

Although development of resistance to first-line drugs is a main threat to the efficacy of MDT program, the alternative drugs recommended in adults (ofloxacin, moxifloxacin, minocycline) are contraindicated for prolonged use in children under 10 years of age. Thus, no alternative regimens are specifically designed for children.

Because lesions in children may undergo self-healing, in case of doubtful cases, it has been suggested to wait and keep the child under observation (untreated) for a period to see whether the skin lesion is improving or sensory loss is appreciated. However, in endemic areas, whenever prolonged clinical observation is not practicable or in the presence of family members affected with leprosy, even in doubtful cases, treatment is wisely recommended.

In the presence of a single lesion of paucibacillary leprosy, a unique administration of three drugs in combination has been used in children: rifampicin (300 mg), ofloxacin (200 mg), and minocycline (50 mg) (ROM therapy).

Relapses are mainly due to inadequate treatment. Several factors, such as child's refusal to swallowing tablets, side effects, drug hypersensitivity, lack of information, and education among the parents, may contribute to a lower compliance to

treatment. For these reasons, activities of information and education among patients and their parents may be implemented and are reported to improve treatment compliance.

Leprosy reactions are the main cause of neural damage and deformities. Thus, they should be identified and urgently as well as adequately treated to prevent disabilities.

Children with reactions (both type 1 and 2) require the prompt use of steroids (prednisolone 1 mg/kg/day) for prevention of nerve damage and deformity. Clofazimine can also be used for management of both type 1 and 2 reactions, generally recommended at 1.5–2 mg/kg three times daily for 1 month and then reduced by one dose per day each month. The maximum dose of 300 mg daily can be administered. Other drugs which can be used for reactions are hydroxychloroquine, methotrexate, azathioprine, cyclosporine, and nonsteroidal anti-inflammatory drugs like paracetamol, etc. Thalidomide is not recommended for children below 12 years old, due to the lack of safety information, but it might be used—if legally available—in adolescent boys.

34.6 Disability

Disabilities and deformities due to permanent damage to peripheral nerves are the most common cause of social stigma in leprosy. They affect psychologically and functionally both the children and their family members. A significant percentage of children affected with leprosy have their initial presentation with deformities suggesting delay in diagnosis [11]. Hence, an important factor to prevent disability in leprosy patient, other than adequate treatment of neural impairment, is early detection. Rehabilitative measures such as physiotherapy and corrective surgery should also be offered to selected patients.

34.7 Preventive Measures

The practice of removing the newborn from the multibacillary mother has fallen into disuse after the introduction of the MDT.

Nowadays preventive measures for leprosy include early diagnosis and management of active cases as well as their contacts. Household contacts should be evaluated annually for evidence of disease for at least 5 years and should be educated to seek immediate attention if suspicious cutaneous or neurologic changes develop. Because leprosy in children is essentially a disease of school age children, community education about leprosy along with school surveys should also be implemented for early detection and to prevent disabilities.

34.8 Vaccine and Chemoprophylaxis in High-Risk Contacts

See Chap. 28.

34.9 Effect of Childhood Leprosy on Community

Despite global efforts, discrimination and stigma associated with the disease are still present. Children affected with leprosy risk to be deprived of education or subjected to bullying and rejection due to stigma associated with the disease.

The child's psyche must be safeguarded at all costs. There is the common opinion that the sick child should not be informed of the diagnosis. He may be informed at a later age, if affected by a multibacillary form requiring a longer follow-up.

34.10 Conclusions

The high prevalence recorded for childhood leprosy is a strong indicator of recent transmission of the disease and a sign of failure of control programs targeted to the elimination of leprosy. The skill for diagnosing and managing leprosy is diminishing leading to missed and misdiagnosis of leprosy in infants and young children and occurrence of deformities and disabilities. For all the above, effective planning to bring down the incidence of leprosy and its complications in children should become a top priority in programs aiming to achieve the goal of elimination of leprosy.

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Leprosy and HIV/AIDS Co-infection

35

Sinésio Talhari and Carolina Talhari

According to the World Health Organization, a country is considered endemic for leprosy when presenting a prevalence rate of more than 1 case per 10,000 inhabitants. At the end of 2019, the registered prevalence of leprosy globally was 177,175. The number of new cases detected during the year 2018 as reported by 160 countries was 202,185. India, Brazil, Indonesia, Nepal, Bangladesh, and Myanmar had the highest number of new cases during 2019 [1].

HIV prevalence rates are also increasing in many countries where leprosy is endemic. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), in 2019, the number of people living with HIV worldwide continued to grow, reaching an estimated 38 million [2].

In such a scenario, it would be reasonable to expect that the geographical overlap of these two diseases would result in an increasing number of co-infected individuals. However, global data indicate that, contrary to early expectations, there seems to be no significant increase in leprosy and HIV co-occurrence [3]. Most of the larger studies on the subject were done in the early to mid-1990s, examining the rate of HIV seropositivity among leprosy patients [3]. Recently, a study from Manaus, a major Brazilian city where both leprosy and HIV are endemic, indicated higher leprosy prevalence among HIV-positive individuals when compared with the general population [4].

At the beginning of the AIDS epidemic, it was a well-accepted notion that if the new disease would have an impact on leprosy, it would be by boosting the number of multibacillary cases, as indeed observed for other mycobacterioses, especially tuberculosis. Another possibility was that the different incubation period for tuberculoid (2–5 years), as compared with lepromatous leprosy (5–15 years), could shift

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the balance towards tuberculoid disease, since patients might die of AIDS-related infections before manifesting lepromatous disease. However, five major pre-HAART African studies reported that the ratio of lepromatous to tuberculoid leprosy was not significantly affected by HIV co-infection in countries where both diseases are endemic [3].

A subgroup of AIDS patients may present with apparent clinical deterioration despite the T-CD4+ cell count improvement induced by highly active antiretroviral therapy (HAART). This immunopathological inflammatory phenomenon has been called immune reconstitution inflammatory syndrome (IRIS) [5, 6]. Tuberculosis, *Mycobacterium avium* complex infection, cryptococcosis, histoplasmosis, and other infectious and noninfectious diseases such as sarcoidosis have been associated with IRIS [6]. Leprosy has also been reported in association with IRIS (Fig. 35.1) [4–6]. A case definition has been recently proposed for leprosy-associated IRIS which includes the following: (1) leprosy and/or type 1 leprosy reaction (T1R) presenting within 6 months of starting HAART, (2) advanced HIV/AIDS infection, (3) low T-CD4+ cell count before starting HAART, and (4) T-CD4+ cell count increasing after HAART has been started [6]. Leprosy-associated IRIS usually develops within 6 months after initiating HAART [3, 6]. However, there have been reports of periods longer than 10 months [4, 7, 8].

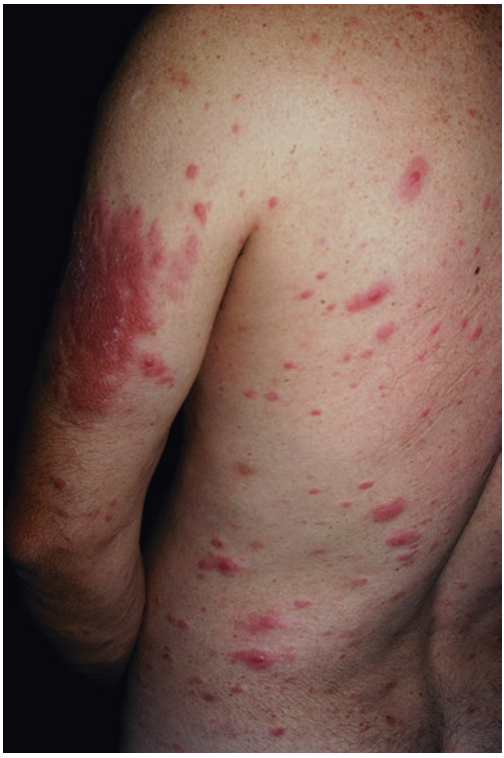

Fig. 35.1 Borderline leprosy patient presenting infiltrated lesions on the trunk and upper left limb as manifestation of IRIS

An interesting aspect of the pathogenesis of leprosy in AIDS patients with low T-CD4+ cell count is what has been called the granuloma paradox [3]: an apparent preservation of the ability to form granulomas among these patients [9], in clear contrast with what is observed in *M. tuberculosis* and HIV co-infected individuals. It has been shown that histopathological features of leprosy appear to be maintained in co-infected patients [10–12].

Massone et al. suggested that cell-mediated immune responses to *M. leprae* were preserved at the site of disease and that in the absence of CD4+ cells, CD8+FOXP3+ and CD20+ cells may be involved in granuloma formation [12]. Another study demonstrated that co-infected and non-co-infected biopsy tissues showed similar levels of IL-1β and IL-6 expression for type 1 leprosy reaction [13].

In previous reports [7, 8], three patients initially diagnosed with AIDS and borderline lepromatous (BL) leprosy shifted to borderline tuberculoid (BT) leprosy (Fig. 35.2) following initiation of HAART/multidrug therapy, indicating unstable clinical and histopathological pictures. Whether the shift was caused by IRIS or an upgrading T1R is an interesting question. The changes in the histopathological

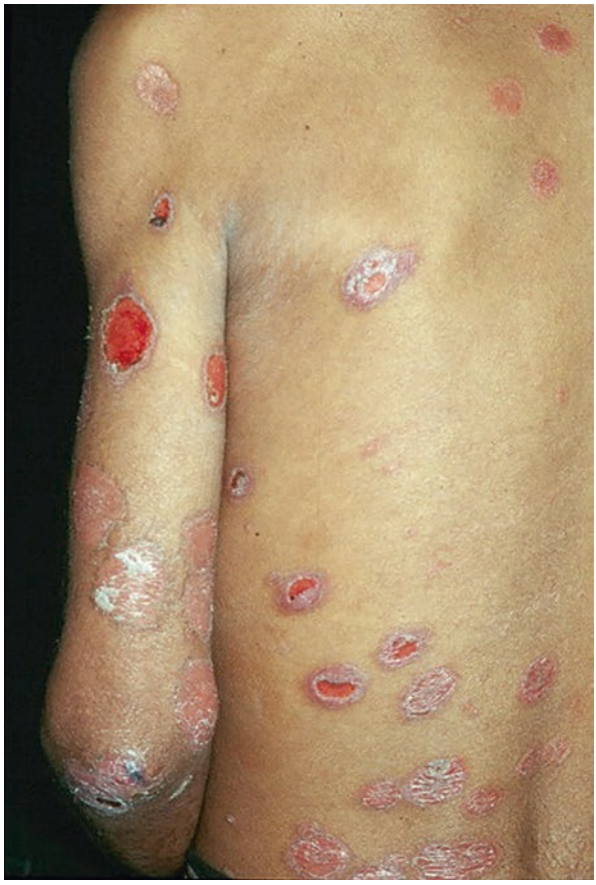

Fig. 35.2 Disseminated and ulcerated lesions on a patient with borderline tuberculoid leprosy as manifestation of IRIS

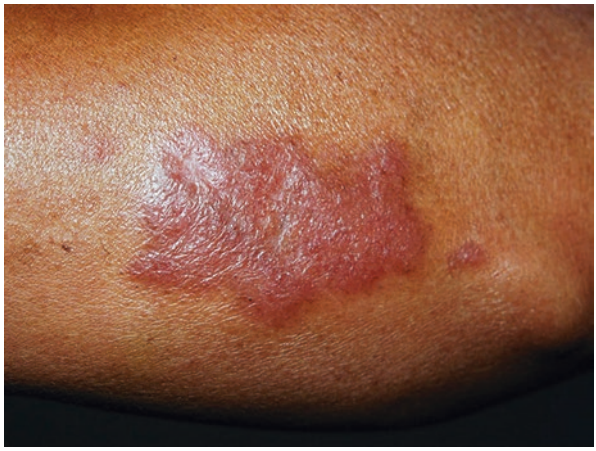

Fig. 35.3 Borderline tuberculoid leprosy in a patient with leprosy reaction type 1: an infiltrated plaque on the upper limb

aspects of these co-infected patients were also followed. After initiating HAART and MDT, the previously seen foamy histiocytes containing numerous acid-fast bacilli were replaced by granulomas consisting of lymphocytes and epithelioid cells with scant or no acid-fast bacilli [7, 8]. These findings clearly demonstrate a true impact of HIV infection, HAART, and MDT on leprosy granuloma formation, contrasting with the granuloma paradox initially proposed [3], reinforcing the need for careful follow-up of co-infected patients so that changes in clinical and histopathological findings are not missed.

The most often observed clinical form of leprosy in co-infected patients is BT (Fig. 35.3) [3, 4, 6–10]. Besides typical cutaneous (Fig. 35.4) and neurological manifestations of leprosy [3, 6, 9, 10], co-infected patients may present with hyperkeratotic eczematous and ulcerated lesions (Fig. 35.1) [4, 6, 7]. Therefore, diagnosing leprosy may be challenging for a significant proportion of AIDS patients.

One particularly challenging aspect of leprosy-HIV/AIDS co-infection is correct diagnosis of neurological manifestations. Involvement of peripheral nerve in leprosy is well-known; however, in a HIV/AIDS background, it may be confounded by neuropathy associated with HIV itself or with stavudine and other nucleoside-analog reverse-transcriptase inhibitors [4]. Another study suggested that leprosy patients with leprosy neural involvement had a greater damage gradient attributable to HIV disease [14].

Once leprosy is defined as the cause of nerve damage in *M. leprae* and HIV co-infected patients, an additional challenge is to differentiate between relapse, neural IRIS, and silent neuropathy. In these patients, clinical findings such as peripheral nerve enlargement (Fig. 35.5), sensory loss, muscular force impairment, and electroneuromyography results are important tools for diagnosis of leprosy [4].

According to published data [3, 4, 6–10], *M. leprae* and HIV co-infected patients respond to MDT as well as immunocompetent individuals, without the need for prolonged treatment courses. As previously stated, most of the post-HAART reports of dually infected patients are of BT leprosy, often presenting for the first time with

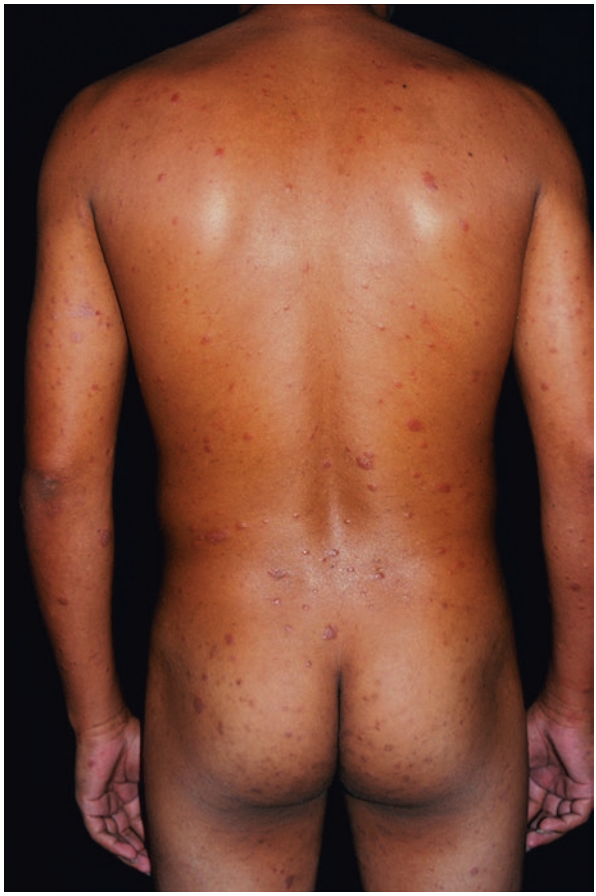

Fig. 35.4 Disseminated and infiltrated lesions on a borderline lepromatous leprosy patient

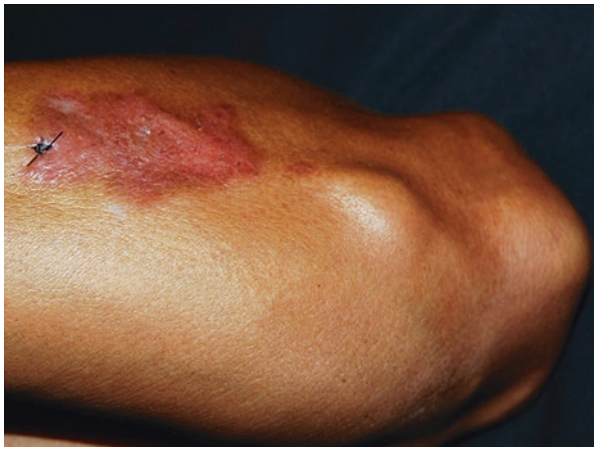

Fig. 35.5 Same patient as Fig. 35.3, 3 months after finishing multidrug therapy: an infiltrated lesion and peripheral nerve abscess as manifestation of IRIS

T1R [3, 4, 6–10]. This fact poses a challenge regarding whether it is safe to give HAART and steroids to an immunosuppressed patient presenting AIDS, leprosy, and IRIS/T1R. Moreover, co-infected patients may need prolonged steroid therapy, even after finishing MDT. Early steroid therapy may be used in AIDS patients to prevent atrophic scars and disability secondary to nerve involvement [4, 7].

It has been suggested that, even though leprosy-HIV/AIDS co-infection does not manifest homogeneously across affected populations, immunological features seem to be shared by certain subgroups. In this context, a clinical classification of *M. leprae* and HIV/AIDS co-infected patients was proposed, including the following:

(1) *M. leprae*–HIV true co-infection: this group is composed of HIV-positive individuals who do not fulfill AIDS criteria and who are not therefore under HAART, behaving similarly to immunocompetent subjects.
(2) Opportunistic leprosy disease: composed of AIDS patients not receiving HAART, presenting usually multibacillary leprosy; this group would be composed by individuals manifesting leprosy as an opportunistic mycobacteriosis, as expected in immunosuppressed individuals.
(3) HAART-related leprosy: including AIDS patients presenting all clinical forms of leprosy, related or not to IRIS. Combined HAART and multidrug therapy might cause upgrading shift within the leprosy clinical spectrum, as may be revealed by long-term follow-up [4].

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Epidemiology of Leprosy

36

Pieter A. M. Schreuder and Salvatore Noto

36.1 Epidemiology of Leprosy

Despite a long history of recognition and study, leprosy remains a poorly understood major infectious disease of man. Particularly its pathogenesis and mode of transmission are still unclear. In the last three decades, large-scale leprosy control activities have been carried out in many parts of the world, and millions of patients have been treated, yet the effectiveness of these efforts in reducing the incidence of leprosy is debated [1–5].

WHO collects statistics on a regular basis from all member states on the prevailing leprosy situation. The burden of leprosy is mostly expressed based on epidemiological and operational data. The burden of the disease from the point of view of the patient (and his/her family) is rarely taken into account. Long-term effects like disability-adjusted life years (DALY) are not considered (not even collected) when planning financial support for leprosy control programs.

In this chapter some aspects of the operational and descriptive epidemiology of leprosy will be discussed, namely: the basic epidemiological indicators for monitoring leprosy, the geographical distribution of the disease, and its trends by World Health Organization (WHO) region and selected countries.

36.1.1 Indicators

The main objectives of the leprosy control program are to cure people with leprosy, to stop the transmission of the infection, and to prevent disabilities. Health

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indicators are measures (mostly quantitative) aimed at summarizing the health situation and the performance of the health system, measuring and monitoring progress toward the achievement of objectives, and facilitating the evaluation of policies and initiatives undertaken by the leprosy control program. Indicators should be easy to measure and should directly show how far a result or objective is being achieved.

Reliability of data is based on proper collection, calculation, and interpretation of indicators. Unfortunately, available global leprosy statistics present problems [2, 5]. Particularly information may be missing about completeness of countries' data, about how cure rates or proportions of new patients with WHO disability grade 2 are calculated, and, above all, about operational and policy factors that are crucial to the analyses of trends.

The International Federation of the Anti-leprosy Associations (ILEP) suggests the following point [6]:

1. *Reliability of data*: was the basic information collected properly from the person with leprosy or from the medical records?
2. *The denominator*: an important part of any rate or proportion is the denominator; it is the population from which the cases are drawn. In a proportion or a rate, the numerator should be a subgroup of people contained within the denominator.
3. *Validity of the measures*: even if the individual pieces of information have been collected properly, are we getting an accurate overview of the situation? Validity can be reduced by several factors: difficulties in measuring important statistics; link between indicators and operational factors; changes of definition; and presence of confounding factors.
4. *Trends versus one-off analyses*: The trend that most indicators show over a long period—such as several years—is much more informative than a single reading.
5. *Presentation of the data*: For instance, figures, though usually much easier to understand than tables, can sometimes give an erroneous impression because of the scale of the axes.

36.1.1.1 Basic Epidemiological Indicators for Monitoring Leprosy

There are several epidemiological and operational indicators monitoring leprosy control activities. The most important are prevalence, incidence, proportion of new cases presenting with grade-2 disability, and treatment outcome. In simple words prevalence and incidence tell how many patients there are (standard measures of disease occurrence); a high proportion of new cases with grade-2 disability points to delay in diagnosis; and treatment outcome tells how many patients have successfully completed treatment among those diagnosed. To interpret these indicators, it must always be specified: the criteria used for diagnosis (e.g., adherence to the cardinal signs or only "clinically"; inclusion of indeterminate leprosy or postpone the diagnosis till one of the cardinal signs is positive), the definitions of case, cure and defaulter, the population, and the time. A case of leprosy is a person presenting clinical signs of leprosy who has yet to complete a full course of treatment. A patient is defined cured by the WHO when he has successfully completed his course of treatment. Defaulters are defined those patients that were not able to complete the course of treatment within the given time.

36.1.1.2 Ratio and Rate

The value obtained by dividing one quantity by another is called a ratio. The difference between a proportion and a ratio is that the numerator of a proportion is included in the population defined by the denominator, whereas this is not necessarily so for a ratio [7]. A rate is a ratio whose essential characteristic is that time is an element of the denominator and in which there is a distinct relationship between numerator and denominator. In other words, a rate is calculated for a stated period of time and when the numerator is included in the denominator.

36.1.1.3 Prevalence and Prevalence Rate

Prevalence is the number of cases of a particular disease in a defined population at a specified time. This is also called simple numerical prevalence and provides a useful and straightforward measure of the caseload. However, in order to compare prevalence at different times and places, it is necessary to express the data as proportions (prevalence rates), i.e., the number of cases of disease at a specified time divided by the population in which these cases occur. The numerator (cases) and denominator (population) figures refer to the same population. Over the past years, conventionally, the prevalence rate of leprosy has been expressed as the number of cases per 10,000 population.

$$\text{Prevalence} = \text{the number of patients registered for treatment on 31st December of a given year}$$

$$\text{Prevalence rate} = \frac{\text{Prevalence}}{\text{Population in the given area}} \times 10{,}000$$

36.1.1.4 Registered Prevalence and Registered Prevalence Rate

True leprosy prevalence (all leprosy patients in need of treatment) is made up by the patients in the community not yet diagnosed plus those diagnosed and on treatment. The data about the former group are often not available (except in case of a population survey), so statistics commonly refer to the second group only, that is to say, to the number of cases registered for treatment. This is the "registered prevalence" and is conventionally reported at the end of a given year. Registered prevalence is the nearest available indicator to true prevalence. It is influenced by factors like case finding activities, policies for maintaining registers, and duration of the disease (*prevalence = incidence times duration of disease*). In the past decennia (since the start of the leprosy elimination campaign), these factors have changed and influenced considerably leprosy statistics based on registered prevalence.

36.1.1.5 Incidence and Incidence Rate

Incidence is the number of new cases (only the new cases) of a particular disease that occurs in a defined population over a defined period of time. The time period often used in leprosy statistics is conventionally 1 year. In order to compare incidence over time and between areas, it should be expressed as a proportion (rate)

relative to the population in which the new cases occur. The source of the denominator figure (population) should be stated. Measure of occurrence of new cases of leprosy, expressed as incidence, is the most effective index of transmission of the disease. It reflects the current risk of developing leprosy within a specified population. Since the purpose of control programs is to prevent disease, their aim should be to reduce incidence. Thus, incidence statistics are more useful in monitoring the success of a control program than are prevalence statistics.

36.1.1.6 Detection and Detection Rate

Despite their value, measures of leprosy incidence are difficult to get. In fact, many new cases may not be recognized for some years after clinical onset, and repeated total population surveys are necessary to obtain true incidence. Alternatively, the number of newly detected and registered cases is frequently used as an estimate of incidence. This indicator is called detection (or case detection or new case detection). It is the nearest available indicator to incidence. Detection is the number of cases newly detected and never treated before during a given year. Detection rate is the number of cases newly detected during a given year divided by the given population. Since detection rate is used, it is important to recognize that it is influenced by the type and intensity of case-finding activities. In many endemic countries, active case finding activities, like contact examinations, have been reduced as a consequence of national leprosy elimination policies.

Annually new cases detected can be grouped separately and expressed as a proportion by age group (below 15 years of age), by the presence or absence of deformities (grade-2 disabilities), by sex, and by classification (paucibacillary (PB) versus multibacillary (MB)). Detection rates are calculated on an annual basis.

$$\text{Detection} = \text{the number of patients detected from 1 January to 31st December of a given year}$$

$$\text{Detection rate} = \frac{\text{Detection}}{\text{Population in the given area}} \times 100{,}000$$

36.1.1.7 Proportion of Children Among Newly Detected Cases

This is the number of patients under 15 years old among the newly detected patients during 1 year. It is expressed as a percentage. High proportion of children among the new cases is considered an indication of high transmission of leprosy in the given population.

$$\text{Proportion of children} = \frac{\text{B}}{\text{A}} \times 100\%$$

A = all newly detected cases during the given year.

B = number of patients under 15 years old among A.

36.1.1.8 Proportion of Cases with Grade-2 Disability Among Newly Detected Cases*

This is the proportion of people with WHO disability grade-2 among the newly detected cases during 1 year. It is expressed as a percentage. High proportion of cases presenting with grade-2 disability at the time of diagnosis can indicate a late diagnosis. This can be partly due to patient's delay in reporting to the health service or to doctor's delay in making the right diagnosis in time.

$$\text{Proportion of cases with disability} = \frac{\text{B}}{\text{A}} \times 100\%$$

B = number of patients with disability at the time of diagnosis among A.

A = newly detected cases during 1 year who have had a disability assessment.

A cohort of MB patients has a higher proportion disability grade-2 compared to a cohort of PB patients. The proportion of PB and MB patients among new patients differs between areas and over time. As such it would be more informative if the proportion grade-2 disability of a cohort of MB patients is compared with the proportion grade-2 disability of another (area, time) cohort of MB patients. The number of new patients with grade-2 disabilities is also an indicator of the need for physical and social rehabilitation and at the same time stresses the need for prevention of new and additional disabilities. It would greatly improve the monitoring process if also grade-1 disabilities were recorded and reported, and if the eye-hand-foot (EHF) scores for monitoring prevention of disability of patients were introduced [8].

36.1.1.9 Proportion of Females Among New Cases

In 2019 this was 39% and more outspoken among MB cases compared to PB cases. In general, a consistent finding is that the proportion of females among MB cases is mostly much lower than the proportion of males among MB cases. Do women in developing countries seek health care late for any health-related issues including leprosy? Or are women in general less affected by leprosy? In some areas we see an increase in female PB cases after the menopause.

The proportion of males among MB cases is especially outspoken in the more polar classifications (BL/LL). As MB patients in general have higher disability rates compared to PB patients, one will find that males have higher disability rates than females.

36.1.1.10 Proportion of Multibacillary Patients Among New Cases

The proportion of MB cases differs from area to area. In Cebu, the Philippines, there is a very high MB proportion. In contrast to this, in many |African countries, we see a low MB proportion. If a high MB proportion signifies a decline in transmission, as the incubation time of MB cases is much longer, is not sure.

36.1.1.11 Rate of New Cases with Grade-2 Disabilities per 100,000 Population per Year

This is a new indicator proposed by WHO [9]. Its place as an epidemiological indicator in leprosy has still to be determined. Leprosy is not evenly distributed and comes in clusters.

36.1.1.12 Treatment Outcome

It is essential to know the "result of the treatment" of the patients that have been diagnosed and entered in the treatment registers. Were they cured? How was their disability score at release from treatment compared to the start of treatment? Did they abandon the treatment? In what proportion? Without this information a quality assessment of the case-holding activities and of the overall performance of the leprosy control activities is difficult.

After release from treatment (RFT), patients can develop new and/or additional disabilities, and the disease can relapse. Most programs do not have a regular and planned follow-up of patients RFT (and often not even of those patients most at risk like those patients with already existing disabilities). Without such information it would be difficult to assess the overall performance of the leprosy control programs and plan new activities.

Data about treatment outcome can be obtained by analyzing cohorts of patients having started treatment during a given year. The size of each cohort should be large enough to be meaningful (arbitrarily at least 30). A group of patients with the same diagnosis and classification, PB or MB leprosy, and that were registered in the same period (that is to say, a cohort) is chosen. The results of their treatment in terms of cure, default, etc. are analyzed.

Cure rate is the proportion of patients that has been cured among the patients supposed to have been cured in the same cohort. It should be as close to 100% as possible. It would have been wiser to speak of "treatment completion rate" as the authors of this chapter suggest. We actually do not know if a patient is cured after completing treatment.

PB patients who have successfully completed their treatment—6 monthly doses within a period of 9 months. MB patients who have successfully completed their treatment—12 monthly doses within a period of 18 months. In case of a different treatment regimen, the definition has to be readjusted accordingly.

$$\text{Cure rate}\left(\text{PB}\right)=\frac{\text{PB patients who successfully completed the treatment}}{\text{PB patients who started treatment in the same cohort}}\times 100\%$$

$$\text{Cure rate}\left(\text{MB}\right)=\frac{\text{MB patients who successfully completed the treatment}}{\text{MB patients who started treatment in the same cohort}}\times 100\%$$

Defaulter rate is the proportion of patients who has not completed the treatment during the given time, among the patients who started treatment in the same cohort.

36.1.2 The WHO Enhanced Global Strategy 2016–2020

The Global Leprosy Strategy 2016–2020 "Accelerating towards a leprosy-free world" was officially launched on 20 April 2016. The overall goal is to further reduce the burden of leprosy while providing more comprehensive and timely care following the principles of equity and social justice. Its main targets are defined as:

- *Target 1*: Zero G2D among pediatric leprosy patients.
- *Target 2*: Reduction of new leprosy cases with G2D to less than one case per million population.
- *Target 3*: Zero countries with legislation allowing discrimination on basis of leprosy.

36.1.2.1 Core Programmatic Indicators:

1. Annual new case detection
2. Annual new case detection rate (per 100,000)
3. Prevalence
4. (Point) Prevalence rate (per 10,000)
5. Proportion of grade-2 disability cases among new cases
6. Proportion of pediatric cases among new cases
7. Proportion of MB cases among new cases
8. Proportion of female cases among new cases
9. Proportion of foreign-born cases among new cases
10. Number of relapses in a year
11. Treatment completion/cure rate of MB cases
12. Treatment completion/cure rate of PB cases
13. Percentage of contacts screened among the household contacts registered

36.1.2.2 Indicators for Monitoring Progress: Comparing Global Strategy 2016–2020 with Strategy 2011–2015:

What was new in the WHO Enhanced Global Strategy 2016–2020 compared to the Global Strategy (2011–2015) are the programmatic indicators (9) proportion of foreign-born cases among new cases and (13) percentage of contacts screened among the household contacts registered. Not mentioned in the new core programmatic indicators are, for example, the proportion of patients who develop new/additional disability during MDT and the proportion of patients who develop new/additional disability after RFT.

The draft Global Leprosy Strategy for the period 2021–2030 is in line with the road map on neglected tropical diseases. The goal is elimination of leprosy by 2030, which includes the following targets:

- 120 Countries with zero autochthonous cases
- Number of new cases reduced to about 63,000 worldwide
- Rate of new G2D cases reduced to 0.12 per million population

- Rate of detection of new child cases reduced to 0.77 per million child population

To sustain advances toward these targets, active leadership by countries is necessary. These should be supported by a meaningful engagement of persons affected by leprosy, accelerated efforts by all partners, and an uninterrupted supply of multidrug therapy medicines and rifampicin free of charge.

36.1.3 Geographical Distribution and Trends of New Case Detection of Leprosy by WHO Region and Selected Countries

36.1.3.1 Global Situation in the Year 2019

The global number of new cases of leprosy detected in 2019 was 202,185. Their distribution by WHO region is reported in Fig. 36.1. Leprosy is still an important public health problem in three regions, namely, Southeast Asia, the Americas, and Africa. The Southeast Asian region reports the highest number with 143,787 new cases. They represent 71% of the global total. In this region India reports the highest number in the world with 114,451 cases (Table 36.1); they represent 80% of the regional and 57% of the global new case detection (NCD). No data are available for the European region.

36.1.3.2 Global Trend

Ministries of Health from endemic countries report NCD of leprosy to WHO yearly. The number of countries reporting varies making comparisons between 1 year and another unreliable. However, the numbers of new cases detected and reported to WHO are the main indicator of the global leprosy burden and also the main indicator of disease incidence. At global level data are available from 1985 to 2019 (Fig. 36.2). The analysis of the global annual NCD of leprosy shows that this indicator has been relatively stable between the years 1985 and 1997 varying between 550,000 and 700,000 cases. Two major peaks were reported in 1998 and 2001. Between 2001 and 2005, there was a sharp decrease (strongly influenced by the Indian figures). Since 2006 this indicator of global NCD of leprosy excluding India is gradually, but slightly, declining (Fig. 36.3).

The number of new cases indicates the degree of continued transmission of infection. Global statistics showed in 2015 that 94% of new leprosy cases were reported from 14 countries reporting more than 1000 new cases each and only 6% of new cases were reported from the rest of the world. Pockets of high endemicity still remain in some areas of many countries, including countries reporting less than 1000 new cases. Some of these areas show very high notification rates for new cases and may still witness intense transmission.

36.1.3.3 Regional Trends: Southeast Asia

Annual regional data are available from 1991 to 2019. The Southeast Asian region annual trend is very similar to the global one (Fig. 36.4). Two major peaks occurred in 1998 and in 2001. Since then a steady decrease took place up to 2006, and then the indicator levelled off. Figure 36.5 shows the annual leprosy NCD trend in India.

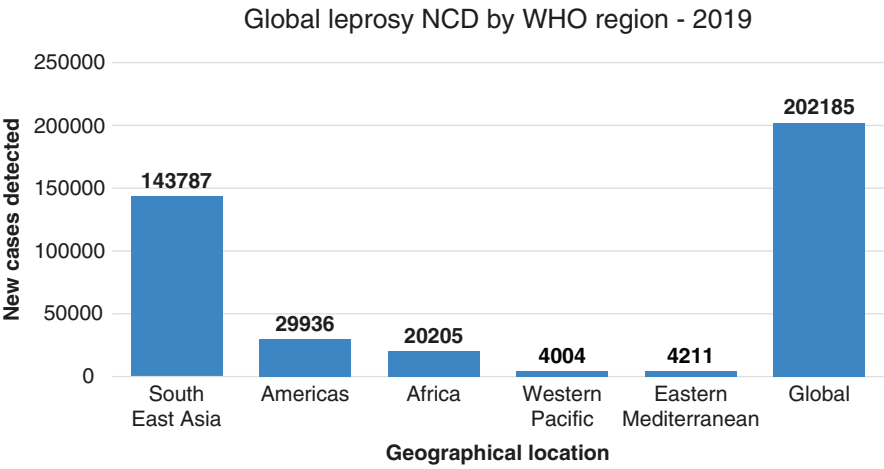

Fig. 36.1 Global leprosy new case detection (NCD) by WHO region 2019 (excluding European region)

Table 36.1 Leprosy new case detection at global, WHO selected regions and countries levels

NCD								
Year	Global	S-E Asia	India	Americas	Brazil	Africa	W–P	E–M
1980	NA	NA	NA	NA	14,515	NA	NA	NA
1981	NA	NA	NA	NA	17,133	NA	NA	NA
1982	NA	NA	NA	NA	16,994	NA	NA	NA
1983	NA	NA	NA	NA	18,798	NA	NA	NA
1984	NA	NA	NA	NA	18,854	NA	NA	NA
***1985	550,224	NA	477,000	NA	19,303	NA	NA	NA
1986	573,790	NA	507,000	NA	18,497	NA	NA	NA
1987	595,145	NA	419,000	NA	19,726	NA	NA	NA
1988	553,597	NA	474,000	NA	26,615	NA	NA	NA
1989	550,743	NA	466,000	NA	27,844	NA	NA	NA
1990	571,792	NA	481,000	NA	28,765	NA	NA	NA
*1991	613,016	492,292*	517,000*	30,532**	30,874**	41,989	NA	NA
1992	667,133	559,765	547,000	33,699	33,396	38,024	NA	NA
***1993	615,830	495,344	494,000	38,364	34,251	39,654	NA	NA
1994	560,646	456,882	427,000	36,623	33,190	47,900	12,737	6504
1995	529,376	428,652	425,571	36,842	36,263	46,516	12,135	5231
1996	566,567	457,921	415,302	43,783	40,505	46,489	12,613	5761
1997	684,961	565,416	524,411	43,159**	45,125**	56,507	13,573	6306
1998	804,357	689,069	634,901	47,218	42,444	51,530	10,617	5923
1999	738,112	621,620	537,956	45,599	42,389	55,635	9501	5757
2000	719,219	606,703	559,938	44,786	41,305	54,602	7563	5565
2001	763,262	668,658	617,993	42,830**	44,608**	39,612	7404	4758
2002	620,638	520,632	473,658	39,939	47,506	48,248	7154	4665

(continued)

Table 36.1 (continued)

NCD								
Year	Global	S-E Asia	India	Americas	Brazil	Africa	W–P	E–M
2003	514,718	405,147	367,143	52,435	49,206	47,006	6190	3940
2004	407,791	298,603	260,063	52,662	49,384	46,918	6216	3392
2005	299,036	201,635	169,709	41,952	38,410	45,179	7137	3133
2006	265,661	174,118	139,252	47,612	44,436	34,480	6190	3261
2007	258,133	171,576	137,685	42,135	39,125	34,468	5863	4091
2008	249,007	167,505	134,184	41,891	38,914	29,814	5859	3938
2009	244,796	166,115	133,717	40,474	37,610	28,935	5243	4029
2010	**228,474**	**156,254**	**126,800**	**37,740**	**34,894**	**25,345**	**5055**	**4080**
2011	226,626	160,132	127,295	36,832	33,955	20,213	5092	4357
2012	232,857	166,445	134,752	36,178	33,303	20,599	5400	4235
2013	215,656	155,385	126,913	33,084	31,044	20,911	4596	1680
2014	213,899	154,834	125,785	33,789	31,064	18,597	4337	2342
2015	210,740	156,118	127,326	28,806	26,395	20,004	3645	2167
2016	217,968	163,095	135,485	27,356	25,218	19,384	3914	2834
2017	210,671	153,487	126,164	29,101	26,875	20,416	4084	3550
2018	208,641	148,495	120,334	30,957	28,660	20,590	4193	4356
2019	202,185	143,787	114,451	29,936	27,863	20,205	4004	4211

These data give only a gross indication of the global and regional leprosy trend. The countries reporting to WHO are different from 1 year to another. This makes comparisons imprecise. Available data show an imprecision for Southeast Asia and India for the year 1991* (numbers should be higher for Southeast Asia); for Americas and Brazil for the years 1991, 1997, and 2001** (numbers should be higher for Americas). The 1985–1993*** series of global data refers to 32 selected countries only [10, 11], [12–18]

E–M, Eastern Mediterranean; *NA*, data not available; *NCD*, new case detection; *W–P*, Western Pacific

Here too there are two major peaks reported in 1998 and 2001, a steady decrease up to 2006 and then the levelling off of the indicator.

It is the trend in India that has determined the shape of the Southeast and global graphics. In leprosy something "exceptional" has happened in India between 1996 and 2006. The increase in NCD between 1996 and 2001 and the decrease reported from 2001 to 2006 are faster than expected for a chronic disease like leprosy. Among the many operational factors that may have determined this particular trend are increased, "exceptional," efforts in case detection activities with clearance of the backlog cases (proportion of cases with onset of the disease in previous years). These could explain the fast increase in NCD, the fast decrease of the indicator, and its levelling off to values nearer to the true incidence of the disease in the 2006–2019 period.

36.1.3.4 Regional Trends: Americas, Africa, Western Pacific, and Eastern Mediterranean

– In the American region, the annual NCD of leprosy has been relatively stable between the years 1996 and 2009 varying between 40,000 and 50,000 cases

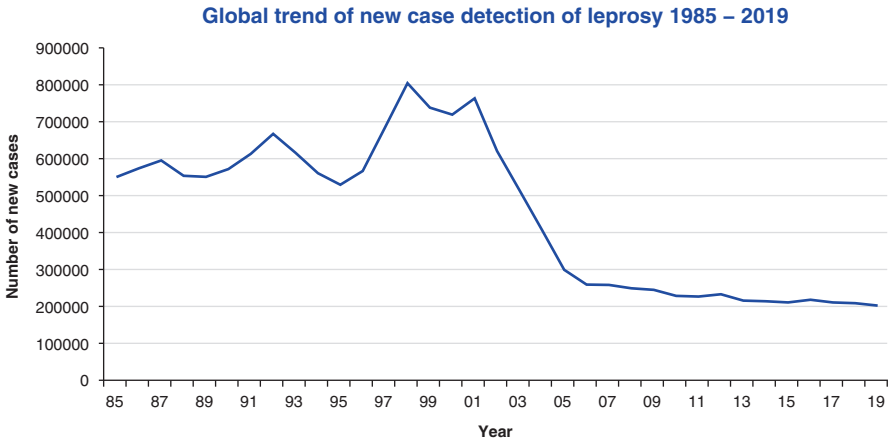

Fig. 36.2 Global trend of new case detection of leprosy 1985–2019. The 1985–1993 series of data refers to 32 selected countries only

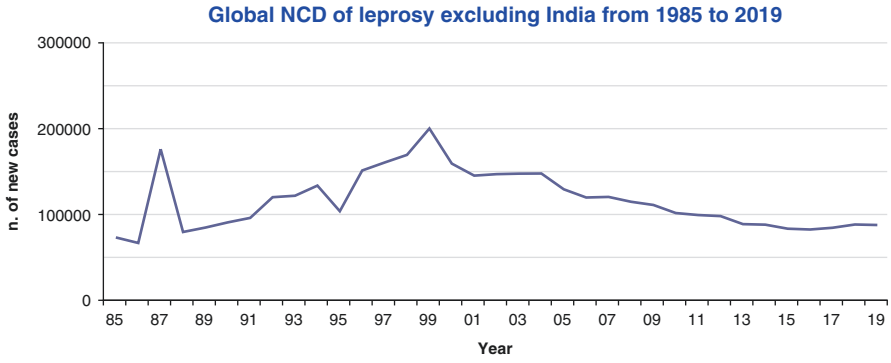

Fig. 36.3 Global annual new case detection of leprosy excluding India, from 1985 to 2019

(Fig. 36.6); figures are now decreasing. In this region 92% of the cases are in Brazil (Fig. 36.7).

- In the African region (Fig. 36.8), the last peak with 48,248 cases was reported in 2002; then figures started decreasing till 2011, and since more or less stable.
- In Western Pacific region, a peak of new case detection of leprosy, with 7137 cases, was reached in 2005; since then the indicator is declining.
- In Eastern Mediterranean the annual number of new leprosy cases has been around 3000–4000 cases annually since 2003, but varies rather widely (Table 36.1).

36.1.3.5 Conclusions

Over the last decennia, international and national anti-leprosy policies have changed greatly, strongly influencing operational factors which not always lead to quality

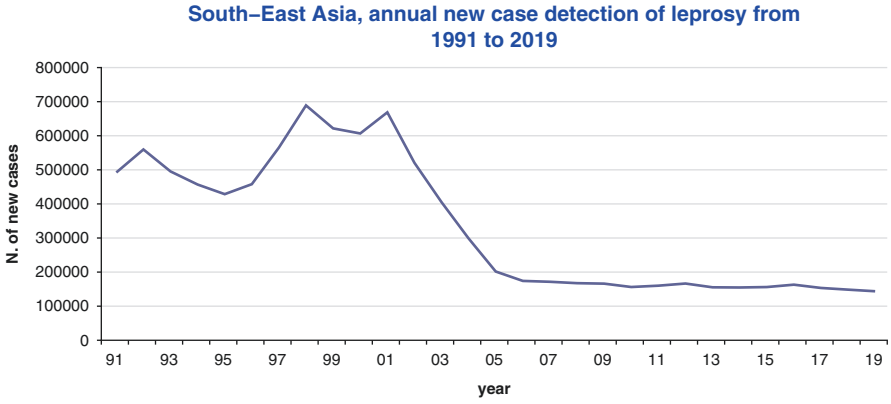

Fig. 36.4 Southeast Asia. Annual new case detection (NCD) of leprosy from 1991 to 2019

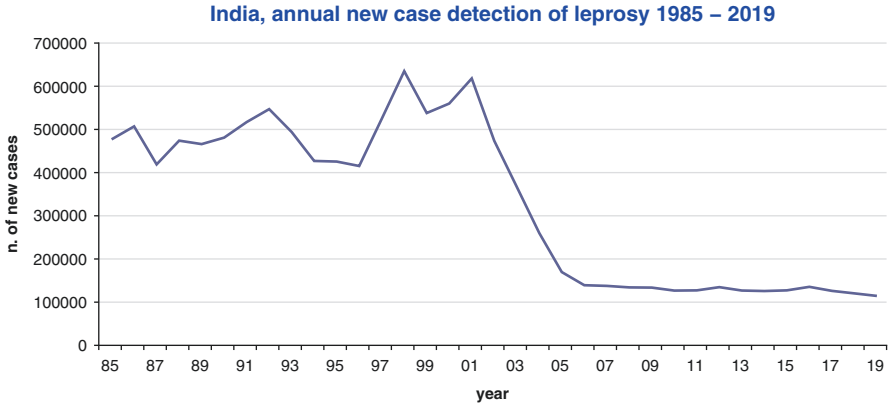

Fig. 36.5 India. Annual new case detection of leprosy from 1985 to 2019

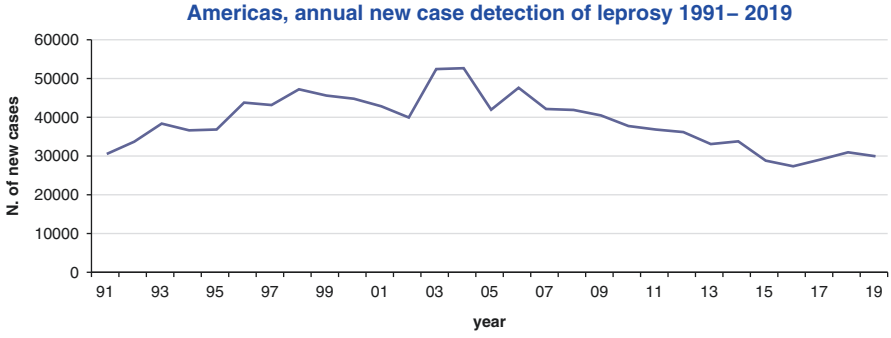

Fig. 36.6 Americas. Annual new case detection of leprosy from 1991 to 2019

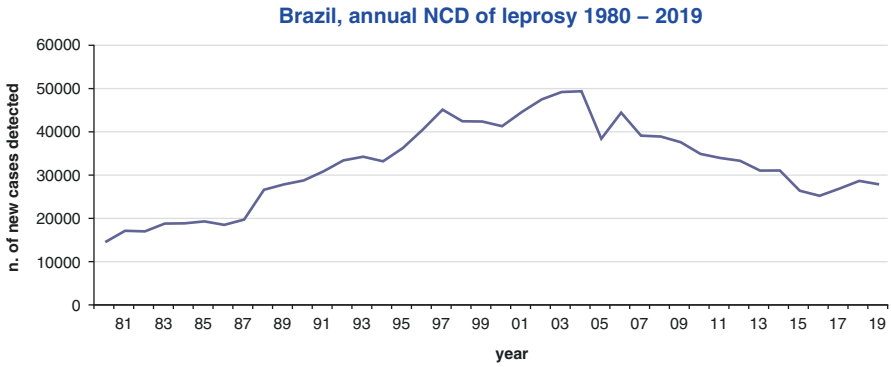

Fig. 36.7 Brazil. Leprosy annual new case detection trend 1980–2019

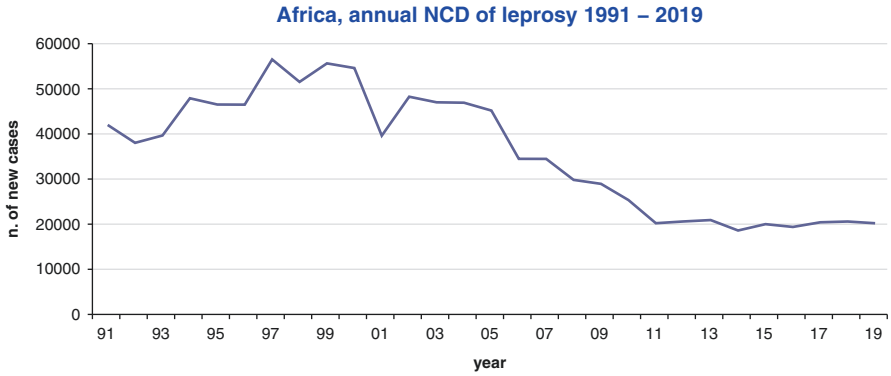

Fig. 36.8 Africa. Annual new case detection (NCD) of leprosy from 1991 to 2019

leprosy services and reliable leprosy statistics. Only detailed, country-level knowledge of these operational factors (see the Indian example) would allow confident interpretation of the data. However, a slight, gradual rate of decline in NCD of leprosy started in the period 2006–2010 and continues up to today.

Over the past 15 years, we have seen a kind of turnaround in leprosy control: from attention to prevalence (the WHO strategy named "elimination of leprosy as a public health problem" was unwarranted based on this indicator) to incidence (reducing transmission), from only passive case reporting to providing funds for active case-finding activities to promote early detection and prevent nerve damage. Renewed attention should go to detection and treatment of silent neuritis, treatment of reactions (reversal reactions and ENL) and prevention of further disability, upgrading skin smear services, attention to possible drug and multidrug resistance in leprosy and the importance of post-MDT chemoprophylaxis in the case of LL patients. Leprosy Post-Exposure Prophylaxis is being implemented in several countries.

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Leprosy Control

37

Sunil Deepak and Giovanni Gazzoli

37.1 Introduction

"Leprosy control" means planning and organization of health services at different levels to reduce the incidence of leprosy and its consequences in a given population. Leprosy control strategies and services are planned at national and sub-national levels by Ministries of Health. The national strategies of leprosy control are also guided by regional and international strategies prepared by the Global Leprosy Programme of the World Health Organization (WHO/GLP). Leprosy is a part of the Neglected Tropical Diseases (NTD) in WHO, which creates opportunities for creating synergies between leprosy and other infectious disease control programs.

Leprosy control strategies in a country include different components, such as:

- Making a national plan including strategies for public awareness and health education, diagnosis, treatment, complications, drug supply, prophylaxis, reporting, monitoring, and evaluation
- Setting criteria for diagnosis and treatment of persons with leprosy and its complications
- Defining the roles of different levels of the health services, including the role of different health professionals in leprosy diagnosis, treatment, and rehabilitation
- Defining norms for conducting surveys and examination of persons for new cases of leprosy, including contact examination and prophylaxis
- Preparation and distribution of information and training materials

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- Organizing training and capacity building of health professionals at different levels
- Setting up appropriate referral services
- Setting up case registers and data collection systems
- Organizing periodic evaluations to evaluate the efficacy of leprosy-related services
- Networking with other services and programs such as services related to supply of protective footwear, assistive products and community-based rehabilitation (CBR)
- Setting up mechanisms of participation of organizations of persons affected with leprosy in leprosy control services

37.2 Leprosy and Concepts of Disease Control, Elimination, and Eradication

WHO Conference on "Global Disease Elimination and Eradication as Public Health Strategies," held in Atlanta in 1998, agreed on the definitions of control, elimination, and eradication of infectious diseases.

Control of an infectious disease is defined as "the reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction."

Elimination of an infectious disease is defined as, "reduction to zero of the incidence of infection caused by a specific agent in a defined geographical area as a result of deliberate efforts; continued measures to prevent re-establishment of transmission are required."

Eradication of an infectious disease is defined as "permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; continued intervention measures are no longer needed."

There is another related definition, that of *extinction of an infectious disease*, that is defined as, "the specific infectious agent no longer exists in nature or in the laboratory."

In 1991, World Health Assembly had adopted the goal of "eliminating leprosy *as a public health problem* by reducing its prevalence to less than 1 case per 10,000 population." This goal, also known as "the leprosy elimination goal," was reached at global level in 2000 and should not be confused with definitions of control, elimination, and eradication of infectious diseases given above. In terms of the definitions given above, the "leprosy elimination goal" corresponds to "control of leprosy."

37.3 Historical Overview of Leprosy Control Programs

Over the past six decades, leprosy control programs have undergone many significant changes. Around the early 1950s, use of dapsone became common in most leprosy-endemic countries. Dapsone controlled the infection but did not cure it.

Thus, leprosy control strategy continued to be isolation of affected persons in leprosy colonies and villages, where they were treated with dapsone [1].

In 1982 WHO introduced the multidrug therapy (MDT) [2]; initially, it consisted of two regimens, paucibacillary (PB) and multibacillary (MB), divided on the basis of the bacteriological index (BI). PB cases were those with BI < 2+ at any site, and MB were cases with BI ≥ 2+ at any site. In 1988, the WHO Expert Committee on Leprosy [3] changed the microbiological criteria for their classification: paucibacillary cases included only smear-negative patients.

During the 1980s, many leprosy endemic countries launched national leprosy control programs, based on domiciliary treatment with MDT. In 1991, World Health Assembly decided the "leprosy elimination goal." In the following years, the norms for leprosy control were simplified including fixed duration of treatment for all cases of leprosy and a new method of classification into PB and MB, not on the basis of BI, but on the basis of the number of skin lesions.

The "leprosy elimination goal" was reached in 2005. Since then, through elaboration of Global Strategies, WHO Global Leprosy Programme (GLP) has been guiding the norms of national leprosy programs. A new Global Strategy for Leprosy Control (GSLC) for the period 2016–2020 [4] was proposed by WHO in 2016, while the new Guidelines for diagnosis, treatment, and prevention of leprosy came out in 2018 [5].

Over the past 15 years, the annual number of new cases has stabilized at global level around 200,000. To further reduce the disease transmission, the new Guidelines include post-exposure prophylaxis with a single dose of rifampicin (PEP/SDR) among the contacts of all new cases. It recommends a uniform MDT composed of three drugs (dapsone, clofazimine, and rifampicin) for all cases—for 6 months for PB cases and 12 months for MB cases.

37.4 Goal and Guiding Principles of Leprosy Control

The goal of leprosy control is to provide high-quality services based on principles of equity and social justice, for reducing the leprosy burden in communities.

The guiding principles of leprosy control include organizing services that ensure early detection, free treatment with MDT, adequate referral services for acute complications, and continued care for chronic consequences of the disease. It also includes PEP/SDR among the contacts and a partnership with leprosy-affected persons to end discriminatory laws affecting them. It recommends that the quality of leprosy control services should be monitored through trends of new cases with grade 2 disabilities. The three pillars of leprosy control are:

- Stop leprosy and its complications.
- Stop discrimination and promote inclusion.
- Strengthen government ownership, coordination, and partnership.

The objectives of leprosy control in the Global Strategy are:

- Zero Grade 2 Disability (G2D) among pediatric leprosy patients
- Reduction of new leprosy cases with G2D to less than one case per million population
- Zero countries with legislation allowing discrimination on basis of leprosy

37.5 Leprosy Control Through Primary Healthcare Services

In most countries, the leprosy control activities are organized as a part of the primary healthcare (PHC) services. For example, community health workers are trained to identify suspected cases of leprosy and refer them to the nearest primary healthcare centers, where trained health professionals (depending upon the country, these could be paramedical workers or nurses or doctors) can confirm the diagnosis and start the treatment with MDT.

Community-level health workers distribute the monthly medicines and ensure that the persons complete the treatment, as well as look out for any complications. They are also responsible for community awareness, health education, and information to combat stigma and prejudice against leprosy, common in most countries.

Providing leprosy-related services through PHC can increase the coverage of leprosy control and allow persons to receive treatment and care closer to their homes. The services are provided in integrated settings, where persons also receive care for any other health problems, and this helps in combating stigma and discrimination [6].

However, the coverage of PHC services and adequate training of the staff involved in leprosy-related work are two key issues to be considered for ensuring good quality of care. Lack of primary healthcare services, lack of staff, or high turnover of staff can have negative impact on leprosy control services. To overcome some of these problems, some leprosy programs also work in collaboration with community health programs run by non-governmental organizations.

Leprosy control services through primary healthcare system require adequate support from referral services for supervision, on-the-job training, bacilloscopy and confirmation of diagnosis for difficult cases, and treatment of complications such as reactions.

37.6 Voluntary Reporting of New Cases

In the past, countries adopted different strategies for early case detection, including school surveys and general population surveys. According to GSLC, the promotion of voluntary self-reporting is crucial to case detection, while active case finding methods, including large-scale campaigns, are generally recommended only among areas with higher endemicity and/or for hard-to-reach areas.

Effective voluntary reporting of new cases requires that population at risk in endemic areas is aware about the early signs and symptoms of leprosy and knows

that primary healthcare services can provide diagnosis and free treatment. At the same time, having community-level health workers who are aware of early signs and symptoms of leprosy also helps in early identification of new cases.

Percentage of persons with grade 2 disabilities among the new cases and especially among children is a good indicator to monitor the effectiveness of voluntary case reporting. Increasing proportion of persons with grade 2 disabilities among the new cases means that voluntary case reporting is not functioning properly. In such situations, GSLC suggests organization of active case-finding campaigns in areas of higher endemicity and contact management.

37.7 Contact Examination and Post-Exposure Prophylaxis

Since contacts of both PB and MB cases of leprosy are at much higher risk of having leprosy compared to general population, most leprosy control programs organize a checkup of family contacts of all newly diagnosed cases of leprosy. GSLC suggests voluntary self-reporting of contacts and home visits for contact examination only in areas of high endemicity.

However, different leprosy control programs may define "family contacts" differently. For example, many leprosy control programs consider persons living inside the same household as "contacts" to be examined. Some other leprosy control programs also consider some neighboring households as contacts.

WHO/GLP recommends the use of single-dose rifampicin (SDR) as preventive treatment for contacts of leprosy patients (adults and children 2 years of age and above), after excluding leprosy and TB disease and in the absence of other contraindications. This intervention should be implemented by programs that can ensure (i) adequate management of contacts and (ii) consent of the index case to disclose his/her disease [5].

37.8 Countries and Areas With Higher Disease Burden

In most endemic countries, active cases of leprosy are not distributed uniformly over the whole territory but are usually found in clusters. During 2019, 16 countries reported significant number of new leprosy cases: Brazil, India, and Indonesia reported >10,000 new cases each, while 13 other countries (Bangladesh, Democratic Republic of the Congo, Ethiopia, Madagascar, Mozambique, Myanmar, Nepal, Nigeria, Philippines, Somalia, South Sudan, Sri Lanka, and the United Republic of Tanzania) each reported 1000–10000 new cases [7].

Depending upon the areas with higher number of new cases and the new case detection rates, these areas need specific strategies of leprosy control to ensure that leprosy diagnosis and treatment facilities are available through primary healthcare services, and services for care of complications and referral are organized in such a way that they are accessible to all those in need.

Most urban areas in endemic countries need different leprosy control strategies compared to the rural areas. For example, persons living in urban slums, low-income

areas, and peripheries often include poor emigrants who may frequently change residence or who face additional barriers for accessing health services. The health services in urban areas are fragmented between hospitals, private health practitioners, traditional healers, and government services, so organizing diagnosis and treatment services requires some understanding of these different factors.

Specific groups of persons in urban areas such as poor persons, street and pavement dwellers, and sex workers face additional barriers in accessing health and social services and thus require specific consideration for leprosy control programs.

37.9 Areas With Low Disease Burden of Leprosy

Most of the 115 countries that reported new cases of leprosy in 2019 have a low disease burden due to leprosy. Countries and areas with low disease burden pose different challenges for the organization of leprosy control services for ensuring timely diagnosis and regular treatment of new cases.

Health workers in areas with low disease burden usually see few or no new cases of leprosy in a year. Thus, even if they were trained in diagnosis and treatment of leprosy, they tend to forget this knowledge because of lack of opportunities for using it. Conducting repeated refresher courses for the health personnel in such situations may not be cost-effective. Some countries are attempting training through mobile apps in such situations [8].

Areas with low disease burden can organize leprosy control services through identification of referral or specialized centers for disease conditions that are not very common, accompanied by a communication plan to inform the health centers to recognize suspected cases and refer them for confirmation of diagnosis and starting of treatment.

37.10 Leprosy in Specific Groups

Isolated populations and difficult to access areas or areas where the coverage of health services has gaps require specific strategies. The Global Strategy advises that the strategy adopted should give emphasis to strengthening and sustaining health services at community level.

For deciding strategies, the specific population groups, the range of services available, and any gaps in answering the needs related to leprosy control should be identified. This should lead to the development of a plan, which focuses on building partnerships with various stakeholders, through activities such as recruiting local community volunteers and strengthening capacity of local health workers [4].

Specific groups in a population including children, poor women, and minorities, who may face additional barriers to access existing services, require specific strategies. Leprosy program managers may need to monitor data and eventually undertake studies to understand the situation of these specific groups and the barriers they face, to design strategies for reaching these groups.

37.11 Role of Referral Services

Leprosy control services integrated in the primary healthcare system require the support of efficient referral services to be effective. According to WHO, leprosy diagnosis and management of reactions may require specialist support. Similarly, persons with leprosy-related disabilities often require lifelong protective tools to prevent worsening of disabilities and may need reconstructive surgery and assistive devices. Thus, partnerships with specialized centers are important [9].

General referral services such as reconstructive surgery, orthopedics, assistive products, ophthalmology, etc. have a significant role in rehabilitation of persons with disabilities due to leprosy. In the past, many vertical leprosy programs were providing these services but with decreasing disease burden, such specialized services meant only for persons affected with leprosy are rare. Thus, leprosy control services need to link with general referral services to ensure that persons affected with leprosy can access them.

37.12 Role of Persons Affected With Leprosy and Their Organizations

The Human Rights Council of United Nations has recommended that "Persons affected by leprosy and their family members, have the right to be actively involved in decision-making processes regarding policies and programmes that directly concern their lives" [10].

WHO has produced guidelines for promoting the participation of affected persons in leprosy control programs [11]. According to these guidelines, people who have personally experienced the disease are important partners in their treatment. Ensuring that persons affected by leprosy are the central focus of the program will have profound implications for the way that services are planned, delivered, and evaluated.

These guidelines identify different areas in which persons affected with leprosy can play an important role. The primary issues are stigma and discrimination; equity, social justice, and human rights; and gender. The central theme of the guidelines is to recognize the expertise of individuals who have had the disease and, through partnership, enable these individuals to support the delivery of leprosy services. For example, persons with personal experience of planter ulcers or loss of sensation can be very effective in reaching out to other affected persons about the importance of a regular routine of prevention of disabilities and self-care activities.

At the same time, the guidelines recognize that facilitating the participation of persons affected by leprosy is not simple. It is essential to document the process and results, provide feedback on lessons learned, and disseminate the results to a wider audience to expand the process.

37.13 Rehabilitation Needs of Persons Affected With Leprosy

There are different groups of persons affected with leprosy who have specific rehabilitation needs. The WHO Global strategy for 2016–2020 advises that the persons affected with leprosy should be integrated in the CBR programs dealing with persons with different kinds of disabilities. At the same time, leprosy programs need to network with other programs such as the Assistive Technology 2030 initiative promoting access to quality assistive products for persons with disabilities and elderly persons [12].

Leprosy services should collaborate with any existing CBR programs and participate in the training of their personnel to ensure the inclusion of the persons with disabilities due to the disease in the rehabilitation activities. Leprosy services can also work with CBR programs to identify any barriers faced by leprosy-affected persons in accessing those programs and devising strategies to overcome barriers. Leprosy services can inform leprosy-affected persons about the different services offered. CBR Guidelines published jointly by WHO, UNESCO, ILO, and IDDC (2010) include a separate module on inclusion of persons with disabilities due to leprosy in the CBR programs [13].

37.14 Indicators for Monitoring Effectiveness of Leprosy Control Services

WHO/GLP suggests a long list of indicators for monitoring the leprosy situation in a country [9], including the following:

- Annual new case detection and new case detection rate (per 100,000 population), aggregated by sex and age group.
- Point prevalence of leprosy (per 10,000 population)
- Proportion of newly diagnosed leprosy patients with G2D, disaggregated by sex
- Number of G2D among pediatric cases
- Number and/or proportion of relapses among all leprosy notified cases
- Proportion of MB cases among new cases
- Completion rate of MDT in all cases, disaggregated by sex
- Availability of web-based, case-based reporting system allowing disaggregation by age, sex, place of residence, and other relevant criteria (e.g., foreign born)
- Proportion of contacts screened among registered contacts
- Proportion of patients evaluated for disabilities at the end of treatment, disaggregated by sex
- Proportion of patients having reactions among new cases, disaggregated by bacillary load (PB or MB)
- Proportion of reactions occurring after treatment among the total number of reactions

Leprosy control services need to provide national guidelines about patient cards, case registers, contact registers, disability registers, etc. for collecting information for program monitoring in a uniform manner. Specific forms are necessary for the transmission of leprosy data from periphery to the central levels, so that program managers can assess the program needs (such as for supply of MDT and medicines for treating reactions) and quality of leprosy services through specific indicators.

37.15 Other Issues

There are some other significant issues related to organization of leprosy control services, such as the following:

37.15.1 Informatiotn and Communication

Since new case detection is almost exclusively through voluntary presentation of suspected cases, leprosy control services need to work together with programs of health promotion and prevention of diseases to ensure that information about early signs and symptoms of leprosy and free treatment in the primary healthcare centers is available in communities in endemic areas.

Areas with higher burden of leprosy may need specific information and communication materials as well as interventions through public media. Awareness about easy curability of leprosy through MDT and campaigns against social stigma and prejudice are also significant components of information and communication strategies.

37.15.2 Training of health personnel

Analyzing the needs of different levels of health personnel involved in leprosy control services, preparing training materials to answer those needs, and organizing training courses are also responsibility of leprosy control programs. Through Internet, countries can access specific training materials from organizations such as International Leprosy Association (ILA), ILEP, Infolep, and IDEA. Internet also allows free access to scientific journals such as *Leprosy Review* as well as email-based resources like Leprosy Mailing List.

37.15.3 Research

Research on areas such as new anti-leprosy drugs, surveillance on development of drug resistance, and use of chemo-prophylaxis increasingly requires collaboration between scientists and leprosy programs. Regional offices of WHO as well as international organizations such as ILA and ILEP can facilitate these interactions.

37.16 Conclusions

With changing epidemiological profiles of leprosy, leprosy control services need to face new challenges. After an initial decrease, the number of new cases of leprosy is stable, and a significant number of cases remain in different parts of the world. A WHO report on the leprosy situation at the end of 2019 [7] noted that the targets decided in the Global Strategy for the period 2016–2020 for reducing the number of persons with G2D among the new cases and among children will not be reached. Even for the target of repealing discriminatory legislations from countries, 22 countries still had such laws. Thus, the leprosy control programs continue to face a lot of challenges.

Post-exposure prophylaxis with single-dose rifampicin has been introduced to reduce the disease transmission and the number of new cases. This process and its impact need to be closely monitored. There is also a growing need to identify new strategies for providing quality leprosy control services in situation of low endemicity.

The future of leprosy control for answering the needs of leprosy-affected persons has to be in integrated settings, through primary healthcare and through collaboration with other programs such as CBR and other infectious diseases control programs and through greater role of affected persons in their own care. Mobile telephony and Internet-based new technologies are becoming increasingly important in sharing information and capacity building of national leprosy control programs.

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The Leprosy Mailing List

38

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38.1 Introduction

The Leprosy Mailing List (LML) began in 2001 as an email-based moderated discussion forum circulated among a few friends who had experience of working in leprosy control programs. Gradually, it expanded among persons with an interest in leprosy from all over the world. It is an independent, free forum run on a voluntary basis by small editorial team—P. Schreuder (moderator), S. Noto (founder), B. Naafs, and S. Deepak.

The LML is open to everyone who has an interest in leprosy. To join the list, individuals should send an email to the moderator (editorlml@gmail.com).

The list has around 600 subscribers, among whom there are persons affected with leprosy as well as different health personnel including professionals with long experience of working in leprosy programs. The subscribers represent a wide variety of backgrounds including leprosy control, research, public health, laboratory, dermatology, tuberculosis, ophthalmology, neurology, infectious diseases, non-governmental organizations, and scientific journals.

38.2 History

During the initial years, the LML received support from two Italian academic institutions—Centre for Training and Research in Public Health in Caltanissetta and San Martino University hospital in Genoa, while its archives were hosted by the Italian leprosy relief organization (AIFO).

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Since 2013 the management of the list is completely on a voluntary basis. Its recent archives are accessible to public through a Google group archive (https://groups.google.com/g/leprosymailinglist) and through a blog (http://leprosymailinglist.blogspot.com/).

38.3 Norms and Functioning

The LML is seen as a safe space for sharing new information, raising doubts, and debating ideas, including controversial issues, in a mutually respectful manner. It does not have a formal peer-review process for publishing contributions from members; however, individuals can share their views through articles explaining their arguments along with data and references.

Members can send contributions such as papers, letters, comments, and reports to the moderator, who edits them in a common format, and if needed, asks for clarifications from the authors, before circulating them in the list to all the members. All contributions to the LML are accepted as far as they are relevant to leprosy. When in doubt about a contribution, it is discussed jointly by the editorial team for taking a decision.

38.4 Issues Discussed in LML

The list brings together persons working in the field who can share doubts about cases under their care and ask for opinions from more experienced persons. Thus, clinical issues are the most popular. It is also a space for sharing information regarding new publications such as the WHO guidelines. Occasionally, researchers use the list to reach out to persons they would like to involve in their surveys or to identify specific field programs.

A review of messages shared on LML during the last 12 months shows that a total of 190 messages were shared in 2020. The most discussed issues on the list included strengthening the evidence-based practices in leprosy control, the impact of coronavirus (Covid-19) epidemic on leprosy services and drug supply, the duration of treatment of lepromatous leprosy, and the surveillance of development of drug resistance in leprosy.

38.5 Considerations About Role of LML

Over the past three decades, the prevalence of leprosy has reduced significantly, while the yearly incidence continues to be relatively stable. Today, it is seen as a niche area, and many specialized leprosy journals have stopped publishing. With integrated leprosy programs in the countries, professionals with wide experience in the disease are fewer, and specific expertise about the impact of advanced disease and its complications may not be available in many countries. Though only a few

countries report significant numbers of new cases per year, the number of countries with lesser incidence continues to be large. According to the epidemiological report of the World Health Organization [1], in 2019 16 countries reported more than one thousand new cases, while another 99 countries reported lesser number of cases. In this situation, LML provides a valuable forum for discussions and for sharing of information and expertise.

In an international consultation on defining the global leprosy strategy for the period 2021–2030, held in October 2020, the Global Leprosy Programme of the World Health Organization identified different challenges facing leprosy control. These included limited engagement of the stakeholders and dwindling leprosy expertise across countries [2]. This again confirms the importance of the role played by LML.

LML is an open forum; one is welcome to put his/her considered opinion, preferably backed up by references, even when this opinion is not shared by "official" organizations and governments.

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Part IX

Buruli Ulcer: General Section

History and Geographic Distribution of Buruli Ulcer

39

Françoise Portaels and Gerd Pluschke

39.1 Introduction

Cutaneous ulcers caused by *Mycobacterium ulcerans* were discovered more than 80 years ago, at nearly the same time, in two antipodal regions: in 1937 in southeast Australia and in 1942 in tropical Africa. At the time, these discoveries did not generate much interest in the medical/scientific world [1]. However, anyone interested in how a medical curiosity is transformed into a topic of worldwide interest should study the initial report of MacCallum, Tolhurst, Buckle, and Sissons, published in 1948, on their observations and pioneer findings in *M. ulcerans* infections [2]. In spite of the increased interest in this new disease, it remained largely ignored for decades by many national public health programs.

It was only in 1998 that WHO launched the Global Buruli Ulcer Initiative (GBUI), following the visit of its Director-General, Dr. Hiroyoshi Nakajima, to Côte d''Ivoire. Nakajima was impressed by the debilitating tropical disease that destroys the skin of its victims: Buruli ulcer (BU). The first international conference on BU was organized by WHO in July 1998. The GBUI was established to coordinate BU control and multidisciplinary research efforts in partnership with member states, academic and research institutions, nongovernmental organizations, and other foundations.

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The history of BU may thus be divided into two periods: before 1998 and after 1998.

39.2 Historical Overview

39.2.1 What Was Done Before 1998

The etiologic agent, *M. ulcerans*, was discovered in Bairnsdale, Victoria State, in a temperate zone of southeastern Australia. Searle, Clay, and Alsop, general practitioners in the Bairnsdale area, and Torode in Colac recognized indolent ulcers with undermined edges in the late 1930s. Biopsy specimens of these ulcers contained acid-fast bacilli (AFB). Unfortunately, these perceptive observations were never published.

The first patient reported by MacCallum et al. was a 2½-year-old boy hospitalized on 29 June 1940 in a private clinic in Bairnsdale, with an ulcer on his leg. A biopsy from the margin of the ulcer teemed with AFB.

M. ulcerans infection existed in central Africa for many years before the first published report by MacCallum et al. These infections were probably often considered a form of "tropical phagedenic ulcer" (TPU). Sir Albert Cook (1897) was perhaps the first expatriate physician to record a description of chronic necrotizing ulcers with undermined edges he saw in Uganda. During the years 1923 to 1964, Ralph E. Kleinschmidt, a missionary physician in northeastern Congo, also observed undermined ulcers rich in AFB [3].

Of particular relevance to BU was the long experience in Africa of treating TPU because of its importance for economic activities such as mining and various kinds of plantations. Programs for fighting TPU clearly stimulated interest in other cutaneous ulcerative diseases, including BU. In 1942, Prof Pieter G Janssens was involved with the TPU problem at the Kilo-Moto medical service in the far northeastern corner of the Congo (Ituri Province). He observed chronic necrotizing ulcers containing AFB and affecting mostly children in the Kakerifu encampment situated between the Kibali and Nzoro rivers. Janssens noted that *M. ulcerans* infection, although having some similarities with TPU, was a disease apart. In 1957, he became Director of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, and was the first to bring BU and its importance as a tropical disease in Africa to the attention of the ITM. Before he died, at the request of F. Portaels, Janssens agreed to document his rich experience in the discovery of BU in Congo [1].

Unlike Janssens in Africa, scientists in Australia had ready access to both sophisticated laboratory facilities and BU patients and, thus, were able to contribute significantly to the early understanding of the disease and culture of the etiologic agent [2].

The most significant contributions to the knowledge of BU in Africa came from Uganda and the Democratic Republic of the Congo (DRC). The disease was named "Buruli ulcer" after the geographic area of the first large epidemic investigated in Uganda in 1961, in a county named "Buruli" (now called "Nakasongola"), near Lake Kyoga [4].

The Uganda Ministry of Health and the Makerere Medical School instituted the Uganda Buruli Group (UBG), with a mandate to investigate the BU situation and to advise the authorities on strategies for managing this significant public health problem. The UBG described the clinical aspects of the disease; emphasized the importance of early treatment, preferably in the pre-ulcerative stage [5, 6]; and produced important details on the epidemiology of BU [7]. The UBG also observed that BU was strongly associated with slow-flowing and stagnant waters; however, the Group was unable to isolate *M. ulcerans* from the environment but cultivated many other mycobacterial species [8]. Similarly, our attempts to culture *M. ulcerans* from more than 1000 environmental specimens collected in DRC between 1970 and 1974 failed. Many other environmental mycobacterial strains were cultivated, some of them new to science [9].

From 1965 to 1973, in Lower Congo (DRC), Wayne M. Meyers was responsible for leprosy patients in Kimpese. During this period, he also treated many BU patients. Meyers was the first one to succeed in cultivating *M. ulcerans* in vitro from clinical specimens in a rural hospital and to successfully treat ulcerated BU lesions without surgery. The efficacy of oral rifampin in patients with early ulcerated lesions was demonstrated in 1971 and heat therapy in 1974 [10, 11]. Based on extensive clinical studies and detailed interviews of patients or their families, the role of trauma in transmission of *M. ulcerans* to humans was postulated [12].

In 1991, Dr. Augustin Guédénon, dermatologist and Director of the anti-leprosy control in Benin, contacted the Mycobacteriology Unit of the ITM to inform us of the increased importance of BU in his country. At that time, the real significance of the disease compared with tuberculosis and leprosy was not realized. With the motivation of Guédénon, and thanks to carefully archived materials of Sister Julia Aguiar and her extensive experience in diagnosing and treating BU, a descriptive study based on data from the records of 867 patients treated at the "Centre Sanitaire et Nutritionnel at Zagnanado" (Zou Department) was conducted. The patients came from four departments in southern Benin called at that time Atlantique, Mono, Ouémé, and Zou. The total number of BU patients detected exceeded those of leprosy and tuberculosis in some sub-prefectures [13].

It became clear that implementation of a Benin National Anti BU Program proved essential for education of populations and healthcare workers. In close collaboration with Benin, various aspects of BU, including its geographic distribution, incidence and prevalence, mode of transmission, pathogenesis and immunity, clinical manifestations, differential clinical diagnosis, laboratory diagnosis, and treatment, were studied [14]. For the first time, direct detection and identification of *M. ulcerans* in clinical specimens from Benin were performed using PCR [15].

The majority of microbiologically confirmed known BU foci in Africa were identified and described before 1998, in chronological order: Democratic Republic of Congo, Gabon, Uganda, Republic of Congo, Nigeria, Ghana, Cameroun, Côte d'Ivoire, Liberia, Sierra Leone, Benin, and Togo. Microbiologically confirmed cases were also described on other continents before 1998, in chronological order: Australia, Mexico, Malaysia, Papua New Guinea, Peru, French Guiana, and Japan [1].

Most of the data that we have available now on the clinical aspects and the epidemiology of BU were available before 1998, primarily because of investigations carried out in Uganda, DRC, West Africa, and Australia. Although the disease remained uncommon in Australia until the end of the twentieth century, John Hayman, a pathologist from Victoria, published several articles on clinical features, histopathology, and epidemiology of the disease [16–18].

Natural infections in mammals have been described for the first time in koalas from Australia. The lesions were clinically identical to those observed in humans [19].

Clinical trials published in 1969 and 1976 showed that the protective effect of BCG vaccination was short lasting and thus of limited value for BU control [5, 20]. A more effective BU vaccine has not become available so far. The first cases of BU patients with subclinical HIV co-infection have been described in 1992 [21]. Since then, accumulating evidence is indicating that HIV infection increases the risk of developing BU and that HIV co-infected BU patients tend to develop more severe and more frequently multifocal pathologies.

One of the most important advances in laboratory diagnosis of BU was the discovery of a repetitive DNA restriction fragment from *M. ulcerans* and the development of a specific and very sensitive PCR assay for the rapid diagnosis of *M. ulcerans* in clinical specimens [22] and the detection of *M. ulcerans* DNA in the environment for the first time [23]. This high-copy-number insertion sequence was designated IS*2404* [24].

39.2.2 Achievements Since 1998

Important findings and achievements were attained since the creation of the GBUI in 1998. The number of publications on BU has literally exploded. Of the approximately 1500 articles devoted to BU, 15% of them were published before 1998 (in 50 years) and 85% until the end of 2020 (in 22 years).

Of particular interest was the creation, beginning in 1998, of National Control Programs against BU, programs for education of populations and healthcare workers, resulting in better case finding, correct laboratory diagnosis, better treatment of the disease, and better understanding of its epidemiology and pathogenesis.

The majority of known BU foci in Africa and elsewhere were described before 1998. Since 1998, only six new countries have been added to the list of endemic countries where microbiologically confirmed cases were discovered, five in Africa (Burkina Faso, Central African Republic, Equatorial Guinea, Kenya, and South Sudan) and one in Asia, China where the first confirmed case of *M. ulcerans* subspecies *shinshuense* was described in 2000 [25].

Since the creation of the GBUI, significant progress has been made in the field of BU treatment and laboratory diagnosis. These advances are developed in Chaps. 45 and 41.

Significant progress has also been made in the field of scientific research, partly thanks to international collaborations stimulated by the GBUI.

In 1999, George et al. isolated a cytotoxic factor from *M. ulcerans*. The chemical structure of this toxin was deciphered. This polyketide-derived macrolide, required for the virulence of *M. ulcerans*, named mycolactone, destroys tissues by apoptosis and necrosis and suppresses host immune responses [26, 27]. In 2004, Stinear et al. demonstrated that *M. ulcerans* carries the giant plasmid pMUM001 that harbors genes encoding the polyketide synthases required for mycolactone synthesis [28]. The loss of this plasmid, after several in vitro subcultures, makes the strain non-pathogenic [29]. The possible role of plasmids in the virulence of *M. ulcerans* had already been pointed out in 1989 [30].

A real-time PCR assay for quantification of *M. ulcerans* DNA was developed in 2003 [31], and two multiplex real-time PCR assays for the detection of *M. ulcerans* in clinical and environmental samples were developed in 2007 [32].

The first complete 5.8-Mb genome sequence of a Ghanaian *M. ulcerans* isolate was published in 2007 and showed >98% nucleotide sequence identity with the genome of *M. marinum* [33]. However, in addition to the acquisition of the virulence plasmid, *M. ulcerans* has accumulated multi-copy insertion sequences, many pseudogenes, and multiple DNA deletions. The reductive evolution indicates that *M. ulcerans* has evolved from a generalist to a niche-adapted specialist. All mycolactone-producing mycobacteria represent a single clonal group, which has diverged into several ecovars [34]. Among *M. ulcerans* isolates from human lesions, two principal lineages have been identified: the classical lineage responsible for BU in Africa, Papua New Guinea, and Australia and the ancestral lineage isolated from patients from Asia, Mexico, and South America [35]. Comparative whole genome analyses have furthermore revealed that, in many African BU endemic regions, local clonal complexes of *M. ulcerans* have developed [36, 37]. The strong spatial segregation of these complexes is speaking against the existence of highly mobile reservoirs.

The possible role of insects in the epidemiology of BU was evoked for the first time in 1999 [38]. Aquatic insects (Hemiptera) were suspected to be vectors of BU in Africa [39], and mosquitoes were suspected to play a role in the transmission of BU in southeastern Australia [40]. Case-control studies in Africa and Australia have also suggested insects may play a role in transmission [41, 42]. The first cultivation of *M. ulcerans* from a water strider, an aquatic insect that does not bite humans, was also reported. Hemiptera should, however, be considered as passive reservoirs [43].

While the local incidence of BU caused in Africa and Australia by classical lineage strains is high, cases caused by the ancestral lineage in Asia, Mexico, and South America occur only sporadically, which may reflect differences in environmental reservoirs of the two ecovars. For short-term visitors to BU endemic areas in Victoria, Australia, the mean incubation period for BU was estimated to be 135 days, with 34 days recorded as the shortest and 264 days as the longest [44]. Sero-epidemiological studies are indicating that in BU endemic areas of Africa, only a small minority of individuals exposed to *M. ulcerans* are developing clinical BU disease and that exposure to *M. ulcerans* intensifies at an age of about 4 years [45].

The major role for mammals in the ecology of *M. ulcerans* in Australia was highlighted in 2010 [46], and two domestic animals were recently found infected by *M. ulcerans* in Benin suggesting that animals may also play a role in the ecology of *M. ulcerans* in Africa [47]. However, the environmental reservoir of *M. ulcerans* and its exact mode of transmission still remain unknown. Over 70 years ago, Tolhurst and Buckle wrote the following: "Whatever the reservoir of the organism, the method of transfer to man has still to be elucidated" [2]. This just proves that "there is nothing new under the sun!"

39.3 Geographic Distribution

Cases of BU have been reported in 34 countries [48]. Of these 34 countries, cases have been confirmed microbiologically in 27 countries.

There are laboratory-confirmed *M. ulcerans* infections in the following **tropical** countries:

- *Africa*: Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Kenya, Liberia, Nigeria, Republic of Congo, South Sudan, Togo, and Uganda
- *The Americas*: French Guiana, Mexico, Peru, and Suriname
- *Asia*: Malaysia
- *Oceania*: Papua New Guinea and northern Australia

The **nontropical** countries with confirmed BU are southern Australia, China, and Japan.

Cases in the remaining seven countries (Brazil, Indonesia, Kiribati, Malawi, Senegal, Sierra Leone and Sri Lanka) lack convincing microbiological confirmation, and the clinical features are not in favor of BU.

In recent years, the number of BU cases reported to WHO has decreased in several countries, especially in most of the highest prevalence African countries (Ghana, Côte d'Ivoire, and Benin) [49]. This decrease was also observed in French Guiana [50] and on the Bellarine Peninsula in Australia [51].

Reasons for decrease remain unknown, but several hypotheses have been proposed: environmental changes; improvement of living conditions (access to safe water); the increasing use of antibiotics for the treatment of BU, which may impact the human reservoir [52]; and the increasing confirmation of cases by PCR reducing the overdiagnosis of the disease that may have occurred previously [53]. It is however unlikely in countries such as French Guiana or Australia where dermatologists are BU experts since several decades [50]. With a decline in surveillance activities, underreporting may be an issue in some African BU endemic areas.

Some "so-called" epidemics of BU may be due to the lack of clinical experience in the differential diagnosis of BU and the lack of laboratory confirmation of the

cases. For example, Guinea reported to WHO, between 2002 and 2017, a total of 1480 cases, but none of them were laboratory confirmed. During the same period, South Sudan reported 1014 cases to WHO, but only a few cases were confirmed by laboratory tests [54]. In Uganda, the disease was believed to have disappeared in 1976 [20]. However, a survey carried out in 2003 revealed 117 suspected cases in the Nakasongola district (formerly Buruli district), but none of them were confirmed by laboratory tests [55].

Conversely, the number of cases has increased on the Mornington Peninsula in Australia [51], and an increasing number of cases has been reported to WHO in Nigeria [55]. Reasons for increase in Nigeria may be partly related to the increasing awareness of BU and better detection of the disease.

39.4 "To Be or Not to Be" a Buruli Ulcer Case, "That Is the Question"!

Any clinical feature of BU can be mistaken for another skin condition, particularly in areas where other skin diseases are frequent (see Chap. 43). Studies on the differential diagnosis of BU seem to indicate that its clinical diagnosis may sometimes be more difficult than usually recognized even in experienced hands.

Thus, microbiological confirmation remains essential to confirm (or to invalidate) BU. It is generally based on only one test, IS*2404*-PCR, which is the most sensitive test among the presently available laboratory tests (see Chap. 41). However, false-positive or false-negative PCR results may be due to laboratory errors. In view of this, an External Quality Assessment Program (EQAP) of PCR has been established by WHO. The third EQAP round has revealed that 20% of the participating laboratories had false-positive results, probably due to DNA contaminations [56].

Consequently, laboratory errors or the absence of microbiological confirmation may be responsible for inadequate treatments of patients, non-reliable epidemiological data, and the description of "new" (unlikely) BU foci despite clinical features not being in favor of BU!

It is essential to ensure that all laboratory tests be accurate and reliable. Internal quality control and external quality assessment systems are detailed in Chap. 41.

To avoid a misdiagnosis caused by false-positive or false-negative results, it is recommended that two different tests have positive results before a definitive diagnosis is made. The development of rapid point-of care diagnostic tests would also be a precious tool for the differential diagnosis of BU (see Chap. 41).

Overdiagnosis or underdiagnosis of BU? "*Errare humanum est, perseverare diabolicum*" ("*To err is human but to persist in error is diabolical*") says an old Latin proverb. Good laboratory practices, self-criticism, and collaboration with a multidisciplinary team should allow us to limit bias due to human errors and get a more reliable picture of the actual geographical distribution and real burden of BU worldwide.

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Microbiology of *Mycobacterium Ulcerans*

40

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40.1 Properties of *M. ulcerans*

Mycobacterium ulcerans, the etiologic agent of Buruli ulcer (BU), is a slowly growing acid-fast bacillus, growing optimally at 30° to 33°C on common mycobacteriologic media such as Löwenstein-Jensen medium. The organism is microaerophilic, often requiring 6- to 12-week incubation to achieve isolation in primary culture. Subcultures are generally positive within 3–4 weeks of incubation. Colonies suggestive of *M. ulcerans* appear yellowish and rough and have well-demarcated edges. African and Japanese strains are more yellowish than Australian strains, which are cream in color.

Tests for phenotypic identification were described elsewhere [1], but, presently, the identification of *M. ulcerans* is done by genetic tests (see Chap. 41).

MacCallum and associates were the first to isolate *M. ulcerans* in culture in 1948 from a patient in Australia [2].

The epidemiology of BU is strongly associated with wetlands, especially with slow-flowing or stagnant water bodies which implicates that the source of the organism is related to the environment. After a multitude of attempts to cultivate the organism from the environment over half a century, the first cultivation of *M. ulcerans* from an aquatic environment (from a water strider) was reported in 2008 [3].

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40.2 Genetic Diversity of *M. ulcerans*

40.2.1 Characteristic Features of *M. ulcerans* Genome

The genome of *M. ulcerans* strain Agy99 isolated from a BU patient from the Amansie West district of Ghana was the first to be fully sequenced and published. It comprises two replicons, a 5.6 Mbp circular chromosome and a circular 174 kpb giant plasmid (pMUM001) [4]. The chromosome contains 4160 protein coding sequences (CDS) and has a G+C content of 65%, large numbers of insertion sequence elements (ISEs) (209 copies of IS*2404* and 83 copies of IS*2606*), and two bacteriophages phiMU01 (18 kb, 18 CDS) and phiMU02 (24 kb, 17 CDS). There are 45 genes encoding tRNA and a single rRNA operon [4].

The plasmid contains three large genes (*mlsA1*, 51 kb; *mlsA2*, 7.2 kb; *mlsB*, 43 kb) that encode the polyketide synthases (PKSs) required for the synthesis of mycolactone, the primary virulence factor of *M. ulcerans* [5]. The plasmid also contains four copies of the insertion sequence IS*2404* and eight copies of IS*2606* [6].

M. ulcerans and *M. marinum* are genetically closely related as they share more than 4000 orthologous and syntenic CDS and have an average sequence identity of 98.3% [4, 7]. Comparative genome analysis of *M. ulcerans* Agy99 with the clinical *M. marinum* strain M gives indication that pMUM001 and ISEs represent the main differences between the two genomes and that acquisition of these elements is a landmark in the evolution of *M. ulcerans* from a *M. marinum* progenitor that acquired the elements through horizontal transfer [4, 8].

Although *M. ulcerans* has acquired foreign genetic elements, its genome has diminished in size compared to *M. marinum* as a result of multiple rearrangements and deletions of large sections of the chromosome mainly brought about by the transposition activities of the ISEs. Thus there is about 1046 kb of DNA deleted from *M. ulcerans* genome that are present in *M. marinum* [4, 9]. In all there are 157 regions in *M. marinum* that are absent from *M. ulcerans*. These regions form *M. ulcerans* regions of difference (MURDs). Notable MURDs include secondary metabolism, intermediary and energy metabolism, and PE/PPE genes [4].

Another remarkable feature of the genome of *M. ulcerans* is the accumulation of pseudogenes, and it is estimated that about a quarter of the predicted ancestral protein coding genes have undergone mutation and are therefore inactive [4]. These mutations have resulted in the loss of many virulence factors and immunogens in *M. ulcerans* compared to *M. marinum*. Prominent among which is the drastic reduction in cell surface proteins PE and PPE. Compared to *M. marinum* which has 170 PE and 105 PPE, *M. ulcerans* has only 70 PE and 46 PPE genes. This accounts for 45% of the MURDs. A total of 111 of the predicted pseudogenes of *M. ulcerans* were created by insertion of ISEs [4]. The reduction in the PE/PPE proteins has resulted in the depletion of the related ESX secretion systems and their effector proteins. The ESX loci encode type VII secretion systems [10] and are required to export members of the ESAT-6 (6 kDa early secretory antigenic target) protein family and specific effectors, such as EspA (ESX-1 secretion-associated protein A).

While the genome of *M. marinum* contains two copies of the esxB–esxA gene cassette, members of the *M. ulcerans* ancestral lineage (isolates of patients from the Americas and Asia) have retained only one copy of this gene cluster. Both copies are deleted from the genome of *M. ulcerans* strains belonging to the classical lineage (isolates of patients from Africa, Papua New Guinea, and Australia) [11]. The resulting reduction in abundance or complete loss of strong B- and T-cell immunogens may help *M. ulcerans* to evade host immune responses and may confer a survival advantage in host environments that are screened by immunological defense mechanisms [11].

In the same vein, *M. ulcerans* has also lost capacity to produce phenolic glycolipids (PGL), cell wall components, antigens, and major virulence factors for several mycobacterial pathogens that can also modulate innate immunity. *M. marinum* synthesizes PGL through glycosylation of phenolphthiodiolone, a polyketide-derived intermediate. *M. ulcerans* also produces phenolphthiodiolone but cannot make PGL as a result of inactivation of the glycosyl transferase genes involved in the synthesis process [4, 12].

M. marinum has the *crtI* locus that encodes for the production of phytoene dehydrogenase, an essential enzyme for the biosynthesis of light-inducible carotenoid pigments which protects the bacterium from damage following exposure to sunlight [13]. Although, the *crtI* gene is also present in *M. ulcerans*, it is inactive due to the insertion of a premature stop codon in the gene, suggesting that the pigments are not required for survival, presumably because *M. ulcerans* inhabits dark environments and is not exposed to sunlight [4].

The complements of genes encoding enzymes involved in aerobic respiration are preserved in both *M. ulcerans* and *M. marinum*, and both organisms are thus capable of growth under aerobic conditions. *M. marinum* however retains capacity for anaerobic respiration utilizing pathways that involve the coupling of fumarate oxidation with nitrite reduction or through nitrite reduction via the *nirBD* nitrite reductase and NarK transporter. *M. ulcerans*, on the other hand, has lost the fumarase dehydrogenase system as well as the *nirB* and *narK* loci (which are pseudogenes) and consequently cannot undergo anaerobic respiration [4].

Comparative whole genome analysis has revealed that *M. ulcerans* evolved from a *M. marinum* progenitor through acquisition by lateral transfer of a virulence plasmid and ISEs. Transposition activities of ISEs have resulted in deletions of large segments of the chromosomes, rearrangements in the genome, inactivation of genes, and accumulation of pseudogenes [4, 7]. Thus, ISEs are important in leading the genome reduction drive in *M. ulcerans*. Analysis of isolates also has revealed a high level of genetic homogeneity in *M. ulcerans* leading to a clonal population structure for this pathogen [14].

These features are characteristic of bacterial populations that have passed through an evolutionary bottleneck suggesting there has been constriction of population size during adaptation to a new niche environment [15, 16]. Although the niche of *M. ulcerans* is yet to be determined, the loss of genes expressing potent T-cell antigens and genes required for pigment synthesis, anaerobiosis, and intracellular growth suggests that the bacterium has adapted to a dark, extracellular environment

where slow growth rate, loss of immunogens, and production of an immunosuppressive molecule provide a selective advantage [4].

By providing clues on the environmental niche of *M. ulcerans*, whole genome comparison can aid in the development of targeted and appropriate measures for the primary prevention of BU. Comparative genomics can also potentially lead to the identification of new targets for development of rapid diagnostic tests to augment early detection and treatment of cases, which is the current strategy for BU control.

40.2.2 Strain Typing and Molecular Epidemiology

The high level of genetic homogeneity among *M. ulcerans* isolates is a major impediment to unravelling the environmental reservoir as well as the route through which *M. ulcerans* infects humans and animals [17]. For several decades, investigators have applied high discriminatory genotyping techniques including VNTR [17–19], RFLP [20], IS*2404*- Mtb2-PCR [21], *2426* PCR [22], and MLST [14] to uncover genetic diversity in *M. ulcerans* for molecular epidemiology investigation of BU. However, these methods only resolved *M. ulcerans* on the basis of their continent of origin with no or limited genetic differences on continent and country basis. The level of clonality was highest among African isolates [17–22].

Inspired by the high resolution afforded by single nucleotide polymorphism (SNP) typing, a set of 65 SNP loci was used to investigate the phylogeography and transmission pathways of *M. ulcerans* in endemic communities of the Densu river basin in Ghana [23]. The study identified ten *M. ulcerans* haplotypes with a particular type (founder haplotype) distributed widely across all the endemic communities studied, while the other haplotypes formed local clonal complexes that were confined to individual endemic foci with no evidence of mixing of haplotypes (Fig. 40.1). Comparison of SNP profiles with those of neighboring and distant isolates showed that the Densu basin haplotypes formed a clade which also comprised an isolate from Togo while the Amansie west isolates clustered together with an isolate from Ivory Coast to form a separate clade. A third clade comprised isolates from Benin, Congo, Angola, and Ivory Coast. Grouping of such a diverse *M. ulcerans* collection has been suggested to represent a phylogenetic bias, a drawback of analysis based on a limited set of SNP loci that may be remedied by expanding the repertoire of SNP loci or interrogating the whole genome [24].

It has now become obvious that for highly monomorphic species such as *M. ulcerans*, comparative whole genome sequence analysis may be the only option available for indexing high level of diversity useful for micro-epidemiological and phylogeographic investigations.

Whole genome sequence comparison approach applied to *M. ulcerans* at the district and village levels in the Cameroon [25] and Ghana [26], respectively, has provided additional information on the nature of distribution of *M. ulcerans* and has also enabled us to form new ideas on how this pathogen could be spreading in communities.

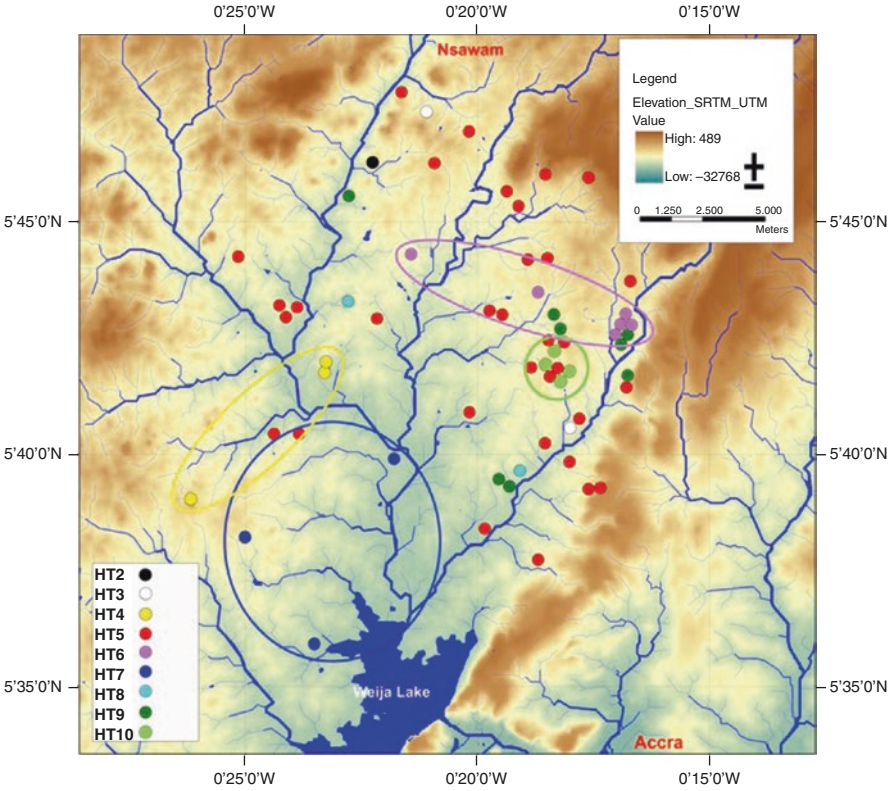
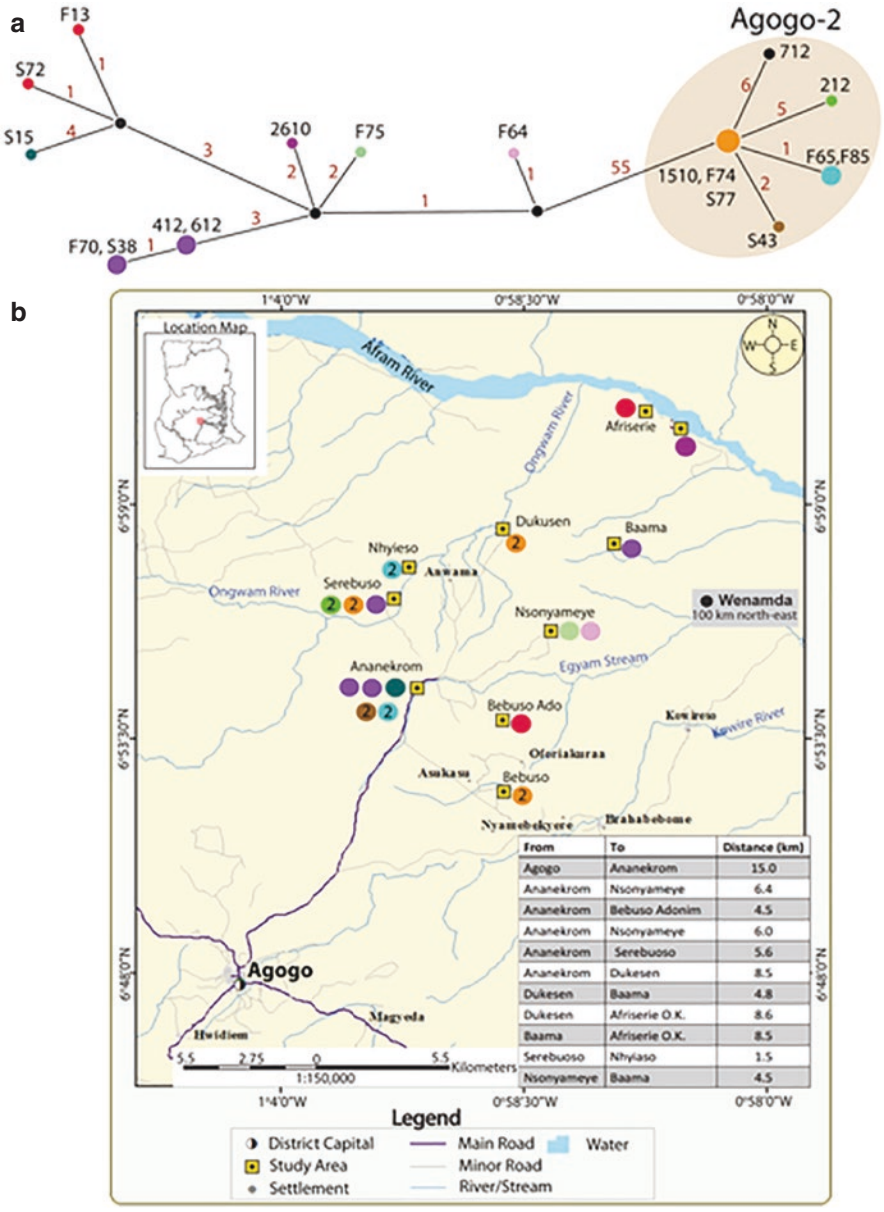


Fig. 40.1 Map of the Densu river basin, showing the homes of patients from whom the strains have been isolated between 2001 and 2006 (colored dots). Haplotypes 2 (*black*), 3 (*white*), 4 (*yellow*), 6 (*purple*), 7 (*dark blue*), 8 (*light blue*), 9 (*dark green*), 10 (*light green*) are unevenly distributed, whereas haplotype 5 (*red*) co-localizes with all other haplotypes. The background map was created using elevation data from the Shuttle Radar Topography Mission (SRTM). Water bodies were classified using optical data from Landsat ETM and radar data from TerraSAR-X

Analysis of the Mape and Nyong river basins in Cameroon uncovered a similar observation as the SNP analysis of the Densu basin where *M. ulcerans* strains form clonal complexes with rare exchanges of strains between distinct endemic areas suggesting focal transmission events occurring within confined endemic foci.

Isolates from Mape, a relatively more recently emerging BU endemic area, were found to be less diverse than populations from longer-standing disease foci of the Nyong basin.

In Ghana, the Asante Akim North study was the first to employ whole genome sequencing to explore the molecular epidemiology of BU at a local scale (clinical isolates from a 30 km^2 region). The study uncovered two *M. ulcerans* clusters, namely, the Agogo 1 and Agogo 2 clusters (Fig. 40.2). The Agogo 1 cluster which comprised closely related isolates from local and neighboring districts of Amansie West and also Ivory Coast represents a local clone that has spread and persisted in



From	To	Distance (km)
Agogo	Ananekrom	15.0
Ananekrom	Nsonyameye	6.4
Ananekrom	Bebuso Adonim	4.5
Ananekrom	Nsonyameye	6.0
Ananekrom	Serebuoso	5.6
Ananekrom	Dukesen	8.5
Dukesen	Baama	4.8
Dukesen	Afriserie O.K.	8.6
Baama	Afriserie O.K.	8.5
Serebuoso	Nhyiaso	1.5
Nsonyameye	Baama	4.5

Fig. 40.2 (**a**) Median-joining network graph showing the genetic relationship between 18 *M. ulcerans* clinical isolates comprising the Agogo-1 and Agogo-2 genotypes (shaded), inferred from whole genome sequence alignments. Node sizes in the graph are proportional to the frequency of genotype occurrence and have been color-coded accordingly. Edges are labelled in red with the number of mutational steps between each node. (**b**) Map of Asante Akim North District study area, showing the location of endemic villages and the origin of each of the 18 BU cases, with a colored circle corresponding with the genotype displayed in the network graph in (**a**). The number "2" within some colored circles indicates an Agogo-2 genotype

the area for some time. The Agogo 2 was more diverse from all other Ghanaian *M. ulcerans* genotypes (138 SNPs) suggesting a likely introduction of isolates from outside Ghana. Further genome comparative analysis identified a strain originating from Ibadan, Nigeria, differing in 29 SNPs from the Agogo 2 cluster, as the closest match among *M. ulcerans* panels assembled from West and Central Africa. The observation of a mixing of local circulating genotypes with isolate clones from distant locations has been reported in Australia [27].

Analysis of the genotype distribution showed for the first time no spatial clustering of genotypes at the local scale with multiple genotypes occurring simultaneously in one village and complete intermixing of Agogo 1 and Agogo 2 clusters among the population (Fig. 40.2). Some patients living in different villages (each separated by distances in excess of 10 kms) were infected with identical *M. ulcerans* genotypes. The suggestion that people moving in the communities become infected from a common point source was deemed unlikely explanation for this observation as further investigations failed to establish epidemiological link between the patients. The possibility of the bacteria or a reservoir spreading it to be widely distributed across the region and infections are being acquired locally seems to be consistent with the whole genome sequence data. The case for local infection is further strengthened by the observation of a 2-year-old infant who had BU but had never travelled outside the village in which she resides.

Comparative whole genome analysis showed for the first time that *M. ulcerans* focal distribution pattern breakdown and multiple *M. ulcerans* genotypes may circulate within a local setting. Multiple genotypes in an area may be the result of accumulated mutations in local clonal complexes over time or the introduction of different genotypes into an area. Also, each *M. ulcerans* genotype may spread equally widely across the region, and the lack of genetic variation among isolates suggests that the spread of *M. ulcerans* throughout a region has occurred relatively rapidly with insufficient time elapsed for mutations to accumulate [26].

It has been suggested that because *M. ulcerans* transmission and microevolution generally occur at a local level, the source of the bacterium is somewhat fixed within a local region, indicating that animal reservoirs of *M. ulcerans* are unlikely to be highly mobile [23].

These whole genome sequence data however give new perspectives on the behavior of possible reservoirs and subsequent transmission mechanisms of *M. ulcerans* and show for the first time that *M. ulcerans* can be mobilized, introduced to a new area, and then spread within a population. Potential reservoirs of *M. ulcerans* thus might include humans or perhaps *M. ulcerans*-infected animals such as livestock that move regularly between countries [26].

40.3 Comments on the Taxonomic Position of *M. ulcerans*

Taxonomy, from the Greek words “taxis” (arrangement) and “nomas” (law), is the science of biological classification. Its purpose is to provide useful ways for identifying and comparing organisms. Classification is the arrangement of organisms into

groups (taxa); nomenclature refers to the assignment of names to taxonomic groups. Throughout the ages, man has given names to living organisms, and that tradition goes back to the very early times, as shown in the very first pages of the Bible. Indeed, at the beginning of the Genesis, we can read that: "The LORD God formed every beast of the field, and every fowl of the air; and brought [them] unto Adam to see what he would call them: and whatsoever Adam called every living creature, that [was] the name thereof" (Genesis, chapter 2, Verse 19).

Once the names are given to "taxa," the characters making it possible to identify them must be described. The choice of these characters is not fixed forever; it can change in the course of time; names too! Taxonomy is thus a dynamic science, in constant evolution.

Two major periods may be distinguished in prokaryotic taxonomy, one characterized by the utilization of phenotypic tests and one characterized by the use of genotypic characteristics. The phenotypic period started at the end of the nineteenth century and the genotypic period during the last decade of the twentieth century and continues to the present.

M. ulcerans can be identified by phenotypic and genotypic tests [1]. In 1997, a specific and sensitive method based on PCR amplification of an insertion sequence, IS*2404*, was developed to identify *M. ulcerans* [28]. Ten years later (in 2007), the genome of *M. ulcerans* was sequenced [4]. In 2012, comparative analysis of whole genome sequences of *M. ulcerans*, other mycolactone-producing mycobacteria (MPM) (*M. pseudoshottsii*, *M. liflandii*, and some *M. marinum* isolates from fish), and *M. marinum* has shown that *M. ulcerans* and all MPM are specialized variants of a common *M. marinum* progenitor and that all MPM differ from *M. marinum* by the acquisition of the pMUM plasmid and introduction of insertion sequences into the chromosome. Based on these findings, it was proposed to consider all MPM *M. ulcerans* ecovars [8]. Although it is correct from a genetic point of view, *M. ulcerans*, *M. pseudoshottsii*, *M. liflandii*, and *M. marinum* have, however, different phenotypic characteristics and, above all, are pathogens of different hosts: *M. ulcerans* is mainly pathogenic to humans and some mammals, while the other MPM are fish and frog pathogens.

Taxonomy should remain pragmatic and clinically meaningful [29]. Although it is sound from a taxonomic point of view, classification of all MPM under a single species should be avoided as it may be very confusing from a medical and epidemiologic point of view.

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Part X

Buruli Ulcer: Patient's Examination

Laboratory Investigations in Buruli Ulcer

41

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41.1 Introduction

The microbiological confirmation of Buruli ulcer (BU) is central in the management of the disease. Laboratory confirmation of BU has become increasingly important in recent years due to the decline in BU incidence in several endemic countries. Currently, laboratory diagnostics for BU include culture, direct smear examination for acid-fast bacilli, histopathology, and PCR targeting the insertion element IS*2404* [1, 2]. A detailed description of each of these techniques is included in the World Health Organization (WHO) manual on the laboratory diagnosis of BU [3]. Moreover, several innovative diagnostics are in development.

Although typical changes can be detected in histopathological sections, this technique is currently not available in most BU endemic African countries and requires invasive sampling techniques, sophisticated equipment, and highly

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experienced scientists. Those who are interested in histopathological features of BU and the various methods used for their diagnosis can consult the WHO manual where they are described in detail [3].

41.2 Sampling

It is of great importance that samples are taken correctly for the laboratory analyses to be successful. Three techniques are used to collect specimens: swabbing, fine-needle aspiration (FNA), and biopsy (punch or surgical).

In **ulcerative BU, swabs** are used, while for **early nonulcerative lesions, fine-needle aspirations** are most appropriate [3]. For histopathology, **biopsies** need to be taken which is usually only done during a surgical intervention or on specific indications [3]. Biopsies require trained health workers. Swabbing and FNA can be carried out at the remote decentralized healthcare levels where most BU patients consult during routine management or active case-finding activities. However, FNA needs to be performed under aseptic conditions by experienced healthcare workers.

For diagnostic purposes, samples should be collected before treatment. Given the heterogeneous distribution of mycobacteria in lesions, **at least two clinical specimens** should be collected from each lesion.

For nonulcerative plaques and edematous forms, the patient or the patient's relative should be asked to indicate the site at which the lesion first appeared since this is the site that is most likely to yield a positive diagnostic result.

Fine-needle aspiration (FNA) can be performed on any nonulcerative lesion, such as papules, nodules, plaques, and edemas, and on ulcers that do not have undermined edges (i.e., where scarred edges would prevent the collection of swab samples). FNA is a minimally invasive procedure and should be done in the estimated center of the nonulcerative lesion. For ulcers without undermined edges, FNA should be performed in the most indurated area around the wound. Samples for FNA should be taken using fine-gauge needles (21–23 gauge by 25 mm in diameter) attached to a syringe. The optimal aspiration technique is described in the WHO manual on the laboratory diagnosis of BU [3]. This technique ensures that sufficient material is obtained. FNA can be used for direct smear examination directly after aspiration and can be stored and transported in a liquid transport medium for PCR analysis and culture.

Swab sampling is most useful for ulcers that have undermined edges [4, 5]. At least two swabs should be obtained from beneath the undermined edges of ulcers. The center of the ulcer should not be swabbed because *M. ulcerans* is generally not present there after the central necrotic tissue has sloughed off. The technique to use for swabs is described in the WHO manual on the laboratory diagnosis of BU [3]. If microscopy can be done locally, one swab is obtained to prepare a smear for Ziehl–Neelsen (ZN) staining to observe acid-fast bacilli. A second swab is then collected and stored dry (if the swab is stored for less than 24 h) or in semi-solid transport medium [6] and is sent to the reference laboratory for PCR and culture.

Punch biopsy is not the first choice for obtaining a sample, although it may be used in limited special circumstances. Punch biopsies (3 or 4 mm in diameter) must

be taken after local anesthesia; surgical biopsies may be taken under general anesthesia during surgical excision. Biopsies should be taken from the center of nonulcerative lesions or from the necrotic margin of ulcers that border healthy (viable) tissue. However, for histopathology, samples from the edges of nonulcerative lesions may be most suitable. Tissue biopsies should contain the entire thickness of infected tissue, including subcutaneous adipose tissue. For routine confirmation of BU, swabs and FNA are sufficient. The indications for punch or surgical biopsies are:

- To establish the (differential) diagnosis of BU
- To investigate the cause of a paradoxical reaction
- To determine whether treatment has failed despite successful administration of quality-assured antibiotics
- To establish whether cancerous changes have occurred.

If radiological evidence of osteomyelitis is present or if osteomyelitis is discovered during surgery, **bone samples** should be collected to confirm *M. ulcerans* involvement.

Before being transported to a laboratory, specimens must be labeled with the appropriate information (the patient's name and/or ID, the date the specimen was obtained, the sample code), following standard practice. A permanent marker should be used to write labels. In addition, all paperwork (e.g., standardized WHO forms) must be properly filled out and forwarded with the sample to the laboratory. When microbiological analyses can be performed within 24 h, clinical specimens should be kept without additives in a sterile container at 4 °C until analysis.

When microbiological analyses cannot be performed within 24 h or when refrigeration facilities are not available, a transport medium should be used. It is recommended that FNA samples be transported in a liquid transport medium containing a Dubos broth base supplemented with oleic acid, albumin, dextrose, and catalase (OADC, Becton Dickinson) and polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA, Becton Dickinson). Swab and/or tissue specimens taken by punch or surgical biopsy should be transported in a liquid transport medium supplemented with 0.5% agar; this produces a semisolid transport medium [6]. The specimens in transport media should be stored at 4 °C, but they can be sent at ambient temperature to reference laboratories for microbiological confirmation. Procedures for the preparation of transport media are described in detail in the WHO manual [3].

The bacteriological transport media described above also allow samples to be analyzed by PCR [5–7]. Specimens that will be used only for PCR may also be placed in a solution of ethanol and distilled water (in a ratio of 1:1), in phosphate-buffered saline, in TE buffer (Tris hydrochloride plus EDTA), or in a commercial PCR buffer. Note, ethanol may interfere with certain DNA extraction procedures.

Biopsies for histopathological analysis should be placed in 10% buffered neutral formalin (pH 7.4) [8, 9].

41.3 The Laboratory Diagnosis of BU at the Point of Care

41.3.1 Microscopy

Among the four recommended laboratory tests for the confirmation of *M. ulcerans* infection, direct smear microscopy (DSM) for the detection of AFB is the only method currently available at the primary healthcare level where the majority of BU patients access healthcare. There are several staining methods including ZN and auramine O. The ZN method which is widely used for sputum smear microscopy for tuberculosis is also the most used method for BU. DSM is rapid and technically simple to perform, requiring comparatively low infrastructure investments to implement [10]. These features explain the widespread use of DSM as a first-line test for the diagnosis of these two mycobacterial diseases of public health importance in resource-poor economies.

A major drawback of DSM, however, is its low sensitivity, which is estimated to vary in the range 26–67% [1, 2, 11]. Of particular concern is the difficulty of DSM to detect AFB in clinical samples with low bacilli load. A negative result by DSM should therefore be confirmed by a more sensitive test, such as PCR.

Acid-fast environmental contaminants (introduced during washing steps in the staining procedure and in reagent preparation) or the rare presence of *M. tuberculosis* in the skin may lead to misdiagnosis. The positivity rate of DSM may vary with the clinical form of BU [3].

The use of fluorescent auramine O stain increases contrast to AFB allowing them to be seen more easily than the red of ZN-stained AFB, and the increased distinction permits the use of objectives with larger fields of view, thereby decreasing total examination time [10]. These features account for the rapidity and improved sensitivity of this staining method over the ZN method. However, auramine staining has not been extensively used for BU. In one study, the sensitivity of two smear preparations (smears made from suspensions of ground tissue and smears made directly from unground tissue) was compared in combination with auramine and ZN staining methods [10]. Both smear preparation methods and both staining methods were equivalent in any combination.

41.3.2 Innovative Approaches to Diagnose BU at the Point of Care

Technical and logistical difficulties (e.g., sample transportation, cold chain requirements, stable power supply, suitable laboratory infrastructure, and qualified laboratory staff) limit the use of PCR in BU endemic areas. The absence of a simple and rapid test that is appropriate for early diagnosis and use in low-resource settings where the disease is most prevalent remains a major challenge to BU control. Such a test should meet the WHO recommended criteria for an ideal diagnostic test suitable for developing countries: ASSURED. The test should be Affordable, Sensitive, Specific, User-friendly (simple to perform in a few steps with minimal training),

Robust and rapid (results available in 30 min), Equipment-free, and Deliverable to the end user. Currently, three potential rapid tests are in development for deployment in district hospitals and primary healthcare facilities. These include a loop-mediated isothermal amplification (LAMP) assay based on the isothermal amplification of *M. ulcerans*-specific insertion sequences, a fluorescence thin layer chromatography (f-TLC) assay for the detection of the mycolactone toxin, and immunological tests for the detection of *M. ulcerans*-specific antigens.

41.3.2.1 LAMP

LAMP is a nucleic acid amplification technique that occurs at a constant temperature. The technique has several features such as highly efficient amplification, high sensitivity and specificity, low susceptibility to PCR inhibitors, and ease of performance that enables it to be easily adapted for point-of-care application.

Four different LAMP tests have been described for the detection of *M. ulcerans* DNA in clinical samples [12–15]. Three of these are based on the amplification of different regions of the insertion sequence element IS*2404* and have a sensitivity of 60–100% and specificity of 100% compared to IS*2404* PCR [12, 15]. One of the LAMP tests referred to as IS*2404* dry reagent-based (DRB)-LAMP consists of lyophilized reaction reagents (master mix and primers) that can be stored at ambient temperature [15]. This portable format represents a step toward the development of a rapid field applicable LAMP test for the diagnosis of BU.

A DRB format of all three IS*2404*-based LAMP tests has further been optimized using a new LAMP chemistry and portable fluorimeter to improve amplification and readout of results. Laboratory evaluation of the optimized IS*2404* DRB-LAMP tests identified the BURULI primer set [12] (which achieved 100% sensitivity compared to IS*2404* qPCR) as the best performing primer set for the IS*2404* DRB-LAMP assay [15].

A prospective evaluation of this IS*2404* DRB-LAMP test (with the BURULI set of primers) is required to assess its performance at district level of healthcare in endemic countries.

41.3.2.2 F-TLC for Mycolactone Detection

Detection of mycolactone in BU lesions using liquid chromatography and mass spectrometry formed the basis of the use of this toxin for the diagnosis of BU [16]. The thin layer chromatography (TLC) method offers a simple and rapid test for the detection of mycolactone in clinical samples [16]. The test involves extraction of mycolactone from clinical samples and separating it from other lipids on a chromatographic plate according to their retention factor.

Wadagni et al. evaluated f-TLC and showed that f-TLC had a sensitivity of 73.2% and specificity of 85.7% when compared with PCR [17]. The sensitivity was higher than that of microscopy (66%) or culture (41%) and compared favorably with that of histology (82%) [11]. Further improvement in removing background lipids originating from human tissues should improve the sensitivity of the f-TLC technique and facilitate its use as a simple and rapid test for the diagnosis of BU at the district level of healthcare.

41.3.2.3 *M. ulcerans* Antigen Detection Tests

The polyketide toxin, mycolactone, has great potential as target for a specific diagnostic laboratory test for BU. Generation of monoclonal antibodies (mAbs) capable of specific binding to mycolactone [18] has allowed developing an ELISA for mycolactone quantification [19]. The assay sensitivity in the low ng range is comparable to what has been described for f-TLC [20]. However, mycolactone is known to be bound by serum proteins [21], which complicates the use of the ELISA as diagnostic tool for the analysis of serum protein-rich wound exudates or aspirates. The competitive ELISA reagents and protocols are currently used to develop a lateral flow assay as simple point-of-care test.

In an alternative approach, the exposed cell surface protein MUL_3720 has potential as target for the development of a diagnostic antigen capture assay [22]. Analyses with swab samples from BU lesions demonstrated that there is a close correlation between *M. ulcerans* bacterial load and the MUL_3720 capture ELISA readout. Compared to PCR, the sensitivity of the ELISA for long-term stored swab samples was about 50% and the specificity close to 100% [23]. Preliminary data indicate that the sensitivity for fresh samples may be higher.

41.4 The Laboratory Diagnosis of BU at National Reference Laboratories

41.4.1 PCR

Among the four recommended tests for diagnosing BU, PCR combines high sensitivity, high specificity, and rapidity to obtain results [1, 2]. It consists of the amplification of an insertion sequence, IS*2404*, present in the genome of mycolactone-producing mycobacteria, a subgroup of nontuberculous mycobacteria, which have been identified as pathogens responsible for infections both in humans and animals. These comprise mainly *M. ulcerans* and rare strains of other mycolactone-producing mycobacteria (certain *M. marinum* isolates, *M. shinshuense*, *M. liflandii*, and *M. pseudoshottsii*) [24]. In the genome of *M. ulcerans* strains from Africa and Australia, the IS*2404* sequence is present in more than 200 copies, rendering the test highly sensitive.

Samples used for microscopy and culture can also be used for PCR: swabs, tissues, and liquids from fine-needle aspiration; viable bacteria are not needed.

Several steps are needed to perform PCR: DNA extraction followed by amplification/revelation. This technique requires specific infrastructures, equipment, and technical skills that are not available in remote laboratories. Therefore, PCR cannot be used as a point-of-care test. Moreover, reagents are relatively expensive. In BU-endemic countries, there is generally at least one laboratory performing IS*2404* PCR.

DNA extraction procedures allow the release of DNA from the bacteria for amplification before revelation. There are several techniques using commercial kits some of which require specific equipment.

Currently, the technique of real-time PCR, also called qPCR, is most used. Formally, a conventional, gel-based technique has been used. Its sensitivity is relatively low compared to the qPCR. Another advantage of qPCR is the decreased risk of contamination.

In well-organized laboratories, time to results is 24–48 h after receiving the sample. Internal quality control is mandatory and includes all the steps of the technique. An external quality control program is organized by the WHO.

PCR is only suitable for the diagnosis of BU. Since DNA can still be amplified several weeks after treatment initiation, it cannot be used for treatment monitoring.

41.4.2 Culture

Among the four laboratory tests, culture is the only technique which allows confirming the viability of the pathogen. Therefore, if culture cannot be performed immediately, samples should be stored to ensure the viability of the bacteria using a transport medium such as the semi-solid, modified Dubos medium enriched with OADC, supplemented with PANTA and 0.5% agar [6]. All types of specimens may be used for culture.

Prior to culture, a decontamination step is performed to remove bacteria other than mycobacteria and to facilitate the growth of *M. ulcerans*. Several techniques are used, based on the property of acids or bases to destroy other bacteria while preserving mycobacteria. However, decontamination procedures can reduce the likelihood of obtaining a positive culture. An overall contamination rate of 2%–5% is considered acceptable for mycobacterial culture. Decontamination is indispensable for non-sterile specimens (swabs, tissues), while for liquid aspirates, no decontamination is needed.

After decontamination, the suspension is inoculated onto a culture medium, most often a solid medium (e.g., Löwenstein–Jensen, Middlebrook, Brown and Buckle medium). *M. ulcerans* grows at 29–33 °C. Depending on the bacterial load in the sample, culture is positive within 6–12 weeks but can take up to 12 months before positivity. Colonies suggestive of *M. ulcerans* appear yellowish or slightly pigmented and rough and have well-demarcated edges.

For identification, molecular techniques such as IS*2404* PCR or a line probe assay such as the CM/AS Hain kit are used on colonies.

Due to the long time to results, culture is not suitable for routine management of BU patients.

Compared to IS*2404* PCR, the sensitivity of culture has been reported to be 41%, while its specificity was 97% [11].

41.5 Quality Control

Maintaining quality diagnosis has a direct impact on patient care and treatment, prevention, and control of BU in endemic countries. Hence, results of diagnostic laboratory testing must be as accurate and reliable as possible to ensure appropriate clinical decisions.

Quality assurance is a critical component of laboratory testing and involves internal quality control (IQC) and external quality assurance (EQA) both of which monitor the quality system during the pre-analytical, analytical, and post-analytical phases of laboratory testing.

IQC and EQA data can be used to monitor and evaluate the performance of laboratory activities, serving as a critical component in the continuous improvement program of a laboratory.

41.5.1 Internal Quality Control

It is important to note that quality control activities are an integral part of the quality management process of the laboratory as defined in the ISO 15189:2012 standard.

In 1981, the WHO defined the term "internal quality control" (IQC) as "a set of procedures for continuously assessing laboratory work and release of results" (Laboratory Quality Management System Handbook, WHO/CLSI/CDC 2011).

The goal of IQC activities in BU testing is to detect, evaluate, and correct errors due to test system failure, environmental conditions, or operator performance before patient results are reported.

Several components of IQC are usually implemented in combination with regular laboratory management.

The table below summarizes the different IQC involved in BU diagnostic techniques.

Quality control area	Internal QC activities
Laboratory layout and administration	In a PCR laboratory, a strict spatial separation should be maintained between the different steps of the PCR testing procedure
	Every procedure performed in the laboratory must be carried out following standard operating procedures and by trained laboratory personnel
Laboratory equipment	An inventory of all laboratory equipment should be available in the laboratory, and each equipment must be uniquely identified for traceability purposes
Sample reception and accessioning	BU testing should be performed only upon the written request by an authorized person
	Upon arrival at the laboratory, personnel should verify that request forms are completed appropriately, and specimens are labeled properly
Reagents and consumables	All reagents should be labeled with their name, the date they were prepared, and the date they were first opened
	Practice first-in-first-out (FIFO) and first-expired-first-out (FEFO)

Quality control area	Internal QC activities
Laboratory tests	
Direct smear examination (Ziehl–Neelsen/ fluorochrome staining)	For every new reagent batch or reagent prepared, known positive and negative smears are stained with the prepared reagent and read on the microscope
	Smears are prepared from a known acid-fast bacilli-positive and negative sample (control slides)
In vitro culture (Löwenstein–Jensen medium)	After medium preparation, a representative sample of culture medium tubes should be incubated at 35–37 °C for 2 weeks to check their sterility
	Contamination rates should be verified monthly
Polymerase chain reaction (PCR)	Ensure that both positive and negative controls are included in every DNA extraction batch/PCR run

41.5.2 External Quality Control

External quality assessment (EQA) provides a means to monitor quality systems in all phases of the testing process. The term EQA is used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory. This comparison can be made to the performance of a peer group of laboratories or to the performance of a reference laboratory. The term EQA is sometimes used interchangeably with proficiency testing; however, EQA can also be carried out using other processes.

Methods used to evaluate a laboratory's performance are:

1. Proficiency testing
2. Blinded rechecking or retesting
3. On-site evaluation

EQA for microscopy is currently organized by national tuberculosis control programs (NTPs), and hence it is recommended that BU programs should collaborate with these NTPs to ensure that the same quality standards as in tuberculosis microscopy are used to diagnose BU.

With regard to PCR testing, the Institute of Tropical Medicine (Antwerp, Belgium) organized four rounds of EQA [25] after which the WHO transferred the implementation of this activity to the Pasteur Centre of Cameroon (CPC), an institution which serves as the WHO national and regional laboratory for many infectious diseases and as the national reference laboratory for diseases such as tuberculosis and BU. The CPC also serves as coordinating center for the BU laboratory network (BU-LABNET), a network of BU PCR laboratories in the African endemic region which was created in October 2019 and committed to improving the PCR diagnosis of BU. The EQA program will be organized under the framework of this novel network and will involve sending a test panel of negative and positive BU clinical samples to participating laboratories.

41.6 Future Perspectives

Remote and rural communities in low-resource African countries may be highly affected not only by BU but also by many other infectious diseases, and it is well recognized that there are many diagnostic gaps. PCR-based testing requires expensive equipment and reagents, complex infrastructure, and well-trained personnel, restricting its use to centralized diagnostic facilities. Diagnosis and surveillance are challenging when centralized services are involved, as turnaround times between submission of clinical specimens and diagnosis often take extended periods of time due to logistical (transport) problems.

Therefore, there is a great need for the development of a diagnostic BU assay designed for use at the point of care (POC). For nucleic acid-based molecular diagnostics like LAMP, smartphone-based detection combined with microfluidic technologies may on the long run develop into a broadly applicable diagnostic platform [26]. While a variety of other technology platforms are also suitable for the development of rapid diagnostic tests (RDTs), most RDTs for neglected tropical diseases are currently immunoassay-based and suitable to meet WHO's ASSURED criteria. BU is usually a slowly progressing disease, and some delay in receiving diagnostic results is not too critical for clinical decision-making. However, receiving results of a diagnostic test at the POC during a first contact with the patient reduces the need for multiple visits to the health facility, which represent a major financial challenge for many of the impoverished BU patients. For BU and many other infectious diseases, RDTs also have great value as epidemiological tools, as they enable to rapidly screen populations in endemic areas.

Immunoassay technologies generally make use of target antigens (antigen capture and antigen competition assays) or antibodies (serological assays) that are present in the patient sample.

Developing a serological laboratory test for BU has failed. One reason is that there is broad serological cross-reactivity between different mycobacterial species. Antibodies cross-reactive with *M. ulcerans* antigens may be elicited by BCG vaccination or natural exposure to *M. tuberculosis* or other pathogenic or environmental mycobacteria.

In recent years, antigen detection assays have been successfully developed for various tropical infectious diseases. Antigen capture and competition ELISAs for the quantification of *M. ulcerans* targets have been described for the *M. ulcerans* protein MUL_3720 and for mycolactone [19, 22] and may potentially be converted into RDTs. The most used type of RDT is lateral flow tests, which require no equipment and only basic familiarity with the test. Reagents used for ELISA are usually also suitable for lateral flow test development. One weakness of lateral flow assays is the subjective interpretation of readouts, but also here smartphone-based quantification methods are being developed. Another weakness of immunoassay-based RDTs is their limited sensitivity compared to PCR-based assays. It remains to be

validated whether the emerging immunoassays for BU will have sufficient sensitivity when tested with fresh samples at the POC.

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Clinical Features of Buruli Ulcer

42

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42.1 Introduction

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is a necrotizing disease of the skin, subcutaneous tissue, and bone and the third most common mycobacterial disease, after tuberculosis and leprosy [1]. BU is included in the WHO list of 20 neglected tropical diseases (NTDs) [2]. Most of these NTDs are endemic in Africa, and eight of them have cutaneous manifestations [3, 4].

BU is focally endemic in rural wetlands of tropical countries of Africa, America, Asia, and Australia. Cases have also been reported in non-tropical areas of Australia, Japan, and China [1].

BU is presently declining in several African endemic countries such as Benin [5].

Skills in clinical diagnosis of the disease are thus likely to wane. In addition, BU presents a clinical polymorphism which does not always make diagnosis easy, especially for healthcare providers working outside the endemic areas. Moreover, in Africa, the accuracy of the clinical diagnosis may be challenging

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due to the presence of other diseases with similar presentation [6]. It underscores the importance of a differential diagnosis if BU is suspected (see Chap. 43) and the necessity to confirm clinically suspected cases by means of laboratory tests (see Chap. 41). Laboratory tests routinely used to confirm the clinical diagnosis of BU are described in detail in the WHO Manual "Laboratory diagnosis of Buruli ulcer" [7].

The disease is rarely, if ever, contagious. *M. ulcerans* is an environmental mycobacterium associated with wetlands, especially those with slow-flowing or stagnant water.

Exposure of cutaneous tissues to *M. ulcerans* may lead to early clearance of the infection or development of disease soon after infection (primary BU) or of a subclinical or asymptomatic infection (latent BU) that may subsequently reactivate and produce BU disease [8]. An epidemiological model of BU has been published by Portaels et al. [1]. This model is a simplified schematic representation of a much more complex reality whose various aspects remain to be clarified [9].

In African countries, the delay in seeking medical attention may sometimes be very long [10]. Thanks to early detection campaigns and the decentralization of care, in certain endemic regions, this delay can be considerably reduced [3, 11, 12].

Mean incubation periods of primary BU are estimated to be under 3 months [13]. In Australia, in short-term visitors to endemic regions who otherwise live in non-endemic areas, the mean incubation period was 4.8 months [14]. Occasionally, the incubation period may be very short (<15 days). Some patients with such short incubation periods developed the disease after cutaneous trauma without superficial damage to the skin (e.g., bruise or sprain). These cases also suggest activation of latent *M. ulcerans* infection by local trauma [8].

The disease is rarely fatal, but when untreated or improperly treated it can lead to severe physical sequelae and enormous social and economic problems.

The lesions are relatively painless due to nerve damages caused by the toxic lipid, mycolactone, produced by *M. ulcerans* [15]. However, recent research studies performed in Africa have indicated that some patients experience substantial pain as a result of BU, particularly when non-ulcerated lesions become ulcers [16]. In Japan, approximately half of the patients have reported pain [17]. Fever and adenopathy are rare. There may be pain, fever, and even swollen lymph nodes when there is superinfection of the lesion by other bacteria.

All parts of the body may be affected. Lesions tend to form in exposed areas, most commonly on the arms and the legs. Although there is an obvious link between BU and parts of the body exposed to injuries, initial infection of the palms of the hands and the soles of the feet has never been observed.

42.2 Description and Classification of Clinical Features

Clinically, BU can be classified according to the different stages of the disease manifested by different clinical forms or according to the severity of the lesions.

42.2.1 Classification of BU Lesions According to Clinical Stages and Forms

Three stages may be distinguished in the development of the disease, comprising two active stages (nonulcerative and ulcerative stages: stages 1 and 2) and one inactive stage (scars: stage 3).

The active stages present a spectrum of forms, which may in part depend on time to seek medical care, host immune status, inoculum size, inoculation depth, geographic area, and *M. ulcerans* strain virulence [8, 18].

Nonulcerative forms represent the first stages of the disease. Early stages are often ignored by patients and may sometimes heal spontaneously. After variable periods (a few weeks to several months), these forms ulcerate. Delayed admission to the hospital results in an increased frequency of ulcerative forms compared to nonulcerative forms.

42.2.1.1 Stage I: Nonulcerative Forms

There are four nonulcerative forms characterized as follows:

- *Papule* (most often observed in Australia): painless, raised skin lesion, up to 1 cm in diameter with erythematous surrounding skin. May ulcerate early (Fig. 42.1).
- *Nodule* (commonly seen in Africa): subcutaneous, firm, palpable, painless, or only slightly painful although often pruritic, attached to the skin but not to the deep tissue, up to 3 cm in diameter. The lesion gradually increases in size and is sometimes surrounded by an edematous indurate zone. The surrounding skin may be discolored (Fig. 42.2).
- *Plaque*: painless, firm, indurate, raised, clearly demarcated, with irregular edges, dry, more than 2 cm in the largest dimension and covered with reddened or discolored papery skin (Fig. 42.3).
- *Edematous lesion*: a more diffuse, extensive, firm, non-pitting swelling, with ill-defined edges, painless or mildly painful and not perceptibly inflamed. This form may involve large parts of the body. This stage can last several days or weeks, or even months (Fig. 42.4).

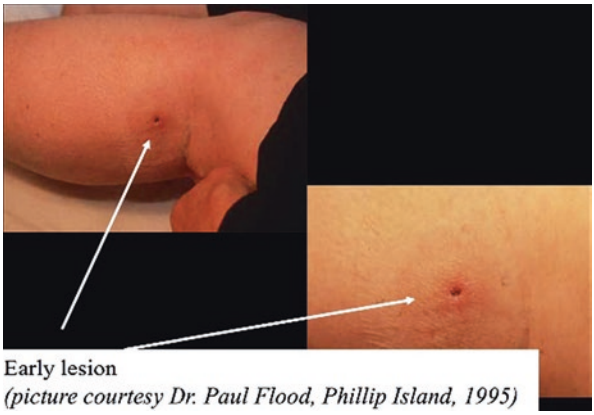

Fig. 42.1 Papular lesions detected in Australia (Credit: P. Flood)

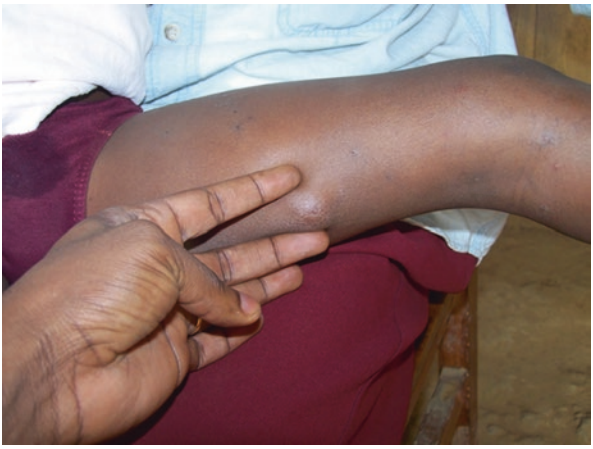

Fig. 42.2 Nodule on the left thigh. It is sometimes necessary for tension to be exerted on the tissues for nodules to be noticed (Credit: D. Phanzu)

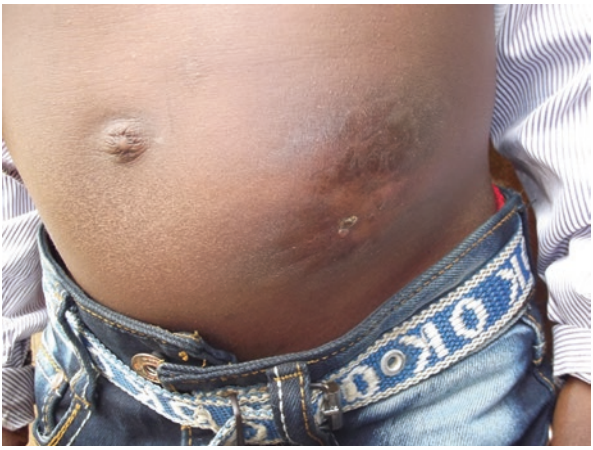

Fig. 42.3 Plaque on the left flank. Indurated plaque limited to the flank. The small circular lesion visible on the plaque could correspond to a point of inoculation (Credit: E. Kayinua)

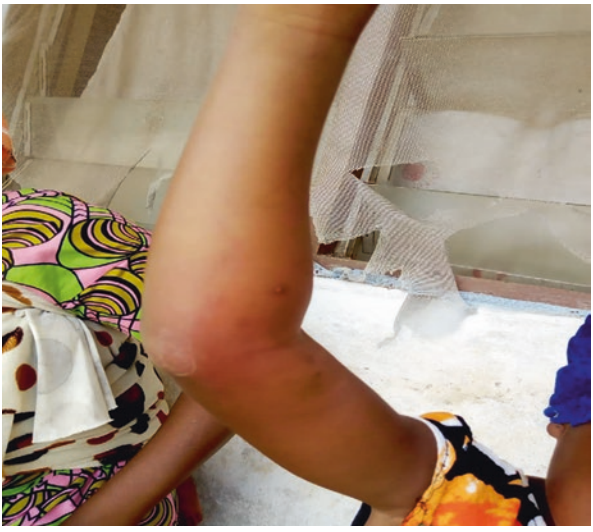

Fig. 42.4 Edema of the entire right arm (Credit: National Buruli ulcer Control Program Benin)

42.2.1.2 Stage 2: Ulcerative Forms

Ulcerative forms are characterized by more or less extensive single or multiple ulcerations, painless or slightly painful, undermined, with a center lined with yellowish-white necrotic slough and devitalized undermined edges surrounded by a zone of induration. The edges are sometimes hyperpigmented. There is no satellite adenopathy. These lesions are chronic and rarely heal spontaneously (Fig. 42.5).

42.2.1.3 Stage 3: Scar Lesions

Atrophic stellate scars, generally following stages 1 or 2, may be observed in poorly managed patients or in patients whose lesions healed spontaneously without surgical or medical treatment. Some severe lesions may heal spontaneously, as observed in 4.7% of patients in Benin [10, 19]. Spontaneous healing was also described in Australia [20].

When a scar is found over a joint, it may lead to very disabling sequelae. As a result of the adhesion and shrinking of periarticular scars, the scope of joint movement is reduced and joints may become ankylosed to the point that they no longer function at all (Fig. 42.6).

Besides non-ulcerative and ulcerative forms described above, some patients present complicated forms which include mixed, disseminated forms and bone involvement.

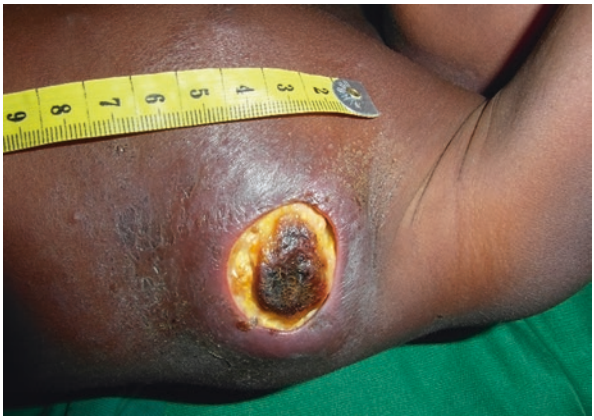

Fig. 42.5 Ulcer on the left lateral side of the trunk

Fig. 42.6 Retracted scar on the right hand following traditional treatment (Credit: F. Portaels)

42.2.1.4 Mixed Forms

Mixed forms are characterized by the simultaneous presence of different forms in the same patient, at the same or different sites.

42.2.1.5 Disseminated Forms

Disseminated forms are characterized by the presence of clinical forms which may or may not be similarly situated at different places on the body. Spreading of the disease to other parts of the body may be by contiguous spread or septic metastasis. It is therefore important to examine the patient all over, looking for new or old lesions. The patient may not always be aware of, for example, scars of healed infections (Fig. 42.7).

42.2.1.6 Bone Involvement

In Africa, osteomyelitis, either contiguous or metastatic, is observed in approximately 10% of patients [10, 21]. Bone involvement has not been observed in French Guiana or in Japan [17, 22].

The bacterium infects the bone by two different routes:

- Contiguous osteomyelitis: Reactive osteitis occasionally develops beneath destroyed overlying skin and soft tissue. Bone becomes devitalized and necrotic, with development of sequestrae.
- Metastatic osteomyelitis is most likely a result of lymphohematogenous spread of *M. ulcerans* from a cutaneous lesion which may already have formed a scar and of which the patient may be unaware.

Both contiguous and metastatic osteomyelitis often result in deformity and amputation [1] (Fig. 42.8).

The frequency of the different active forms varies according to geographic areas, early detection rates, and the delay between onset of disease and admission to a BU treatment center.

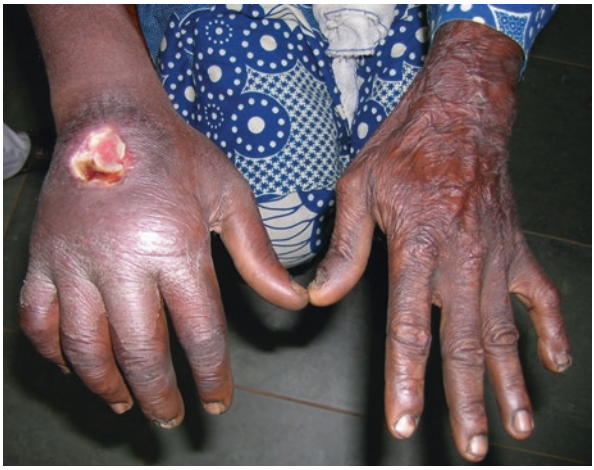

Fig. 42.7 Disseminated form of Buruli ulcer on the back of both hands. The right hand with an active lesion while a scar lesion on the back of the left hand of the same patient (Credit: National Buruli ulcer Control Program Benin)

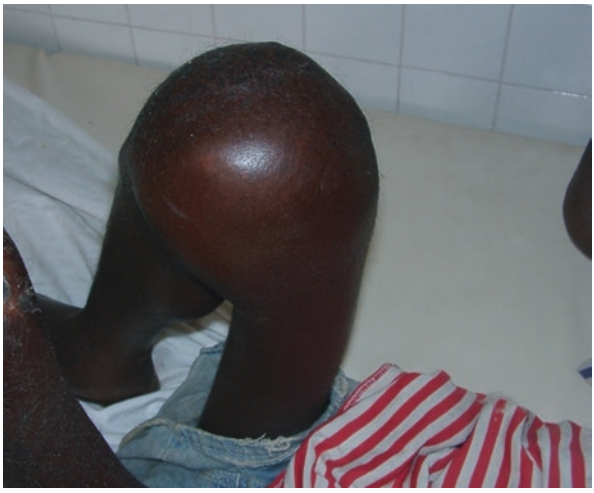

Fig. 42.8 Osteoarticular lesion at the right knee (Credit: National Buruli ulcer Control Program Benin)

42.2.2 Classification of BU Lesions According to the WHO Categories

In addition to the classification of BU lesions into non-ulcerative and ulcerative, for antibiotic treatment purposes, WHO has classified patients in three categories according to the size and severity of lesions [23]:

- *Category I*: Single or small lesions such as nodules, papules, plaques, and ulcers that are less than 5 cm in diameter.
- *Category II*: Non-ulcerative or ulcerative plaques, edematous forms, and single large ulcerative lesions of 5–15 cm in cross-sectional diameter.
- *Category III*: Disseminated and mixed forms including osteomyelitis, extensive lesions of more than 15 cm in diameter, and lesions at critical site (lesions in the head, neck, and face region, genitalia).

According to data published by WHO, the proportion of these different categories in the reported cases is, respectively, 32%, 35%, and 33% [24].

Treatments applied to these categories are detailed in the WHO manual (WHO 2012) [23] and in Chap. 45.

The frequency of some categories is largely dependent on the access to healthcare facilities in some geographic regions. Category I lesions are more frequent in Australia and Japan than in Africa where patients living in remote areas frequently present more advanced stages (category II and III lesions) [17, 25, 26].

Recently, in collaboration with endemic countries, WHO published a score to summarize the criteria for clinical suspicion of BU based on ten criteria [27] (see also Chap. 44).

In summary, clinical criteria for suspecting BU include:

- Presence of a chronically developing lesion (several weeks or months), i.e., a "wound that will not heal".
- No fever or regional lymphadenopathy.
- Typical nodular, papular, indurated plaque or edematous lesion.
- One or more relatively painless chronic ulcers with undermined edges or a depressed scar.
- Swelling over a painful joint, suggesting bone involvement.
- Patient age < 15 years (in Africa).
- Patient living or traveling in an endemic zone.

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Differential Diagnosis of Buruli Ulcer

43

William R. Faber, Ghislain E. Sopoh, and Jim E. Zeegelaar

43.1 Introduction

Several studies have highlighted the fact that the clinical diagnosis of Buruli ulcer (BU) by health professionals is not as simple as one usually thinks, even in endemic countries [1–3]. Kibadi et al. (2010) found that 34% of patients in the Democratic Republic of Congo completing the clinical and epidemiological criteria of BU, as defined by WHO, could not be confirmed by microbiological examinations [4]. Siegmund et al. (2007) showed that even with the most sensitive tests, 22% of suspected cases of BU could not be confirmed [5]. Indeed, each of the clinical forms of BU can be confused with many other conditions. The differential diagnosis of BU depends on the stage of presentation of the disease and the pathologies existing in the area where the patient lives.

Although the disease is named "Buruli ulcer," clinical manifestations without ulceration also exist, and for each of these manifestations, there is a differential diagnosis.

The following clinical forms can be distinguished:

1. Papules
2. Nodules
3. Plaques

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4. Inflammatory edema
5. Ulcers
   (a) Infectious
   (b) Noninfectious
6. Scars
7. Other BU forms
   (a) Mixed forms
   (b) Disseminated or multifocal forms
   (c) Paradoxical reaction

Osseous lesions are described under edematous lesions as they are frequently associated with edemas.

Most differential diagnoses are of infectious origin [3].

For most of the infectious conditions described in this chapter, laboratory tests are available to confirm them. Histopathological investigation may be important for the confirmation of some diseases such as subcutaneous mycoses. These tests are not included in this chapter.

It is important to know if a disease is geographically restricted or has a global prevalence. The differential diagnosis is therefore also dependent on the geographical area as a number of conditions covered in this chapter are geographically restricted.

43.2 Papules and Nodules

These two conditions are described together as their only distinction is the difference in size, a nodule being bigger than a papule.

Definition of a papule: circumscribed solid elevation of the skin <1 cm, heals without scar formation.

Definition of a nodule: firm and painless swelling under or elevated above the skin of about 3 cm maximum in diameter.

43.2.1 Cutaneous Leishmaniasis (CL)

CL is caused by protozoan parasites belonging to the genus *Leishmania*, which is divided into two subgenera, *L.* (*Viannia*) spp. and *L.* (*Leishmania*) spp.

In the Americas, CL is caused by at least eight different species, primarily of the *L.* (*V.*) *braziliensis* and *L.* (*L.*) *mexicana* complexes. Afro-Eurasia CL is caused by four species: *L. major*, *L. tropica*, *L. aethiopica*, and *L. infantum*. The parasites are transmitted to humans via the bite of the female phlebotomine sandfly which has fed on an infected mammal.

The disease occurs throughout the tropical and the subtropical regions. In the past decades, there is a definite increase in the incidence of CL. This is because of factors such as rural to urban migration, development of new agro-industrial

projects, re-locating non-immune communities in endemic areas, movement of army troops into endemic regions and the termination of insecticide spraying, and possibly also climate change. HIV infection does not seem to increase the risk of CL infection, but may influence treatment response.

It is estimated that 1–1.5 million cases of CL occur annually.

The clinical picture of CL varies with the endemic region and depends on the species involved, the immune status of the host, genetics, and probably the transmitting sandfly. The typical lesion is a painless ulcer although it starts with a circumscribed swelling which increases in size before ulceration occurs. They are generally localized on exposed body parts as they are the consequence of a bite of the sandfly [6].

43.2.2 Mycobacterial Infections

Infection with *M. tuberculosis* and a variety of so-called atypical mycobacteria may present skin manifestations.

43.2.2.1 Cutaneous Tuberculosis

Cutaneous tuberculosis is still endemic in some tropical countries. Several presentations of infection with *M. tuberculosis* may start with papules and nodules [7].

- Primary Infection

Primary infection accounts for only 2% of all cases of cutaneous tuberculosis. It is caused by exogenous inoculation of *M. tuberculosis* in the skin of a non-sensitized person. The lesion starts 2–4 weeks after inoculation as a smooth papule or nodule. After 3–8 weeks, non-tender regional lymphadenopathy develops, which may suppurate to form a "cold" abscess, which then may spontaneously drain with sinus tract formation. This process in general heals spontaneously with atrophic scarring in 3–12 months. Primary lesions are mainly located on the face and the extremities in children, but inoculation by injections and surgical procedures is also possible.

- Scrofuloderma

It is due to contiguous spread from a deeper localized infection such as lymph node or in some cases bone. Initially there is an indurated inflammatory area overlying the deeper infection. Due to suppuration, fluctuating nodules develop. In the course of time, cord-like scars or keloids develop. The lesions are mostly localized over the lymph glands in the neck.

- Lupus Vulgaris

It is caused by reactivation of the disease in patients with a high degree of cell-mediated immunity after earlier hematogenic dissemination. The lesions start as

brown-red papules. The most common location is the face; lesions on the legs and the buttocks are common in Asia and Africa.

- Tuberculous Gumma

Tuberculous gumma or metastatic tuberculous ulcer is caused by hematogenic dissemination from a primary focus during periods of lowered resistance. The lesion starts as a subcutaneous nodule or a fluctuant swelling.

A high index of suspicion is warranted because the clinical picture of mycobacterial infection of the skin can be non-specific. Investigation for mycobacteria is indicated in cases of persistent infiltrative lesions or non-healing ulcers.

43.2.2.2 *M. marinum* Infection (So-Called Swimming Pool Granuloma)

As initial reports of cutaneous disease by *M. marinum* were associated with swimming pools, it was called swimming pool granuloma. Infection in swimming pools nowadays is rare due to proper chlorination. The distribution is worldwide, occurring in fresh-brackish as well as salt water, and is prevalent in heated water (for instance, in tropical aquaria) in temperate climates and in pools and the sea in more tropical climates. In principle any water-related activity carries a potential risk for infection. Infection takes place through, in general superficially, traumatized skin.

As infection is preceded by trauma, majority of the lesions are located on the back of the fingers or the hand or around the knee. The initial lesions start as an inflammatory papule after a relatively long incubation period of 2–6 weeks. The papule then gradually enlarges into a bluish-red inflammatory nodule or plaque. There is generally a delay of months to even years before a doctor's opinion is sought because the lesions are painless and enlarge slowly. The lesions may heal spontaneously; this may take months to years. Less often deep infections such as tenosynovitis, osteomyelitis, arthritis, and bursitis occur. *M. marinum* infections are one of the causes of nodular lymphangitis (also called sporotrichoid extensions after the lymphatic spread of sporotrichosis). Clinically, there are nodules and/or ulcerating lesions resulting from spread along the lymphatic vessels. Deep infections and nodular lymphangitis do not heal spontaneously [8].

43.2.2.3 Other Mycobacteria

Other mycobacteria responsible for most cutaneous diseases are *M. fortuitum*, *M. chelonae*, *M. abscessus*, and *M. avium-intracellulare* which also may present with papules and nodules.

43.2.3 Leprosy

It has a variety of clinical presentations depending on the degree of cell-mediated immunity (CMI) of the host to infection with *M. leprae*. On the lepromatous part of the leprosy spectrum, ranging from mid-borderline to lepromatous leprosy, nodular

lesions can be seen. The number of lesions increases and the distribution becomes more widespread toward the lepromatous pole of the spectrum corresponding with the decrease in CMI.

During the type I or reversal reaction, increased inflammation leads to erythematous swelling of the lesions. As leprosy also affects peripheral nerves, this reaction is mostly accompanied by an acute neuritis with painful swelling of the involved nerves. In case of type II reaction or erythema nodosum leprosum, crops of painful erythematous nodules arise on normal-looking skin of especially the extremities and the face.

43.2.4 Non-venereal Endemic Treponematoses (Yaws, Pinta)

These diseases are widespread in many regions of the world, and millions of people, especially children, are at risk. They share evident cutaneous clinical manifestations and a chronic relapsing course. Yaws may nowadays present an atypical form or a milder, "attenuated" form in some regions, with less florid skin lesions, especially in areas with a low prevalence.

A serological test for treponemal and/or non-treponemal antibodies is required to make a definite diagnosis. There is no difference in this respect between the different non-venereal treponematoses.

43.2.4.1 Yaws

Yaws primarily affects children aged under 15 years who live in poor communities with poor hygienic conditions, warm, humid, and tropical forested areas of Africa, Asia, Latin America, and the Pacific islands in remote, often inaccessible, areas. It is spread by skin-to-skin contact.

After an incubation period of 9–90 days, the first (early stage) lesion, so-called mother yaw, appears. These primary lesions are mostly localized on legs, feet, and buttocks. Typically, it is a lesion with a papillomatous surface. Sometimes multiple primary lesions are present. They heal spontaneously in the course of 2–6 months. After or during spontaneous disappearance of initial lesions, relapses of more disseminated lesions can occur, which may be preceded or accompanied by fever, malaise, headache, and generalized lymphadenopathy. These early (secondary)-stage skin lesions often resemble the "mother yaw."

Tertiary lesions can present as nodular, nodulo-ulcerative, and gummas. Severe destruction of skin, bone, and joints can occur.

43.2.4.2 Pinta

Pinta is still prevalent in tropical Central and South America in remote rural regions.

In the early stage, a papule or an erythemato-squamous plaque occurs that is usually localized on the legs, feet, or hands. The initial lesions may become pigmented, hyperkeratotic, and scaly, accompanied by local lymphadenopathy. These initial lesions never ulcerate. After several months or even years, more extensive skin lesions may appear.

43.2.5 Syphilis

Disseminated papular and also nodular lesions are part of the clinical manifestations of the secondary stage which manifests 4–10 weeks after appearance of the primary lesion. In the late tertiary stage, nodules and ulcerative nodules can be seen. In these lesions it is difficult or impossible to demonstrate *Treponema pallidum* due the scarcity of the microorganisms. Treponemal and/or non-treponemal serological tests are required to make a definite diagnosis.

43.2.6 Erythema Nodosum

Erythema nodosum is considered to be an immunologic reaction to a variety of triggers. Well-known causes are sarcoidosis and streptococcal infections. However, in the majority of cases, no cause can be found. It has a worldwide distribution. It is more prevalent in females with a peak in the 15–40 age group.

Clinically it manifests as painful inflamed subcutaneous nodules, generally localized on the frontal part of the lower legs, although it may be localized elsewhere in the subcutaneous fat. It often resolves spontaneously in the course of 6 weeks when it is related to an infection; otherwise it may persist many months. Older lesions are more indurated.

43.2.7 Persistent Insect Bites (Fig. 43.1)

This is defined as a persistent local reaction after bite, sting, or contact with an "insect." Insects are Hexapoda (= six legs) comprising flies, fleas, lice, bugs, beetles, moths, bees, hornets, and ants. But for the definition of persistent insect bite, a reaction to other arthropods as, for instance, ticks and mites is also used. It has a worldwide distribution but is more common in regions with a tropical climate.

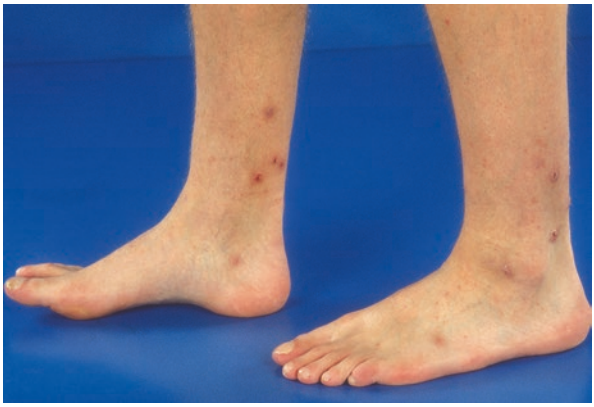

Fig. 43.1 Persistent insect bites

The clinical picture is that of papules and nodules. The duration can extend to weeks till (many) months and runs with spontaneous remissions and exacerbations. Characteristically they are more or less, but frequently severely, itching. Therefore, in the majority of the lesions, excoriations due to scratching are found. As it is a reaction to a contact, bite, or sting, they are localized on exposed body areas. A complication is bacterial infection due to scratching, especially of lesions localized on the legs. The diagnosis is clinical. Treatment is with topical strong steroids.

43.2.8 Subcutaneous or Furuncular Myiasis

Myiasis is infestation of the body tissues of humans and animals by the larvae (maggots) of flies (*Diptera*).

Cordylobia anthropophaga, the tumbu fly, a cause of furuncular myiasis, is widespread in tropical Africa, south of the Sahara. Eggs are laid on sand or soil, especially if contaminated by urine or feces, and also on laundry hanging out to dry. From these eggs, larvae develop that can survive in soil up to 9 days. These larvae can penetrate the skin of a host within 60 s.

Dermatobia hominis is a bluebottle-like fly found in the neotropical areas of the Americas, extending from southern Mexico to northern Argentina. It occurs where temperature and humidity are relatively high, principally in lowland forests. The female fly does not deposit her eggs directly on the host, but uses other insects, such as mosquitoes and blood-sucking flies, as vectors to carry her eggs to the host. When the vector subsequently feeds on a potential host, the eggs hatch and the larvae rapidly burrow into the skin.

After penetration of the skin, boil-like lesions develop gradually over a few days. Each lesion has a central punctum, which discharges serosanguinous fluid. The posterior end of the larva, equipped with a group of spiracles, is usually visible in the punctum, and its movements are usually noticed by the patient. In humans there is often an inflammatory reaction around the lesions. It may be accompanied by secondary infection, lymphangitis, and regional lymphadenopathy.

The larvae can often be expressed by firm pressure around the edges of the lesions, but the punctum may be enlarged surgically in order to remove the larva.

43.3 Plaques

Definition of a plaque: a solid flat elevated skin lesion with a diameter > 1 cm.

43.3.1 Cutaneous Tuberculosis

- Primary infection. This lesion enlarges during the course of several weeks into a plaque.
- Lupus vulgaris.

In the classical form, it extends into plaques with peripheral activity with an irregular border and central healing with atrophic scar formation and depigmentation. The clinical picture may be variable. Besides the plaque form, there is a hypertrophic form with nodules, which may form a hyperkeratotic mass.

Presentations of other mycobacteria may also evolve into plaques.

43.3.2 Leprosy

On the tuberculoid part of the spectrum, solitary or few flat plaques with a papular border and central healing can be found which are the result of enlargement of lesions in the context of a high degree of CMI, whereas at the lepromatous side, nodules can in time enlarge into infiltrated plaques.

43.3.3 Subcutaneous Mycoses

They are characterized by a heterogeneous group of infections that often result from direct penetration of the fungus into the dermis and subcutaneous tissue through traumatic injury. The most common presentations are plaque-like lesions [9].

Mycoses are restricted to certain regions. Most cases are encountered in those living and working in an endemic area with little availability of health resources. Sporotrichosis and basidiobolomycosis have a single specific etiological agent, whereas chromoblastomycosis and mycetoma are clinical syndromes with multiple fungal or bacterial etiologies.

43.3.3.1 Sporotrichosis

Sporotrichosis is caused by *Sporothrix schenckii*, which is a dimorphic fungus that occurs in nature as a saprophyte in soil, decaying organic material, and on surfaces of various plants. Infection results from traumatic inoculation of materials containing the fungus, particularly wood splinters or thorns through the skin. Zoonotic transmission has been described. The organism is particularly found in warm temperate and humid tropical climates and is one of the most common subcutaneous mycotic infections.

Individuals with activities, which expose them to the environment, are at risk. Sporotrichosis has a worldwide distribution. However, most cases at present are reported in South and Central America.

The incubation period is probably a few weeks. It is seen mostly as the lymphocutaneous or sporotrichoid form or less common as a localized or fixed cutaneous form. The primary lesions may appear as papular, nodular, or pustular lesions that develop into a superficial ulcer or a verrucous plaque. During progression, the lymphocutaneous form shows multiple subcutaneous nodules that are formed along the course of the draining lymphatics (sporotrichoid spread). The localized form shows no lymphatic spread and is characterized by indurated or verrucous plaques and occasional ulcers. Dissemination is rare and usually encountered in immunodeficient individuals.

43.3.3.2 Chromo(blasto)mycosis

Chromoblastomycosis is a chronic (sub)cutaneous mycotic disease that occurs more frequently in (sub)tropical areas. The disease is caused by saprophytic fungi that are found in soil and wood. It is most prevalent in tropical and subtropical America and Africa.

The disease occurs typically on the foot or the leg. It occurs mainly in farmers and rural workers who work barefoot. After inoculation of the fungus through the skin, slowly growing scaly wart-like nodules develop and ulceration may also occur. Clinically, it may be indistinguishable from verrucous cutaneous tuberculosis and localized sporotrichosis. It grows slowly and is painless, and lymphatic spread is only seen occasionally. Moderate itching is often mentioned. This may be relevant because scratching may contribute to inoculation of the fungus in adjacent areas. Scratching may also cause secondary infections, which are the most frequent complication of chromoblastomycosis.

43.3.3.3 Mycetoma (Fig. 43.2)

Mycetoma is a chronic granulomatous infection of the skin and the subcutaneous tissue characterized by deformation and increased volume of the involved subcutaneous tissue and, in the advanced stages, destruction of underlining bone structures. It is caused either by true fungi (eumycetoma) or filamentous bacteria (actinomycetoma). Nodules and openings of fistulae through which exudate containing grains *are discharged are* noticed on the skin surface. The grains, also known as sclerotia, are aggregation of hyphae produced by some species of fungi or the bacterial filaments from aerobic actinomycetes. Actinomycetic mycetoma is most common and accounts for 98% of the cases and is caused by aerobic species of the Actinomycetes group and the Streptomyces group. They are filamentous ramified bacteria that, when cultured, create colonies similar to fungi. The maduromycotic (eumycetic) mycetoma is caused by several fungi.

It is mainly a disease of the tropical climates and is more frequently seen in the rural areas where people work in farms under unprotected rudimentary conditions. Probably repeated punctures or abrasion of the skin are required for the inoculation of the organisms. The infection develops very slowly over the years. Its true incidence is unknown. Mycetoma is most often seen on the lower limbs (65%–70%).

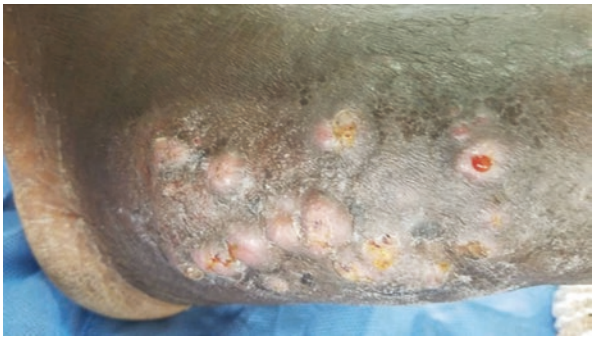

Fig. 43.2 Mycetoma

Clinically, the diagnosis is suspected in the presence of an increased volume of the affected tissue, formation of abscesses, and fistulae exudate containing grains.

43.3.3.4 Basidiobolomycosis

The Basidiobolomycosis is a rare deep mycosis of tropical and subtropical countries.

In early infection, the disease presents as inflamed and slightly painful nodules. In chronic infection, lesions are cold and painless. The edema is nonpitting and hard as wood, and the skin is rigid.

The evolution is slow and gradual, with periods of remissions over several months or years, in the form of an extensive plaque. Untreated infection may be fatal.

43.3.4 Panniculitis

Panniculitis is an (often painful) inflammation of subcutaneous tissue of the skin. Most patients have tender ill-defined red nodules and indurated plaques on the lower legs, thighs, and buttocks.

It includes a variety of diseases with different causes and therefore also different courses of disease. Diagnosis can be difficult and is based on clinical suspicion in combination with results from laboratory tests. A skin biopsy can be helpful for the classification of the panniculitis. The biopsy should include subcutaneous fat; therefore, a deep incisional biopsy is required. Treatment depends on the underlying cause.

43.4 Inflammatory Edema

43.4.1 Cellulitis

Cellulitis is an acute, spreading pyogenic inflammation of the dermis and subcutaneous tissue, caused most commonly by *Streptococcus pyogenes* and *Staphylococcus aureus* and usually complicating a wound, ulcer, or dermatosis.

The area, usually on the leg, is tender, warm, erythematous, and swollen, and demarcation from uninvolved skin is indistinct. Treatment is with antibiotics [10].

43.4.2 Erysipelas (Fig. 43.3)

It is a type of superficial cutaneous cellulitis. It presents with general malaise with high fever and a painful, bright red, raised, edematous, indurated plaque with advancing raised borders, sharply marginated from the surrounding normal skin. The most common localization is the lower leg, but it can also occur on the face.

It is usually caused by group A-hemolytic streptococcus (GAS) (very uncommonly group C or G streptococcus) and rarely by *S. aureus*. Treatment is with antibiotics

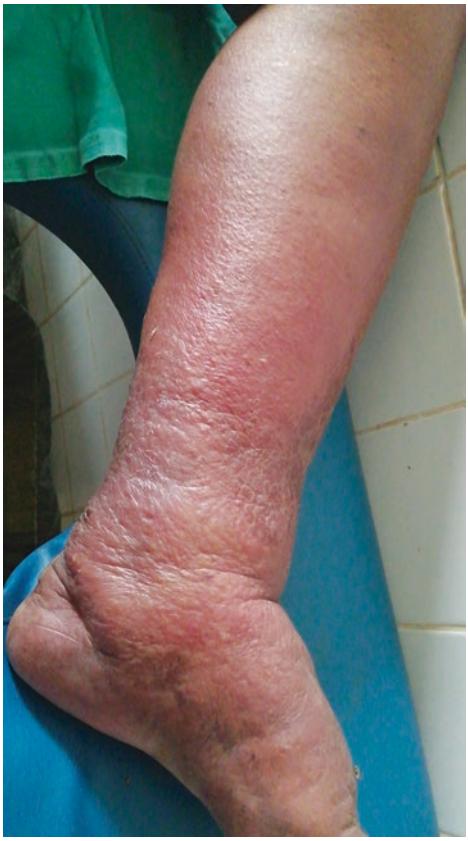

Fig. 43.3 Erysipelas

(penicillin). And as it is accompanied by marked dermal lymphatic vessel involvement, lymphedema may develop. The most common point of entry of the infection is the so-called toe web intertrigo, especially between the lateral toes, mostly due to a fungal infection, and treatment of this condition prevents a majority of erysipelas.

43.4.3 Necrotizing Soft Tissue Infections

Necrotizing soft tissue infections are infections of any layer of soft tissue compartment associated with necrotic changes and include necrotizing forms of cellulitis, myositis, and fasciitis. These infections are characterized clinically by fulminant tissue destruction, systemic signs of toxicity, and high mortality. It can be caused by a wide spectrum of organisms. It is associated with underlying conditions as, for instance, diabetes mellitus (DM), immune suppression, and obesity.

Clinical findings are initially swelling, erythema, pain, tachycardia; and it develops progressively with tense collateral edema, ecchymoses/blisters/bullae/necrosis, crepitus, and/or subcutaneous gas and is accompanied with disproportionate pain.

Treatment consists of early and aggressive surgical exploration and debridement of necrotic tissue, together with broad spectrum empiric antibiotic therapy and hemodynamic support [11].

43.4.4 Purulent Infectious Myositis (PIM)

Formerly known as tropical pyomyositis, PIM is an aggressive pyogenic infection of skeletal muscles which is also described from temperate climate zones. It affects all age groups, although young males are the most susceptible group. It is increasingly documented in persons infected with HIV.

It is usually a progressive febrile disease with pain in the affected muscle(s), and severe, life-threatening forms have been described, especially in immunosuppressed patients and children. Early diagnosis can be difficult due to lack of overlying skin changes [12].

Three clinical stages have been described:

1. *Invasive stage*. It is characterized by a subacute onset of variable fever and minimal systemic symptoms and a painful firm swelling, with or without erythema (as the infection is deep seated). The area is tender with a firm consistency. Aspiration yields no pus as this stage is a diffuse phlegmonous inflammatory process. About 2% of patients present in this stage. This stage lasts from 10 to 21 days. This stage may resolve itself, mimicking fibromyalgia or progressing to next stage of suppuration.
2. *Purulent or suppurative stage*. From the second week to third week, abscess forms in the muscle. The beginning of this stage is characterized by high fever and more severe systemic symptoms. Most cases present at this time. The classical signs of abscess, fluctuation, and erythema may be lacking because the process is localized within the muscle fascia. The involved muscle is usually tender, and the overlying skin may be normal or erythematous.

   Needle aspiration yields pus.
3. *Late stage*. High fever, severe pain, local signs of infection, and systemic manifestations of sepsis may be present. It is characterized by septicemia, metastatic abscesses, and multi-organ dysfunction and is associated with high mortality. There is exquisite tenderness of the involved muscle.

Atypical presentations exist. In some patients the invasive stage may be prolonged and the patient may present with pyrexia of unknown origin. Depending on the localization, it may present as an acute abdomen or spinal cord compression or compartment syndrome. When localized to neck muscles, it can be mistaken for cervicobrachial neuralgia. Noninvasive imaging techniques, ultrasound, CT scan, and MRI, are helpful in establishing the diagnosis. Treatment is by percutaneous or open surgical drainage along with antimicrobial therapy guided by culture results. *S. aureus* is the organism most commonly cultured.

43.4.5 Noma

A gangrenous infection of the mouth was a disease of the poor worldwide. But it nowadays especially affects children in underdeveloped countries in whom the constitution is altered by bad hygiene and serious (viral) illness. Most cases are reported from the so-called noma belt, ranging south of the Sahara from Senegal to Ethiopia. An increased incidence has been reported in patients with HIV infection. It starts with an ulcer of the mucous membrane with edema of the face, with salivation, and an unbearable stench; this phase generally lasts for a few days. A gangrenous necrosis rapidly destroys soft tissues and bone leading to gross deformity of the face. Mortality is still around 10%. Management consists of general measures, treatment of associated diseases, antibiotics and, in a later stage, reconstructive surgery [13].

43.4.6 Osteomyelitis

Osteomyelitis is an infection of the bone caused by bacteria, most commonly *S. aureus* but also by mycobacteria such as *M. marinum* and *Salmonella*. It can be the consequence of direct spread from an ulcer, an open fracture, or a surgical operation but also due to hematogenous spread. Clinical symptoms are pain in the bone, tenderness on palpation, and increase in pain by movement and weight bearing and signs as fever, swelling, and erythema. Acute and chronic forms of osteomyelitis exist.

Treatment is by antibiotics and also local measures depending on localization and other characteristics.

43.4.7 Bone Tumors

Bone tumors constitute 6%–10% of all tumors in children and adolescents. They are more frequent in adolescents around the age of 15, compared to younger children. Frequency decreases in young adults and then increases again around the age of 65. Boys are more affected than girls.

Clinical signs are bone pain and swelling of the bone or adjacent soft tissue related either to the extension of the tumor (inflammatory hourly pain) or with a fissure or fracture complication (mechanical schedule pain or mixed, i.e., combining intermittent and mechanical) and neurological signs (pain whose description follows a neurological pathway, suggesting nerve damage). Imaging techniques will aid in the diagnosis [14].

43.5 Ulcers

Definition of an ulcer: Ulceration is the absence of the normal tendency of a wound to heal, giving rise to tissue degeneration. There are many causes of ulceration which may be infectious or noninfectious or a combination.

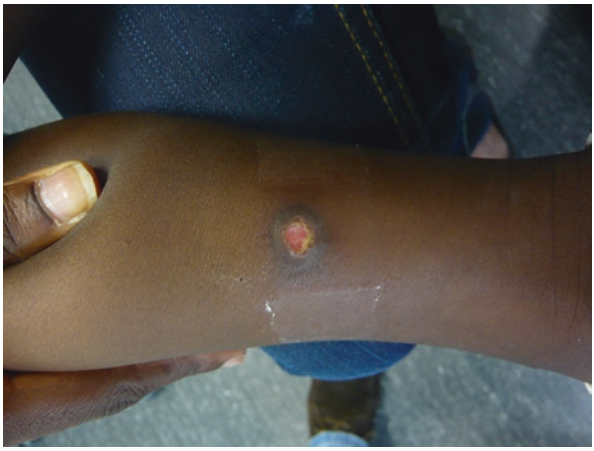

Fig. 43.4 Ulcerating pyoderma

43.5.1 Infectious Ulcers

43.5.1.1 Pyoderma (Fig. 43.4)

Pyoderma includes several clinically distinct types of skin lesions that are caused by *S. aureus* and/or *β-hemolytic streptococci group A*. It is a common cause of (purulent) ulcerative skin lesions in tropical countries.

Superficial skin infection may extend more deeply into the dermis and produce shallow ulcers known as ecthyma. The ulcer is covered with a dark-brown, bloody crust. A tender punched-out ulcer remains once the crust is removed. It is usually found on the dorsal feet, shins, and thighs, but less often on the upper part of the body. There are usually few lesions, but new lesions may develop without adequate treatment.

Ulcerating pyoderma is most commonly encountered as a secondary infection in skin lesions caused by environmental insults, such as insect bites, abrasions, and atopic dermatitis.

The diagnosis is often based on the clinical picture of persistent painful ulceration especially in the lower legs. One should perform a bacterial culture if facilities are available. Susceptibility tests in vitro are preferable. Methicillin-resistant *S. aureus* and tetracycline-resistant streptococci and staphylococci are frequently encountered in many areas of the tropics [15].

43.5.1.2 Cutaneous Leishmaniasis

CL is one of the most important causes of chronic ulcers in some parts of the tropical world.

Lesions are usually painless; if painful there is generally a secondary infection present.

CL in the Americas caused by species of the subgenus *Leishmania* will generally resolve, even without treatment, in 6 months, whereas lesions caused by the subgenus *Viannia* often do not resolve spontaneously. First a nodule develops which

enlarges and eventually ulcerates. A nodular lymphangitis may be present. Mucocutaneous leishmaniasis, although uncommon, may develop in approximately 3%–5% of patients as a complication of new-world CL caused by *Leishmania (V.) braziliensis*, but also occurs with *Leishmania (V.) panamensis*.

Afro-Eurasia CL lesions caused by *L. major* are often nodular, nodulo-ulcerative, and ulcerative. They develop slowly over months and generally resolve in 6 months. Lesions caused by *L. tropica* may persist as an erythematous papule for more than a year. Presentation is often as nodulo-ulcerative plaques with a necrotic base and indurated margin that are frequently covered by a firm adherent crust. The time period for spontaneous resolution is not well-known. It has been reported that leishmaniasis caused by *L. tropica* may affect the nose or the mouth. However, this is probably because of direct extension from skin lesions rather than from the dissemination of the parasite [15].

The initial diagnosis is based on the clinical picture of a non-healing painless ulcer in a patient who lives or visited an area where cutaneous leishmaniasis is endemic. BU and cutaneous leishmaniasis may have similar clinical presentation; therefore laboratory tests are important to differentiate between these two.

Laboratory tests include histopathological examination of biopsies, ulcer smears stained with Giemsa, and culture of the material obtained by needle aspiration or by biopsy. And if available PCR can be used [16].

43.5.1.3 Cutaneous Tuberculosis

- Cutaneous tuberculosis

  Cutaneous tuberculosis, especially primary infection, may show ulceration.

- *M. marinum* infections

  The primary lesion may ulcerate or show a warty surface. And also nodular lymphangitis may present with ulcerated nodules.

43.5.1.4 Subcutaneous Mycoses

Although plaques are the most common presentation, ulceration may occur.

The most common subcutaneous mycoses, which may show ulceration, are sporotrichosis, chromo(blasto)mycosis, and mycetoma.

43.5.1.5 Syphilis

Syphilis has a worldwide distribution in sexually active persons.

The incubation period is 10–90 days. Classically the primary stage is an indurated ulcer (= ulcus durum), but it starts with a papule-nodule before ulcerating. The lesions are typically painless. As it is sexually transmitted, the primary lesion is nearly always localized in the genital area, but extra-genital lesions do occur. In MSM also primary lesions in the peri-anal area can be found.

43.5.1.6 Diphtheria

Cutaneous diphtheria is an infectious bacterial disease caused by *Corynebacterium diphtheriae* or, more rarely, *C. ulcerans*. It is still endemic in many tropical countries and transmitted by direct contact with cutaneous carriers and to a lesser extent via vomit. *C. diphtheriae* produces a toxin which is responsible for the disease diphtheria. The microorganism (both toxigenic and non-toxigenic strains) may be harbored in the nasopharynx, skin, and other sites in asymptomatic carriers. *C. diphtheriae* is often found secondarily in pre-existing ulcers like ecthyma or as superinfection in eczema. In immunized individuals systemic toxic complications such as myocarditis and neuritis are rare. Skin lesions may be an important reservoir of infection. Contacts should be investigated and treated if necessary because there is a potential for secondary transmission. Cutaneous diphtheria is characterized by a chronic, non-healing ulcer with a punched-out appearance and an adherent membrane with a slightly undermined margin. In the first 2 weeks, it is painful; later the lesion becomes painless. After (spontaneous) removal of the adhering membrane, the hemorrhagic base appears. In many cases lesions are less distinctive. Secondary infection in any pre-existing wound and superinfection of eczematous skin lesions are common and often overlooked. Cutaneous diphtheria may persist for 6–12 weeks.

A high level of awareness among clinicians and microbiologists is necessary because cutaneous diphtheria ulcers are non-specific. The initial diagnosis is clinical.

43.5.1.7 Tropical Phagedenic Ulcer

Tropical phagedenic ulcer is a painful rapidly growing ulcer often on the lower leg, commonest in undernourished young people and particularly prevalent in the hot, humid tropical regions.

It may develop on abrasions, scratches, insect bites, or skin diseases such as pyoderma. Multiple factors as nutritional status, presence of fusiform bacilli, and spirochetes may contribute.

Its true prevalence is not known, but it seems to be quite rare these days.

It starts as a small papule or vesicle which becomes necrotic. The small ulcer rapidly enlarges. It is usually a 2–6 cm round punched-out ulcer with well-defined elevated borders. In the chronic stage, the ulcer is non-purulent, indolent, less punched out, and with a fibrotic border and may last for years.

43.5.2 Noninfectious Ulcers

43.5.2.1 Post-Traumatic Chronic Ulcer

Post-traumatic ulcers can be defined as a cutaneous lesion, resulting from acute exposure to energy (mechanical, thermal, electrical, chemical, or radiant). Road traffic injuries and intentional injuries (self-inflicted injuries, interpersonal violence, and war-related injuries) are most important causes of traumatic ulcers.

Injury is a significant health problem throughout the world. About 5.8 million people die each year as a result of injuries. This accounts for 10% of the world''s deaths, 32% more than the number of fatalities that result from malaria, tuberculosis, and HIV/AIDS combined. By far the greatest part of the total burden of injury,

approximately 90%, occurs in low- and middle-income countries [17]. The risk factors for road accidents are increasing in many developing countries. The global burden of disease due to road traffic injuries is expected to move from the ninth position in 2004 to the fifth position by 2030.

A carefully obtained medical history will reveal the origin of the ulcer.

If the ulcer is traumatic in origin, it should be defined in terms of high impact, low impact, repetitive, temperature related, caustic, radiation induced, type of bite, presence of drug abuse, and so on. In chronic ulcers, the age of the wound is important because long-standing wounds can be malignant (Marjolin's ulcer). Previous topical therapy to the ulcer should be delineated, because certain topical agents can contribute to the ulcer's chronicity (e.g., caustic agents such as hydrogen peroxide, 10% iodine, Dakin's solution, and so on).

43.5.2.2 Pyoderma Gangrenosum (PG)

PG is a rare reactive noninfectious inflammatory skin condition with a worldwide distribution that is difficult to diagnose. It is diagnosed clinically by exclusion of other causes of ulcers. Classical PG is the most common form (approximately 85% cases). This presents as an extremely painful erythematous lesion which rapidly progresses to a blistered or necrotic ulcer. There is typically a ragged undermined edge with a violaceous/erythematous border. The lower legs are most frequently affected although PG can present at anybody site. Subtypes include bullous, vegetative, pustular, peristomal, and superficial granulomatous variants. The differential diagnosis includes all other causes of cutaneous ulceration as there are no definitive laboratory or histopathological criteria for PG. As underlying systemic conditions are found in up to 50% of cases, thorough investigation for such conditions should be performed once a diagnosis of PG has been made.

43.5.2.3 Sickle Cell Ulcer (Fig. 43.5)

Sickle cell disease is caused by an abnormality in the gene encoding the β-chain of hemoglobin. When deoxygenated, sickle cell hemoglobin interacts hydrophobically

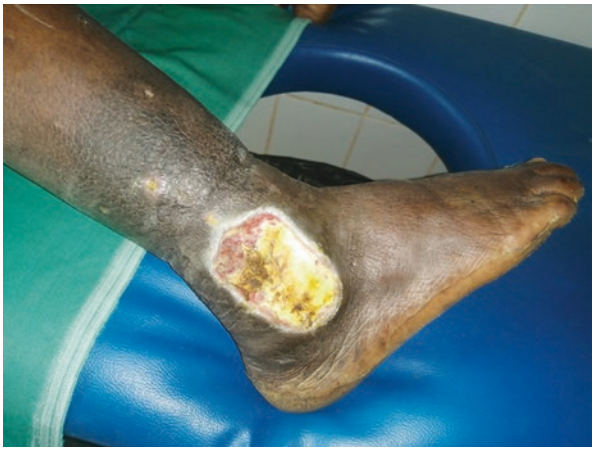

Fig. 43.5 Sickle cell ulcer

with other hemoglobin molecules and tends to aggregate and polymerize, resulting in the characteristic sickle shape. The pathogenesis of leg ulceration in sickle cell is not completely understood. Abnormal adherence of the sickle cell to endothelial cells has been postulated to be important in the initiation and/or progression of vaso-occlusive events leading to infarction of the skin. The sickle cell ulcers are commonly seen in adult males with homozygous sickle cell disease (HbSS) and are unusual in patients with sickle cell hemoglobin-C disease (HbSC) as well as those with sickle cell beta^{+} thalassemia [18].

Ulcers are a common cause of morbidity among North American and Jamaican patients with homozygous sickle cell (SS) disease in whom prevalence of 75% have been reported. The reported incidence from Africa is much lower, incidence varying from 5% to 9.6%. The cause of these differences is not known; however, age distribution of the population and genetic factors might play a role.

The incidence is very low in children under age 10 and markedly increased for those over age 50. Ulcers persist for months to years, heal slowly, and commonly recur. Most ulcers are located in the ankle area over the medial or lateral malleoli. The size of ulcers varies from a few millimeters to large circumferential ulcers. Ulceration is proceeded by prodromal pain and is often spontaneous or after minor trauma. The ulcer has a punched-out appearance with raised margins and a deep base. Radiographs often show some periosteal reaction which makes it difficult to rule out osteomyelitis. Secondary infection is found very often and might delay wound healing.

Pain is often very severe and probably the major problem. It has a great impact on the quality of life in these patients. Diagnosis is made on clinical observation of a painful leg ulcer, mostly in the ankle area in patients having sickle cell anemia. Edema is often present.

43.5.2.4 Vascular Ulcers

There are only very few epidemiological studies available on venous, arterial, or lymphogenic causes of ulcers in the tropics. Studies performed in the tropics reveal a very low or absent prevalence of vascular ulcers.

In the western world, the incidence of arterial disease is still increasing. As many people in developing tropical countries are changing their lifestyle into a more Western one, it is expected that arterial disease will become more prevalent.

Venous ulceration is the end stage of venous hypertension. Clinically edema, lipodermatosclerosis, hyperpigmentation, hyperkeratosis and atrophie blanche may be noticed in chronic venous insufficiency. Ulceration is often not painful.

Arterial insufficiency is occlusion that usually affects the entire femoropopliteal track, but may also affect only small-sized branches which may lead to limited infarction of skin and subcutaneous tissues.

Diagnosis of a venous ulcer is made on signs of venous insufficiency. Venous duplex scan may show superficial and/or deep venous reflux.

Diagnosis of arterial ulcers is made on history of the patient with ischemic disease. Clinical examination may reveal diminished peripheral pulsations. Doppler ultrasound measurements of the peak velocity of the peripheral bloodstream or

measurement of the systolic blood pressure at the feet are a useful parameter to calculate the degree of severity of the peripheral vascular disease. For visualization of the peripheral arterial tree, translumbar and transfemoral arteriography can be performed.

43.5.2.5 Malignant Ulcers

Cutaneous metastases originating from internal cancer or cancer originating in the skin may show ulceration. The three major types of skin cancer are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. These skin cancers are probably as a result of the protective effect of darker skin pigmentation far less prevalent in skin of color compared to the white population. Melanoma distribution in skin of color is often seen in sun-protected sites of the palms, soles, and nail bed.

Most important seems the development of SCC in chronic traumatized or inflamed skin as seen in chronic leg ulcers and traumatic scars. A large study in Nigeria showed a preponderance of SCC of the leg related to neglected, poorly managed, and chronic ulcers or scars from burns or injuries. Albino patients had a higher frequency of both SCC and BCC mostly on the head and neck. A study performed in Malawi showed a surprisingly large number of malignancies in skin ulcers. It is suggested that HIV disease of which the prevalence is very high in this population may be responsible. A three- to fivefold increased risk of developing a nonmelanoma skin cancer has been reported in persons with AIDS.

Signs and symptoms associated with the development of the carcinoma include a change in the scar with formation of a mass or ulcer, possibly with an increase in pain, increasing discharge, foul odor, and bleeding.

In all, non-healing ulceration of the skin biopsy remains the most important definitive diagnostic procedure, and it should be performed on any suspicious lesion or any chronic ulceration, especially those with any recent change in appearance.

43.5.2.6 Neuropathic Ulcers

Neuropathy can occur in several diseases; clinically most important is neuropathy of the feet. Most common causes of neuropathic feet worldwide are leprosy and DM.

Nerve invasion is a unique characteristic of *M. leprae* and results in nerve function impairment. It is estimated that 20%–30% of the leprosy patients have foot problems due to loss of nerve function. As a result, after trauma of the skin, ulceration of especially feet (and hands) may occur.

DM has become a worldwide pandemic, with two thirds of the global diabetic population living in developing countries.

Damage of peripheral nerves causes malfunction of the sensory, the motor, and the autonomic nerve fibers, thus increasing the vulnerability of the foot. Ulcers are usually localized at pressure points which result in deformity due to loss of function of motor nerves. In case of leprosy, ulcers have a chronic course. In case of DM, the clinical course is more acute as there is nerve function impairment in combination with metabolic disturbance and often vascular insufficiency. Also, a (progressive) bacterial infection is far more common. In leprosy as well as in DM, so-called

Charcot feet (= neuro-osteoarthropathy) can develop. This leads to gross deformity of the foot with ulceration on atypical localizations.

Management of neuropathic ulcers can be difficult. Most important is relief of pressure on the ulcer area to facilitate healing.

43.6 Scars

43.6.1 Ulcers

By definition all clinical conditions described under ulcers heal with, in general atrophic, scar formation. The clinical presentation depends on the depth and the size. When the extent of the ulceration is large, it may lead to deformities.

43.6.2 Third-Degree Burns (Fig. 43.6)

Third-degree burns are full thickness burns destroying the epidermis and dermis and may extend into the subcutaneous tissue. In burns up to 70% of patients develop hypertrophic scars.

43.6.2.1 Chronic Osteomyelitis

Deep or extensive ulcers that fail to heal with appropriate ulcer care should raise suspicion for osteomyelitis, especially if they are localized over bony prominences.

Following ulceration but also after surgical intervention, scars, sometimes extensive with deformities, develop.

43.7 Other Buruli Ulcer Forms

Apart from the classical forms, BU can present as unusual clinical forms that can induce misdiagnosis. These are the mixed forms, the disseminated or multifocal forms (see Chap. 42), and paradoxical reaction (see Chap. 45).

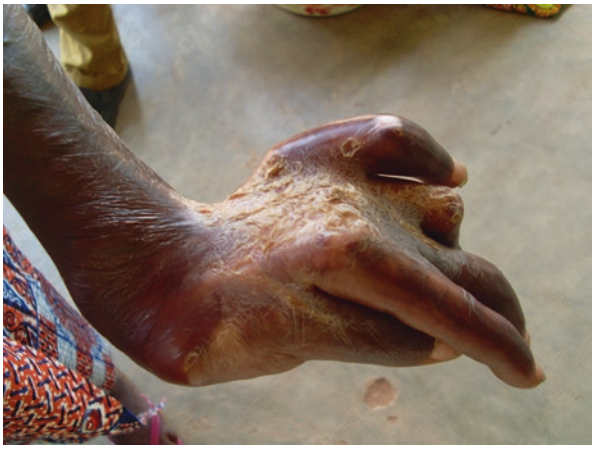

Fig. 43.6 Scar from thermal burn

43.7.1 Mixed Forms

The mixed forms can be defined as a combination of two or more types of primary lesions [19]. In settings where laboratory confirmation or X-ray is not always available, the diagnosis of BU can be difficult as differential diagnosis of deep fungal infections [20] or bone tumor [14].

43.7.2 Disseminated or Multifocal Forms

The disseminated or multifocal forms are defined as several identical or different types of lesions that coexist in the same patient, on the same or more body parts. Disseminated lesions usually present as an acute process, thus implying a differential diagnosis when the patient is seen at first consultation with multiple lesions. When they occur in a patient under antibiotic treatment for BU, they are referred to as paradoxical reaction. Most of the disseminated or multifocal forms at diagnosis are associated with HIV coinfection. However, also some cases without HIV coinfection have been described [21, 22]. The presence of multiple lesions at first consultation in a patient suspected of BU, in the absence of laboratory confirmation, suggests the differential diagnosis of cutaneous tuberculosis or cutaneous manifestation of bacterial sepsis. Cutaneous manifestations of systemic bacterial infections result from the bacteremia or septicemic dissemination or secondary to an infection at a cutaneous entry point [21]. Two main skin lesions are seen: "true" septic skin metastases which can be diagnosed clinically and above all bacteriologically by local samples and "aseptic" skin lesions only allowing a clinical diagnosis. The skin manifestations are polymorphic with ecthyma gangrenosum, subcutaneous abscess, and panniculitis.

43.7.3 Paradoxical Reaction

Paradoxical reactions are defined as new inflammatory lesions following an initial improvement of BU lesion during or after antibiotic treatment, leading to increased inflammation around lesions, extension of an ulcer, or a new lesion on a different part of the body. Paradoxical reactions are sometimes seen on parts of the body where there was no evidence of disease before antibiotic treatment, perhaps as a result of subclinical infection. Cultures of tissue or pus are usually sterile, although acid-fast bacilli can still be seen and PCR for *M. ulcerans* IS*2404* may remain positive. Paradoxical reactions occurred in 21%–22% of the patients, most of them were HIV or HBV coinfected [23, 24]. Paradoxical reactions per se do not give any problem with respect to differential diagnosis, since the diagnosis was already made, and the patient under treatment. However, the management of paradoxical reactions requires therapeutic abstention or, in the event of severe reaction, corticosteroid therapy. Other mechanisms which give similar clinical signs should be considered. This mainly concerns secondary bacterial infection of lesions, or bacterial sepsis [25]. Secondary infections can be misclassified as paradoxical reactions, since they also induce worsening of the lesion. They are all the more to be feared as the

so-called paradoxical reactions occur in patients presenting with immunosuppression. Secondary infections of BU lesions are common and can occur before (60%), during (65%), and after (75%) specific antibiotic treatment [25]. This will lead to worsening of lesions and delayed healing and should therefore be further investigated before drawing the conclusion of paradoxical reaction. The patient will present with pain, fever, anorexia, poor general condition, and other systemic symptoms. Culture from any new lesion should be performed to differentiate a relapse from a paradoxical reaction.

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Part XI

Buruli Ulcer: Patient's Management

Diagnostic Work-up of Buruli Ulcer

44

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44.1 Introduction

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is a neglected tropical disease (NTD) [1] with polymorphic skin manifestations, which slowly evolves to debilitating sequelae when not diagnosed and managed early [2–4]. The wide differential diagnosis makes diagnosis difficult, especially in developing countries [5, 6], where nurses in local clinics play a key role in primary healthcare. A simplified clinical approach and logical reasoning to arrive at a reasonable diagnosis are critical.

We describe the medical approach for clinical examination, the procedure for laboratory confirmation, and guidelines for the discussion of the differential diagnosis in low- and middle-income (LMI) and in high-income countries.

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44.2 Diagnostic Work-Up in Low- and Middle-Income Countries

LMI countries often face limitations in their healthcare systems, with few diagnostic technologies available. Therefore, clinical examination is very important and leads to a diagnostic suspicion, which laboratory testing can confirm. Diagnosis is conducted using an integrated approach, making it possible to consider all clinical diagnoses and to obtain, on the basis of epidemiological and clinical criteria and proceeding by elimination, the most likely diagnosis, which may then be the subject of a laboratory investigation or expert diagnosis. Due to a limited access to medical doctors, especially dermatologists, a first suspicion is made in the community by village volunteers trained to recognize the various skin lesions [7, 8]. Trained nurses at peripheral healthcare centers (HCCs) make the clinical diagnosis based on key epidemiological and clinical criteria. In case of doubt, medical doctors of intermediary or central level confirm the diagnosis. A standardized approach to laboratory investigation improves efficiency.

BU, a disease typically with prominent skin manifestations, requires careful examination of the skin, in addition to general clinical examination of the patient. The patient's examination can be conducted using a three-step process.

44.2.1 Clinical Examination of the Patient

The examination of the skin or dermatological examination is done in three steps.

44.2.1.1 Step 1: History

This step involves asking the patient about the following six elements:

- His/her identity: age, sex, occupation, and place of residence or origin.
- Reason for the visit to the healthcare center.
- Presence of similar cases in family or village.
- History of lesion: trauma (even blunt), sting, or bite, when the lesion was first seen, duration, slow and rapid progression of the lesion, and treatments already taken.
- Signs and symptoms: absence or presence of inflammation (pain, warm to touch, redness, and swelling), as well as other general signs such as fever, anemia, edema, jaundice, itching, and scaling.
- Living conditions: warm and humid environment, proximity to water bodies (lakes or marshes), living, or having lived, in an endemic place, and travel to a known endemic area.

In Africa, around 40% of cases of BU occur in children, with equal sex distribution [9]. Lesions in elderly individuals tend to have another etiology, such as vascular or tropical ulcers, especially when located on the legs [10]. Living in or traveling to a known endemic area (where laboratory-confirmed BU has previously been

documented) is a significant risk factor [11]. *M. ulcerans* is known as a bacterium whose reservoir is in the environment [12, 13]. An area with no history of confirmed cases that shares a common border with a known endemic area is considered at risk and should also be considered carefully when patients are seen coming from such places. The history should ask if there are other people with similar lesions in the area.

Although human-to-human transmission has not been demonstrated for BU, familial cluster have been described [3, 14]. Additionally, enquiries after close relatives could help with the differential diagnosis for other skin conditions, in particular NTDs that can be transmitted by contact, such as leprosy, yaws, or even scabies.

Usually, patients with BU report no antecedent event preceding the lesion. Occasionally, some report a history of trauma or insect bite in the months preceding the first symptoms of the disease [13, 15–17].

The history of the lesion is especially important for diagnosis. The history should review the initial lesion and its characteristics. The absence of spontaneous pain favors BU [2, 18–20]. In contrast, there may be itching or scaling. The lesion usually evolves slowly over several weeks or months (4 weeks or more) [21, 22]. Lesions less than 4 weeks old are unlikely to be BU.

Because of the usual absence of pain, patients may seek for treatment late (approximately 3 months after onset of the first lesions) and after trying self-medication or traditional healers' treatments [7, 23].

44.2.1.2 Step 2: Clinical Examination of the Skin

Clinical examination of the skin should ideally be conducted in adequate conditions, ensuring there is both confidentiality and good light on the patient's body.

The clinician should look closely for and describe the basic lesions generally found in NTDs of the skin, namely, macules or patches, papules, papillomas, nodules, plaques, edema, ulcers or ulcerating lesions, and osteomyelitis with fistulas. The following characteristics of lesions should be specified (in **bold** those characteristic of BU):

- Size (big or small), appearance, color, presence or **absence of tenderness**, and hard or soft consistency of closed lesions.
- Characteristics of ulcers: **undermined**, raised or swollen edges; necrotic and yellowish base; **little or no tenderness**; **presence of a specific smell**; **absence of regional lymph nodes**.
- Number and location of lesions (lesions are most often **single**).

BU presents in two standard clinical forms which have their own evolutionary stages: nonulcerative forms and ulcers [24, 25]. *The nonulcerative forms* can present with four types of clinical lesions: nodule, papule, plaque, and edema. They are the onset forms of the disease. *The BU ulcer* follows the nonulcerative lesions. Several authors have described a characteristic smell of BU ulcers, unanimously recognized as strong, disagreeable, and nauseating, like the smell of rotten fish, cassava, or cheese, mixed with smell of pyocyanic bacteria but distinct from the smell

of putrid wounds [26, 27]. In addition to these standard nonulcerative or ulcerative clinical forms, BU can present as *osteomyelitis, scars, mixed forms, or multifocal lesions*. The clinical forms are described in Chap. 42.

Macules and papillomas are not specific to BU and should point to other conditions, such as eczemas, scabies (macules), or yaws (papillomas). Papules, as presenting sign of BU, are rarely seen in Africa and are more commonly found in Australia [28].

The location of lesions on the upper part of the body favors a BU diagnosis [27]. In older people, there are many other causes of ulcers on the ankle and foot. Thus, location of lesion on the ankle or foot, especially in older people, should first evoke differential diagnoses, such as vascular, diabetic, tropical, sickle cell anemia ulcers, or cellulitis, before BU [5]. Usually in those cases, there is a spontaneous local pain.

44.2.1.3 Step 3: General Clinical Examination

After the skin examination, the third step is an assessment of the general condition of the patient, checking for anemia, fever and blood pressure, weight loss, and limitation of movement or in daily activities.

Most BU patients are in good general health.

44.2.2 Differential Diagnosis

Each clinical form must suggest a differential diagnosis, which could be confirmed or ruled out by combining with other symptoms or laboratory examinations. Table 44.1 summarizes the main differential diagnosis in Africa, according to the clinical lesion.

44.2.3 Clinical and Epidemiological Clues

According to the World Health Organization (WHO), the following features are suggestive of the clinical diagnosis of BU in Africa:

Table 44.1 Main differential diagnosis in Africa, according to the clinical lesion

Clinical lesion	Differential diagnosis
Papules	• Impetigo • Lichen planus • Endemic syphilis
Nodule	• Boil • Lipoma • Cyst • Onchocerca nodule
Plaque	• Deep mycosis
Edema	• Cellulitis • Lymphatic filariasis
Ulcer	• Tropical ulcer • Vascular ulcer • Leishmaniasis

1. Residence in or travel to an endemic area.
2. Age: children <15 years.
3. Clinical lesions: nodule, plaque, edema, ulcer.
4. No spontaneous pain, no fever, and no lymphadenopathy.
5. Anatomical distribution.
   (a) Usually on the upper limb (30%) or lower limb (60%).
   (b) Other parts of the body (10%).
6. >90% of cases present with a single lesion.

Recently, the WHO produced a diagnostic aid, based on a score attributed to the key criteria so far proven to be specific to BU, to help frontline healthcare workers with clinical diagnosis [29], Table 44.2.

Conclusion

☐ Most Likely BU (24–21)	☐ Likely BU (20–17)	☐ Less likely BU (16–14)	☐ Unlikely BU (13–10)

Table 44.2 Scoring of epidemiological and clinical diagnosis of suspected cases of Buruli ulcer

Patient/lesion criteria	Classification	Circle the score
1.1.1. Patient''s age (years)	☐ < 15	3
	☐ 15–49	2
	☐ ≥ 50	1
1.1.2. Patient origin (place of residence)	☐ Known endemic area	3
	☐ Area at risk	2
	☐ Non-endemic area	1
1.3. Characteristic of the lesion	☐ Typical ulcer with undermined edges or typical nodule, edema, and plaque	2
	☐ Ulcer without undermined edge	1
1.4. Number of lesions	☐ Single	2
	☐ Multiple	1
1.5. Evolution of the lesion	☐ Slow (4 weeks or more)	2
	☐ Fast (< 4 weeks)	1
1.1.6. Location of the lesion	☐ Above the knee	3
	☐ Between the knee and ankle	2
	☐ Ankle and foot	1
1.1.7. Age of the lesion	☐ < 3 months	3
	☐ 3–6 months.	2
	☐ > 6 months	1
1.8. Pain (at rest without provocation)	☐ No	2
	☐ Yes	1
1.9. Fever	☐ No	2
	☐ Yes	1
10. Swollen lymph nodes	☐ No	2
	☐ Yes	1
Total		

Table 44.3 Points attributed in the Buruli score of items associated with BU probability [26]

Criteria	Points in Buruli score
Female gender	+2
Age > 20 and ≤ 40 years	−3
Age > 40 years	−5
Pain at rest	−1
Characteristic smell	+3
Yellow color (fibrin)	+2
Undermining	+1
Green color (pus)	+1
Lesion size >5 cm	−1
Lesion hyposensitivity	+1
Locoregional adenopathy	−2
Total score and conclusion	• **<0:** Low probability → look for other diagnosis • **0 to 3:** Intermediate probability → sampling for laboratory testing and wait for the result before treatment • **≥ 4:** High probability → sampling for laboratory testing and initiate treatment without waiting for the result

Other authors suggest other score-based diagnostic criteria, using patient demographic characteristics and clinical signs [26], Table 44.3.

After clinical assessment, BU patients are classified into two groups: new cases and relapses [28], which are described below:

- ***A new case*** is defined as a person presenting with a BU lesion who has not previously received antibiotic treatment for BU.
- ***A relapse case*** is defined as a patient who has previously received antibiotics for BU and who presents with a lesion at the same or another site within 1 year of the end of the last antibiotic treatment. Note that the breakdown of old BU scars does not constitute a relapse [28].
- **Paradoxical reaction** is a recently recognized phenomenon, and some cases of BU previously classified as recurrent lesions are probably paradoxical reactions [30–33]. Such reactions occur during or after antibiotic treatment, with the onset of a new inflammatory process (presenting as a nodule/swelling, plaque, or edema) leading to an extension of the existing ulcer or a new lesion at a different part of the body where there was no evidence of disease before antibiotic treatment, usually with discharge of pus and pain. These reactions are likely to result from a subclinical infection that becomes apparent for the first time due to a vigorous immune response to mycobacterial antigens released during antibiotic treatment (see Chap. 45).

Figure 44.1 summarized the clinical examination steps and BU diagnostic clues for patients presenting with lesions in low- and middle-income countries.

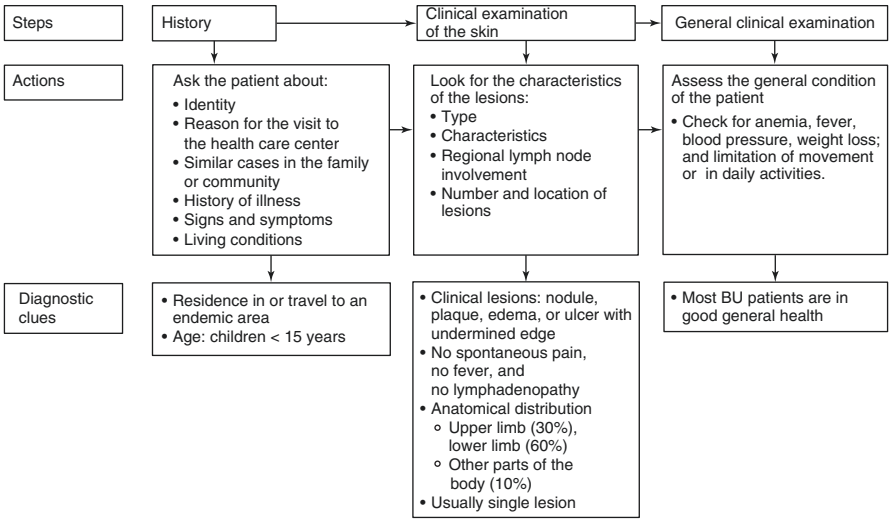

Fig. 44.1 Summary of clinical examination steps and BU diagnostic clues for a patient consulting for skin lesions in low- and middle-income countries. Three steps are necessary for a good clinical examination of a patient consulting for skin disease. At each step, action to carry out and skin NTD's diagnosis clues to search for are summarized

44.2.4 Classification of Patients

In LMI countries, given the low availability of laboratory diagnostic technology and the fact that the sensitivity of clinical diagnosis made by trained health workers in endemic areas could reach 92% [5], treatment can be initiated based on the clinical diagnosis before microbiological confirmation.

The WHO has subdivided BU lesions into three categories, based on the size and location of the lesion and other complications, to help with treatment [28]. They are described in Chap. 42.

44.2.5 Laboratory Diagnosis

The following five laboratory methods increase the likelihood of BU and/or confirm the diagnosis.

- Direct examination of samples under a microscope for acid-fast bacilli (AFB).
- Culture of *M. ulcerans*.
- DNA amplification technique (polymerase chain reaction—PCR).
- Histopathology.
- Fluorescence thin layer chromatography (f-TLC) to detect mycolactone in tissues. It should be emphasized that the sensitivity of the f-TLC technique should be improved to facilitate its use for the diagnosis of BU at the district level of healthcare (see Chap. 41).

At least two positive results are required to confirm the BU diagnosis [34]. They can be performed on swabs, provided an ulcer has formed, or for pre-ulcerative forms, fresh tissue samples and fine needle aspirates are needed. The sampling procedures and laboratory examinations are described in Chap. 41.

The WHO recommends PCR as the gold standard for a confirmatory diagnosis [34]. The combination of clinical diagnosis and two or more laboratory tests performed at different levels of the healthcare system increases the accuracy of the diagnosis. Indeed, the combination of direct smear examination (DSE) performed at the peripheral and central levels slightly increases the sensitivity to 50% compared with that at the peripheral level only (47%). The addition of PCR increases this sensitivity to 69%. Culture performed in addition to the aforementioned three laboratory tests does not add much value, with a sensitivity remaining at 69% [5]. Therefore, several authors proposed a stepwise approach for a BU laboratory confirmation, starting with DSE or f-TLC, followed by PCR only in DSE-negative patients, to reduce costs [35–37]. Note that if the initial PCR is negative, a repeat PCR should be done 1 or 2 weeks later. This approach is summarized in Fig. 44.2.

44.2.6 Summary of Diagnostic Work-Up According to the Healthcare System Level in Low- and Middle-Income Countries

Table 44.4 summarizes the diagnostic work-up at various levels of the healthcare pyramid in LMI countries.

44.3 Diagnostic Work-Up in High-Income Countries: Case of Australia

44.3.1 PCR Replaces Histology for the Diagnosis of Buruli Ulcer in Australia

In Australia culture on special media has been available since 1948 to confirm the etiological diagnosis of suspected BU [38]. However the slow turnaround time meant that histology became the main practical means of confirming a diagnosis of BU in Australia from the 1940s until the late 1990s [39].

In 1995 Melbourne researchers discovered the *M. ulcerans* insertion sequence IS*2404* and were the first to develop a diagnostic PCR based on this sequence for rapid definitive diagnosis of BU. IS*2404* PCR performed on swabs or tissue biopsies replaced histology as the principal mode of diagnosis of BU in Australia. The high copy number of IS*2404* per genome, the high number of bacterial cells typically found within early BU lesions combined with the absence of IS*2404* from other clinically relevant mycobacteria, makes this PCR target ideal for confirming or ruling out BU [40]. Fortunately, PCR for the diagnosis of BU is now widely available even in regional and rural Australia where there are efficient systems to

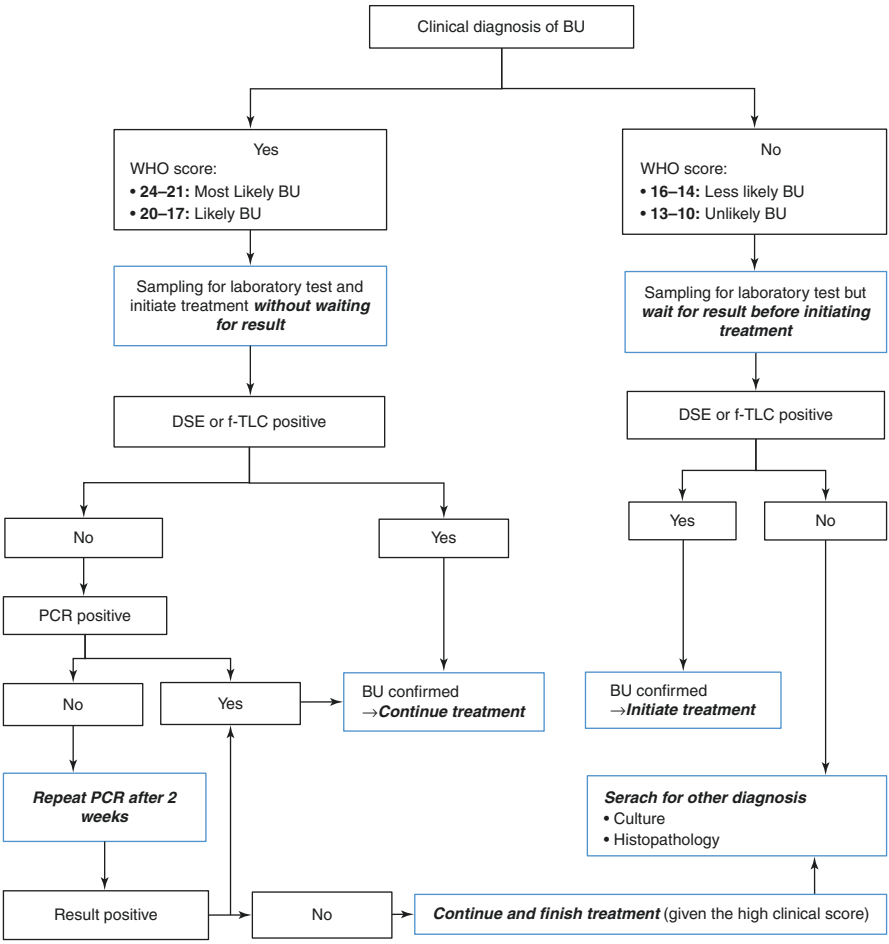

Fig. 44.2 Stepwise approach for BU diagnosis confirmation and treatment initiation in low- and middle-income countries. Following the use of the WHO score sheet, two modalities present: Clinical diagnosis of UB is very likely or probable. Clinical diagnosis of UB is unlikely or not likely. In the first case, the treatment can be initiated without waiting for the results of the biological confirmation. A positive DSE or f-TLC result is sufficient to confirm the diagnosis and initiate the treatment. In the event of a negative result, PCR may be requested, or even repeated if negative. If the PCR comes back negative a second time, it is still necessary to continue and finish the treatment, because of the strong clinical diagnostic suspicion while continuing to search for another etiology. In the second case, take the sample, and wait for the results of the biological confirmation before starting the treatment. If the result of DSE or f-TLC is positive, start treatment. Otherwise, search for another etiology

transport samples for testing to Mycobacterial Reference Laboratories in Melbourne and Brisbane and increasingly to private pathology companies.

Despite this developed country advantage, the diagnostic process in Australia still requires a systematic approach based on history, clinical examination, and

Table 44.4 Diagnostic work-up at various levels of the healthcare pyramid in low- and middle-income countries

Healthcare pyramid level	Activities	Human resource
Community	Clinical suspicion	• Village volunteers • Front-line healthcare workers (HWs), or doctors during community-integrated case-finding activities
	Swab sampling	• Village volunteers • Front-line HWs or doctors during community-integrated case finding activities
	FNA sampling	• Trained front-line HWs, or doctors during community-integrated case-finding activities
	Refer to HCC	• Front-line HWs or doctors during community-integrated case-finding activities
Frontline or peripheral HCC	Clinical confirmation	• Front-line HWs • Doctors during community-integrated case-finding activities or supervision
	Swab sampling	• Front-line HWs • Doctors during community-integrated case-finding activities or supervision
	FNA sampling (except at critical lesion location, such as the neck or eyes)	• Trained front-line HWs • Doctors during community-integrated case-finding activities or supervision
	Laboratory testing (DSE or f-TLC), when available	• Trained front-line HWs (lab technicians)
	Refer to hospital	• Front-line HWs • Doctors during community-integrated case-finding activities or supervision
Reference hospital (intermediary or central level)	Clinical confirmation	• Trained nurses • Doctor (generalists and dermatologists, when available)
	Differential diagnosis	• Doctor (generalists and dermatologists, when available)
	Swab sampling	• Nurses and lab technicians • Doctor
	FNA sampling	• Trained nurses and lab technicians • Doctor
	Biopsy	• Doctor
	Laboratory testing	
	• DSE, f-TLC	• Lab technicians
Reference laboratories (research labs, or teaching hospitals labs)	• DSE, f-TLC	• Lab technicians
	• PCR	• Specialized biologists
	• Histopathology	• Histopathologists

testing similar to that described above. Mistakes leading to delayed diagnosis are still made, most often by clinicians unfamiliar with BU [41]. Diagnostic traps include a failure to understand the changing geographic distribution of BU in south-eastern Australia (Victoria), the long incubation period (median of 5 months, range up to 10 months), and the short exposure times needed to acquire the infection [42, 43]. From the early 2000s, BU has become endemic on the Bellarine and then Mornington peninsulas near Melbourne, and transmission now occurs just a few kilometers from the center of Melbourne. A second Australian focus 2500 km to the north between Mossman and the Daintree river in Queensland is also active. Unlike Victoria, BU in Queensland has remained geographically localized, but incidence varies by year [44]. Other than coastal Victoria and the Mossman focus (Daintree ulcer), BU remains rare and sporadic. In Victoria, almost 40% of newly diagnosed infections are in visitors to endemic areas [45]. For unknown reasons a much higher proportion of cases associated with far North Queensland are permanent residents, and only a small proportion of infections occur in visitors.

The major anatomical location of *M. ulcerans* infection is the subcutaneous fat layer beneath the skin. This can be accessed easily once an ulcer has formed, but in pre-ulcerative edematous or plaque forms, a skin swab per se has poor ability to confirm the diagnosis (Fig. 44.4a). There are occasional reports of false-negative PCR results early in the disease in Australia, but this usually results from in-expertly collected swabs from lesions which are not yet fully ulcerated [46]. It is also theoretically possible for fresh tissue biopsies to yield false-negative PCR results if the biopsy is too small or poorly targeted. However, this has been rare in practice [personal observation], and by far the most common diagnostic pitfall is failure to consider BU. Inexperienced doctors can be misled by the identification of opportunistic colonizing bacteria from ulcer swabs such as *S. aureus*, enterococci, or enteric Gram-negative bacilli. Mycobacteria are rarely visible by routine Gram's stain, and laboratories only perform stains for AFB and culture and PCR for mycobacteria if these investigations are specifically requested.

44.3.2 Clinical Features of BU in Australia

The key initial step in suspecting the diagnosis of BU is therefore to take a careful travel history. Individuals of any age presenting with a subacute progressive skin lesion or ulcer, after even a very brief exposure to an endemic region in the previous 12 months, should have the diagnosis of BU considered. Conversely, the absence of an exposure history weighs strongly against BU (Table 44.5) but does not exclude the diagnosis completely.

Most patients observe a slowly progressive single lesion usually on a limb (see Fig. 44.3 for lesion distribution chart). Lesions are generally but not always painless, may be itchy or scaly, and typically have not responded to empiric oral

Table 44.5 Clinical features suggesting BU in Australia

Feature	Details	Comments
Contact with endemic area <12 months	Yes—but exposure may be brief—e.g., as little as just a few hours; the median incubation period is 5 months	Lack of an exposure history makes BU unlikely
Age	All ages from young children to the elderly	Age-specific attack rate higher in those >75 years [45]
Seasonality	Victoria peak presentation to doctors is in late winter and spring (June to October) In Queensland: peak is in dry season (June to October)	Likely explained by transmission during the summer/wet season; long incubation period, slow progression prior to initial presentation to a clinician
Lesion type	80% present with ulcers most often on an exposed area of a lower or upper limb	Diagnosis more likely to be delayed in pre-ulcerative forms
Lesion location	Single lesion is usual; for Victoria detailed lesion, location maps now available [47]	BU uncommon on face, head & neck, and trunk; rare on the sole of the foot [47]

antibiotics (e.g., cephalexin, flucloxacillin) (Fig. 44.4A1 and A2). Systemic symptoms are rare. A prior insect bite or injury is variably recalled, but few people are able to clearly remember an event 5 months previously, and misattribution to a more proximate even is frequent.

While all age groups are affected, many patients with BU in Victoria are elderly as retired people increasingly choose to move to coastal areas where BU is now endemic [42, 45]. Skin cancers (basal cell carcinoma, squamous cell carcinoma) are sometimes the first considered differential diagnosis in this age group leading to tissue biopsy before the correct diagnosis is suspected (Table 44.6). Once an ulcer develops, BU is usually correctly considered by family practitioners as experience increases and public health education programs raise awareness. Most ulcers are diagnosed rapidly by BU PCR from a standard bacterial swab run around the undermined edge of the ulcer and the remainder by PCR (Fig. 44.4b) on fresh tissue biopsies. Retrospective PCR on paraffin-embedded sections [48] may be helpful when skin cancer was suspected, but histology was not supportive of a malignant diagnosis.

In pre-ulcerative cases, a gelatinous plug often forms before discharging and breaking down. Use of saline and a bacterial swab to gently loosen the plug may yield a suitable specimen provided biological material is clearly visible on the swab at the end of the procedure [personal observation and Fig. 44.4c].

At least 50% of ulcerative BU lesions are smear-positive for AFB. If a patient presents with skin lesion that is smear-positive for AFB, most but not all will be due

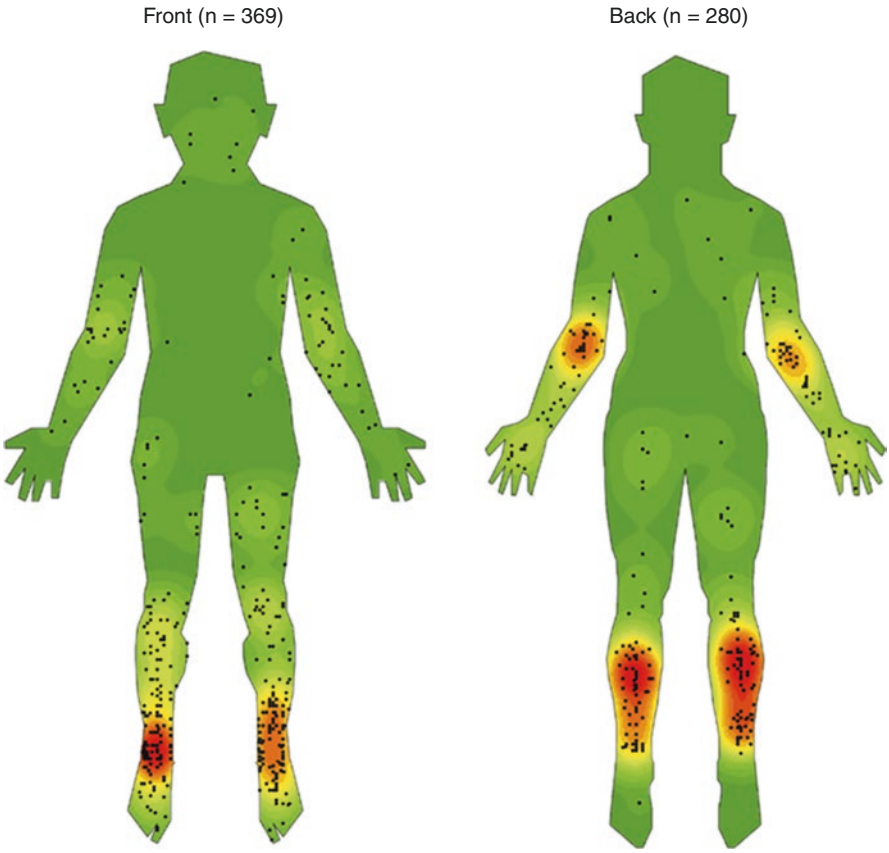

Fig. 44.3 Heat map showing location of lesions from 649 confirmed Buruli infections in children and adults, males and females on the Mornington and Bellarine peninsulas near Melbourne [47]. In red, the most frequent locations; in yellow, those moderately frequent; in green, the less frequent locations. Each black point represents a case

to BU, particularly in Victoria. As in Africa, BU in Australia may also present as acute edematous form which resembles bacterial cellulitis but fails to respond to standard therapy. Osteomyelitis is rare.

Pre-ulcerative and nonulcerative forms may not initially suggest the diagnosis of BU leading to delay and increased morbidity (Fig. 44.4d). Fresh tissue punch or incisional biopsies, provided they sample deeply enough to include subcutaneous tissue, will typically show bland necrosis with large clumps of mycobacteria [38], particularly in early lesions. IS*2404* PCR is almost always positive in these cases.

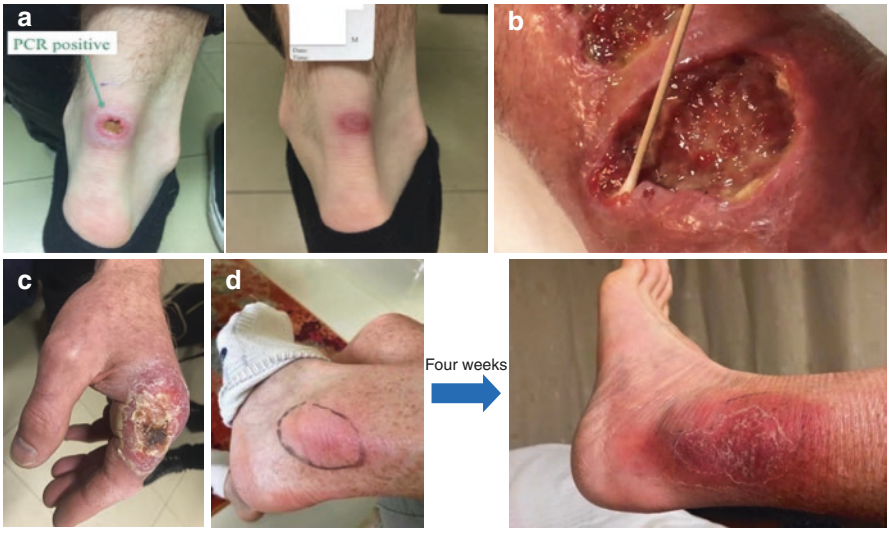

Fig. 44.4 Clinical features of Buruli ulcer and outcomes after treatment in Australia. (**a**) BU small lesion over Achilles tendon (initial and after 8 weeks oral antibiotics). (**b**) Large complex ulcers on the right anterolateral leg above the ankle (actually a single ulcer separated by an undermined bridge of skin). (**c**) Pre-ulcerative BU on the left hand. PCR was positive on a saline moistened dry swab that was used to loosen and the central plug of necrotic tissue. (**d**) Pre-ulcerative BU confirmed by IS2404 PCR and culture on a full thickness skin biopsy

Table 44.6 Differential diagnosis of BU in Australia

Other infections—normal host	
Condition	Contrast with BU
Community-acquired skin infections (Staphylococci/ streptococci)	Often painful, progress over hours to days; more likely to be associated with systemic features; respond to standard antibiotics (Fig. 44.4c)
M. marinum	Contact with water, fish tanks, ponds
M. haemophilum	Cervicofacial disease or nodal disease in healthy children
Cutaneous TB (rare in Australia)	Patient typically born overseas in a high TB endemic country
Leishmaniasis	Shallow, edges less undermined; exposure in endemic country (southern Italy, north Africa, Middle East, South America); more often head and neck
Inoculation event/environmental exposure recalled	
Sporotrichosis *M. fortuitum* *M. chelonae* *M. abscessus*	Often multiple skin nodules without ulceration or minor ulceration in ascending pattern
Burkholderia pseudomallei (melioidosis)	Contact with soil north of tropic of Capricorn; typically, wet season in higher rainfall areas
Immunocompromised host	
M. avium	Often but not always part of systemic illness in immunocompromised host

Table 44.6 (continued)

Other infections—normal host	
Condition	Contrast with BU
Cryptococcus neoformans	Necrotic skin lesions often in association with lung nodule, pneumonitis or meningitis
M. haemophilum	AFB smear-positive necrotic skin nodules/ulcers
Inflammatory conditions	
Pyoderma gangrenosum	Lower limbs; intensely painful
Malignancy	
Basal cell carcinoma Squamous cell carcinoma	Older people; sun-damaged skin
Other conditions	
Diabetic foot ulcers	Lower limbs only, patient is diabetic
Ischemic ulcers	Lower limbs only, often painful, punched out edges
Venous ulcers	Lower limbs only; associated with venous disease, venous dermatitis, obesity, may be bilateral and recurrent

44.4 Conclusion

The great variability in clinical presentation and the broad range of similar conditions make management of BU difficult, especially in LMI countries, where clinicians often have to work with limited laboratory support. Diagnosis requires careful skin examination and general clinical examination of the patient followed by laboratory confirmation. Diagnostic work-up slightly differs in high-income countries such as Australia.

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Treatment of Buruli Ulcer

45

Tjip S. van der Werf, Richard O. Phillips, Roch C. Johnson, and Yves T. Barogui

45.1 Introduction

Since the cause of Buruli ulcer (BU)—infection with *Mycobacterium ulcerans*—was first discovered and reported in 1948 [1], surgical resection has been the mainstay of treatment for more than half a century, when clinical studies were designed and conducted, based on in vitro and in vivo animal experiments. Unlike leprosy, where progress to develop evidence-based pharmacological treatment has been hampered by the fact the *M. leprae* cannot be cultured and tested in vitro for drug susceptibility, *M. ulcerans* has been tested in vitro [2, 3] and in vivo [4, 5]—in animal models [6], for a variety of antimicrobial drug classes, and subsequently also in patients [7–9]. In contrast, in leprosy, the widely accepted multi-drug treatment has been largely based on expert opinion; observational cohort studies but no well-designed, well-powered clinical trials with clearly pre-defined clinical end points

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have been conducted to provide the evidence base for current treatment recommendations [10].

Relapse and failure after surgical resection treatment alone for BU—though to some degree effective—have been well established [11, 12]. Systemic pharmacological treatment for BU, now considered standard of care, has been tested in several clinical trials involving patients with lesions limited to 10 cm cross-sectional diameter [8, 9], and prospective cohort studies have enrolled patients with even larger lesions [13], with marked reduction in treatment failure and relapse, compared to surgery alone. All these studies followed after publication of a small-scale proof-of-principle study in pre-ulcerative *M. ulcerans* infection. This study showed that lesions in patients treated for at least 4 weeks with a combination of streptomycin and rifampin had their lesions sterilized, as evidenced by the fact that these lesions, when surgically excised, were all sterile [7]. Clearly all patients with ulcers need dressings and dressing changes as topical treatment; some topical treatment modalities have been designed and propagated for cure of BU disease. Apart from surgical resection [14], topical therapeutic approaches to seek cure for BU have included heat treatment [15], phenytoin powder [16], and oxidative nitrogen creme [17], and clay [18].

Leprosy should not be understood as merely an infectious disease, but just as well as immune pathology. Patients affected by leprosy suffer from lack of social inclusion and stigma. Apart from anti-infective agents, they need measures to prevent and treat physical disabilities, while also reduction of stigma and social inclusion should be addressed. Likewise, patients affected by BU have sequelae, with contractures, disabilities, and problems with stigma and social inclusion. Apart from the important target to attain relapse-free cure, an important aspect of treatment is therefore also to prevent and manage sequelae. Indeed, many patients have ended up with disfiguring scars and contractures [12], with ensuing functional limitations and stigma [19], causing restriction in social participation. For details regarding the prevention of disabilities, we refer to Chap. 46.

Finally, treatment for BU is much more effective when patients seek medical attention early on in the course of disease. We therefore briefly discuss perceptions, beliefs, and attitudes as well as socioeconomic restrictions that may all cause patient delay. Indeed, unraveling health-seeking behavior and targeting determinants of patient delay are equally important to improve overall treatment outcome.

45.2 Systemic Therapy: Antimicrobial Therapy

Of all treatment modalities, clearly systemic antimicrobial treatment has been most studied, and taken together, antimicrobial treatment recommendations have a strong evidence base [20–22]. The Cochrane library published a systematic review of the entire preclinical evidence on antimicrobial drug treatment [20]. Here, we highlight and summarize the most important evidence of the activity of the different classes of antimicrobials tested, with some new information on novel drugs—and new evidence from a large clinical trial [9]. It is important to realize that antimicrobials

(bactericidal or bacteriostatic agents) either kill or arrest the growth of *M. ulcerans* and its metabolic activities, including the production of the major virulence factor, mycolactone [23]. As mycolactone production ceases, concentrations in affected tissues (ulcers, nodules, plaques, or edematous lesions), as well as in the system, start to decline [24], and its effects gradually wean. Obviously, the observation of ongoing tissue damage during early stages of antimicrobial therapy is confusing for clinicians, and a directly acting therapy against mycolactone molecules still present in tissues and in the system would be a tremendous asset. At this point in time, only in vitro work has shown that directly acting antibodies against mycolactone might one day be added to our therapeutic arsenal [25]. With resolution of immune depression [24], induced through various different mechanisms, Sec61 blockade being dominant [26], tissue repair and wound healing often only start after completion of the 8-week course of antimicrobial therapy. Immune reconstitution may be accompanied by an exacerbated immune response: between 2 and 26% of cases show a transient clinical worsening. This phenomenon is characterized by reduction of bacterial load but increased inflammatory response. Sometimes, inflammatory reactions emerge in the presence of dead bacilli at sites where, initially, no disease activity was noted. Most reports focus on clinically transient worsening with increase in size of lesions compared to baseline; all the above is referred to as "paradoxical reaction" [27]. These reactions that may be to some degree inherited [28] may mistakenly be interpreted as treatment failure. It is therefore important that in designing a study protocol for the evaluation of (combinations of) antimicrobial agents, the dynamics of slow wound healing and possibly transient worsening of lesions following antimicrobial treatment are taken into account. In retrospect, earlier published studies were flawed by design, with inappropriately short follow-up, at a time when the effects of mycolactone on immunity, tissue repair, and healing were incompletely appreciated. The presence of a secreted toxin in culture filtrate of *M. ulcerans* had been reported decades before the chemistry and biological activities of mycolactones were elucidated [29]. Although some patients may develop large lesions that continue to ulcerate for many years, most patients eventually heal [30]. This tendency of spontaneous healing is important to realize; it makes the interpretation of cohort studies without control groups notoriously difficult.

45.2.1 Aminoglycosides

This class of antimicrobial drugs have very low bioavailability and need to be administered parenterally. Aminoglycosides are hydrophilic compounds, and their volume of distribution reflects the lean body mass; excretion is by renal clearance. Their mode of action is by inhibiting bacterial protein synthesis, by binding tightly to the conserved A site of 16S rRNA in the 30S ribosomal subunit, and drug resistance results from genetic changes in *rrs* coding 16SrRNA, *rpsL* coding the S12 ribosomal protein, or *gidB* [31].

Streptomycin, an anti-tuberculosis drug, was repurposed for *M. ulcerans* treatment after *M. ulcerans* isolates from patients in Buruli county in Uganda (now

known as Nakasongola) tested susceptible to this drug in vitro [32]. In vitro and in vivo studies confirmed the bactericidal activity of streptomycin on *M. ulcerans* [4, 5, 33]. Streptomycin-resistant clinical isolates of *M. ulcerans* are uncommon [34]. The clinical impact of streptomycin alone has been difficult to evaluate; streptomycin has predominantly been used in combination with rifampin [7–9, 35–37]. Streptomycin has usually been administered at 15 mg/kg bodyweight; no pharmacokinetic data in patients with *M. ulcerans* infection have been published, and pharmacokinetic/pharmacodynamic modeling has not been performed, but it is likely that dosing should be based on lean body mass; in obese individuals, toxicity can be expected with dosing based on total body weight. Toxicity is a major concern anyway, especially in elderly people who risk vestibular and ototoxic effects [38]. This oto-vestibular toxicity and also renal toxicity are class effects. Amikacin, an aminoglycoside with activity similar to streptomycin [5], has therefore no clear advantage over streptomycin. Several oral treatment schedules have been successfully tested in animal experiments [39], and in Australia, where patients are typically at a more advanced age, streptomycin and amikacin have largely been avoided [40]. Clinical studies have been designed to compare streptomycin-based treatment schedules with oral schedules. Oral agents replace streptomycin, especially clarithromycin [8, 9, 37], and these studies show that streptomycin is no longer needed, as all-oral drug combinations are safer and equally effective [9]; see Fig. 45.2.

45.2.2 Rifamycins

Rifampin was reported to be effective against *M. ulcerans* in vitro as early as in 1972 [41], when surgery was still deemed the only viable option for treatment. Later reports confirmed that susceptibility is excellent in vitro [3, 34] and in animal models [39, 41]. The drug is readily absorbed with excellent bioavailability; distribution is similar to total body weight; drug elimination is by enzymatic metabolism that is saturated at higher dosing, with important auto-induction of drug elimination in the liver; renal clearance is negligible [42]. Its mechanism of action is by interfering with bacterial polymerase, necessary for bacterial cell replication; resistance depends entirely on genetic changes in the *rpoB* gene encoding the polymerase [43]. As *M. ulcerans* is an environmentally acquired microorganism with low or absent antimicrobial pressure, all infections caused by this organism are typically wild-type, and resistance is generally low and effectiveness high [9]. Enhanced drug elimination by induction of hepatic (CYP3A4) enzymes is a concern. Pharmacokinetic studies in BU have been limited to only one report [40]. Clarithromycin exposure is limited by rifampin co-medication; clarithromycin increases rifampin drug exposure slightly.

In clinical trials and observational cohort studies, drug treatment including rifampin at a dose of 10 mg/kg body weight was associated with excellent tolerability, high cure rates, and negligible relapse rates [7–9, 13, 35–37, 44], although in some of the cohort studies, resection surgery was combined with drug treatment [13, 35, 36], making it difficult to evaluate drug efficacy in those studies. In

tuberculosis susceptible to rifampin, dosing up to 35 mg/kg body weight [45] has no important toxicity. Animal experiments suggest that perhaps with increased dosing, shorter duration of therapy for BU might be possible [46].

Other rifamycins include rifapentine [39, 47] that has a considerably longer half-life than rifampin and rifabutin [5] that has higher lipophilicity, longer half-life, and less drug-drug interactions than rifampin [48].

45.2.3 Macrolides

Clarithromycin has been studied most—it is a protein synthesis inhibitor that reversibly binds to the 23S rRNA on the 50S ribosomal subunit. It has been considered a companion (bacteriostatic) drug in combination with more powerful bactericidal agents like rifampin [9]. The dosing has been slightly variable, ranging from 7.5 mg/kg body weight once daily in most studies, to 12 mg/kg body weight [36], to 15 mg/kg body weight once daily in extended release (ER) formulation [9]. Although less of a concern compared to aminoglycosides like streptomycin that is contraindicated during pregnancy, there are still concerns with its use during pregnancy [49]. It is readily absorbed from the intestine and eliminated by cytochrome 450 enzymes, especially CYP3A4 into 14-hydroxy-clarithromycin that appears to have no antimicrobial effect on *M. ulcerans* [40]. Clarithromycin decreases drug elimination of certain drugs—notably also rifampin—by inhibiting P-glycoprotein [50]. Its bacteriostatic effect on *M. ulcerans* has been shown in vitro [2] and in vivo [39]. The clinical experience has been limited to combinations with rifampin [8, 9, 35–37]. An 8-week course of oral rifampin and intramuscular streptomycin appeared highly effective [13, 36], but switching from streptomycin to oral clarithromycin after 4 weeks [8] or even 2 weeks [37] did not affect treatment outcome, compared to patients that followed a full course with eight weeks of streptomycin, combined with rifampin. In a head-to-head comparison, fully oral treatment with the combination of clarithromycin in ER form in the largest clinical trial on drug treatment in *M. ulcerans* infection to date showed similar clinical effectiveness of 8-week fully oral rifampin-clarithromycin ER compared to the rifampin-streptomycin combination [9]. Among secondary end points in this study, median time to healing was better in the clarithromycin ER group (median 16 weeks) than in the streptomycin group (median 24 weeks); paradoxical reactions were similar, but toxicity, especially, oto-vestibular side effects, was significantly more common in the streptomycin-treated group.

Azithromycin has good bacteriostatic activity in vitro [34] and in vivo, summarized in [22], but clinical studies are lacking.

45.2.4 Fluoroquinolones

Ciprofloxacin, ofloxacin, and moxifloxacin have good antibacterial activity in vitro [3]. These drugs act by interfering with DNA replication by inhibiting gyrase that

catalyzes super-coiling of bacterial double-stranded DNA. Resistance is coded by mutations in the genes gyrA and gyrB that encode bacterial gyrase [51]. In Australia, oral treatment has been the preferred approach for antimicrobial treatment, and fluoroquinolones, notably, ciprofloxacin and moxifloxacin, have been recommended and incorporated in treatment schedules, usually in combination with rifampin [44]. Fluoroquinolones have excellent bioavailability and penetrate well into various different tissues including bone. Ciprofloxacin and levofloxacin are predominantly eliminated by renal clearance; dosing interval needs to be extended in patients with impaired renal function. Elimination of moxifloxacin is by metabolic inactivation into inactive metabolites that are excreted into feces and partly by renal clearance. No dose adaptation for moxifloxacin is required for patients with renal failure; comedication with rifampin is a concern as drug elimination of moxifloxacin is enhanced, with predicted reduction of drug exposure by a third [52]. Fluoroquinolones interfere with collagen formation and carry a potential adverse effect on bone formation, tendons, and vascular structures; children may suffer from arthropathy; in the elderly, vascular dissection [53] and rupture of tendons such as the Achilles tendon might occur [54]. For moxifloxacin, which is also a core drug in the treatment of drug-resistant tuberculosis, QTc time may increase with higher drug exposure, with the potential risk of fatal arrhythmia by a mechanism referred to as "Torsade de Pointes." In Africa, where patients are typically younger than in Australia, potential side effects as well as costs of fluoroquinolones have discouraged the use of these drugs. In clinical studies, especially in children with cystic fibrosis, adverse effects appear to be mild [55]. In general, fluoroquinolones are contra-indicated in pregnancy. Fluoroquinolones have not been studied in head-to-head comparisons with other agents, and many patients in Australia receive antimicrobial treatment combined with surgery, which makes it difficult to tease out the effect of these drugs in their own right. Based on in vitro studies [3, 56], moxifloxacin should be considered an effective drug for *M. ulcerans* infection.

45.2.5 Miscellaneous Antimicrobial Drugs

Cotrimoxazole has not been tested in vitro for *M. ulcerans*, although it has a potential role in drug-resistant tuberculosis, with only borderline susceptibility [57]; a small study did not show an appreciable clinical effect [58].

Clofazimine, an anti-leprosy drug, has attracted attention because of its activity against *M. tuberculosis*; it plays an important role in the management of drug-resistant tuberculosis [59]. It has reasonable activity on *M. ulcerans* in vitro [34], and in an animal model, the 6-week combination of clofazimine and rifampin gave relapse-free cure [60]; increasing the dosage of rifampin provided cure with an even shorter duration of treatment [46]. The drug has a long half-life and causes a yellow-orange discoloration of skin, especially in individuals with fair skin color. An early clinical trial with clofazimine monotherapy failed to show benefit [61], although a small observational study later suggested improved outcome [62].

Bedaquiline, a core drug in the management of drug-resistant tuberculosis, appears highly active on *M. ulcerans* in vitro and in vivo [22], but there are no data from clinical interventional or observational studies.

Linezolid, yet another core anti-tuberculosis drug for drug-resistant tuberculosis, with important toxicity (especially, polyneuropathy) with increasing drug exposure [63] has moderate activity on *M. ulcerans* in vitro [22], but there are no clinical studies to support its use in patients with *M. ulcerans* infection.

Telacebec is a compound that has recently drawn much attention due to its extremely high efficacy on *M. ulcerans* in vitro [64] potentially holding a promise for an extremely short treatment duration [22, 65], but further clinical studies are required to assess its safety and efficacy. In pregnancy, only rifampin should be considered fully safe; animal experiments suggest that beta-lactams might be an option [66] for these special patient groups, but to date, no clinical reports have confirmed that this approach might be effective.

45.2.6 Drug Treatment With Combinations of Antimicrobial Agents and Treatment Duration

After a systematic review was published reviewing the evidence emerging from clinical studies [20], the earlier mentioned large clinical trial was published [9]. It showed that fully oral treatment with rifampin and clarithromycin ER resulted in a high cure rate, non-inferior to the combination of rifampin and streptomycin injections. Based on an earlier pharmacokinetic study [40] in the framework of the BURULICO trial [8], at the time of the study design, the choice was made to increase the total dose of clarithromycin by providing 15 mg/kg in an ER formulation. Unpublished data based on a pharmacokinetic analysis of dried blood spots in a limited group of study participants showed that drug exposure was still limited with even slightly lower peak serum concentrations reached (Klis et al, unpublished data); we therefore believe that the double dosing in ER form has no clear advantage over immediate release formulations and that perhaps 10 mg/kg clarithromycin might be an appropriate dose. Importantly, with the treatment schedule used in the trial, there was no evidence of bacteriological failure among the few study participants that failed to have their lesions healed at the pre-defined time point 52 weeks after start of treatment; see Fig. 45.4.

As mentioned earlier, the dosage of rifampin might also be worthwhile to be increased to e.g., 15 mg/kg, as this is clearly to be considered safe and perhaps slightly more effective. A fixed drug combination of rifampin and clarithromycin with slightly higher dosing might be the way forward, also to prevent monotherapy under service conditions. Even though drug resistance is still extremely rare, also considering the fact that there is hardly any antimicrobial pressure on the reservoir of the organism that is clearly environmental, drug combinations are the preferred approach to combat *M. ulcerans* infection; clarithromycin is to be considered as a companion drug to prevent treatment failure during monotherapy. Treatment duration has been chosen based on one single experiment in which pharmacotherapy

with at least 4-week duration resulted in sterilizing effects on lesions that were subsequently excised and cultured [7]. In lesions <10 cm cross-sectional diameter, treatment failure was rare [8, 9], and generally, drug treatment outcome has been beneficial [13, 35–37]. Whether larger lesions require longer treatment duration has remained an unresolved question, and the question whether shorter treatment duration in small lesions is acceptable or even preferable has not been addressed in clinical studies to date. Clinical observations suggest that at least some patients with small lesions do well with less than 8 weeks of treatment [67]. Whether high-dose rifampin or combinations with telacebec facilitate much shorter treatment duration should be explored in future studies.

Fluoroquinolones have been used in observational studies in Australia, with generally good outcome, though in elderly patients, drug intolerance has been a concern [68]. The updated Australian guideline mentions fluoroquinolones as a treatment option [69], but the current WHO guideline does not encourage their use [70]. Treatment during pregnancy remains difficult; in the earlier mentioned trial, one female study participant appeared to be pregnant at week 6 of the study medication which was rifampin-clarithromycin ER in her case; in consultation with the study team, she decided to continue the treatment, with no adverse effects on maternal and fetal outcome, but clearly there is a small but significant risk for fetal damage of macrolides like clarithromycin during pregnancy [49]. Alternative treatment options like surgical resection or topical heat treatment might be an option (see below).

45.3 Topical Treatments

45.3.1 Surgical Resection

Surgical resection has been reported from the 1950s in the then Belgian Congo, now DR Congo [71], and Uganda [72]. As mentioned earlier, surgery alone, even with resection of margins of apparently healthy tissue, still results in residual bacterial load in the resection margins [73], while surgery as monotherapy has been associated with variable but overall, appreciable recurrence and failure rates [11, 12]. Earlier published guidelines have therefore recommended adding antimicrobial therapy to surgery [56], while there has been a shift to recommend all-oral treatment as the first treatment option [69, 70].

The results of the largest ever trial evaluating fully oral antimicrobial treatment compared to the rifampin/streptomycin combination revealed that in neither of the treatment arms, resection surgery was necessary to obtain cure without relapse, assessed 12 months after start of therapy [9]; indeed none of 297 with *2404* PCR-confirmed *M. ulcerans* infection needed resection, and only 4 (2 in each arm) needed skin grafting. This finding questions the ongoing practice in many locales where surgery is still common practice, not only in Africa [74] but also in Victoria,

Australia [75]. Only one study addressed the question of timing of surgery; in the treatment guideline published by the WHO in 2012 [70], surgery timing was suggested to be performed after at least 4 weeks of antimicrobial therapy, but in most centers in Africa, surgery is usually planned around the end of 8 weeks of antimicrobial therapy. In a study conducted in Benin—the only study to date, to evaluate the role of surgery, study participants were randomized for the timing between this time point—at week 8 after start of therapy or, a delay of that decision, until week 14 after start of therapy. Fifty-five (96%) of 57 participants in the delayed-decision group and 52 (90%) of 58 participants in the standard-care group had healed lesions 1 year after start of antimicrobial treatment; 37 (67%) of 55 patients in the delayed-decision group had their lesions healed without surgical intervention, as did 25 (48%) of 52 in the standard-care group (RR 1·40, 95% CI 1·00–1·96). The time to heal and residual functional limitations did not differ between the two groups. Postponing the decision to operate resulted in a marked and significant reduction in the duration of hospitalization and wound care; indeed, delaying decisions to operate was highly beneficial [76]. In summary, the role of surgery has been overestimated in the past [14], and despite advocates to point at potential benefits—albeit for Victoria, Australia that has witnessed an unprecedented outbreak of BU [77], we believe the case in favor of antimicrobial treatment and against surgery to achieve cure for BU disease is really strong now. It goes without saying that limited debridement, skin grafting, and plastic and reconstructive surgery may be beneficial for selected patients, especially those with advanced tissue destruction and contractures; whether osteomyelitis complicated by sequesters needs surgery has not been addressed in the literature.

45.3.2 Heat Treatment

Mycobacterium ulcerans typically grows at temperatures below the core temperature of humans; temperatures above 37°C harm the bacilli, and heat treatment by topical application has been pilot-tested for lesions on limbs [15, 78]. Based on these pilot studies, a larger study enrolled 65 participants with category I-III lesions (for definitions of categories, see Chap. 42). The heating device used contains sodium acetate trihydrate, a phase-change material that can quickly be reheated by boiling water, and cannot exceed temperatures above 56°C. The device is easily rechargeable; it provides a skin temperature at around 40°C, for around 10 h/day. In all, 63 patients were started on topical heat treatment; 52 individuals had PCR-confirmed BU disease; treatment duration varied from 42 to 56 days. Limited debridement surgery was allowed; to the 12 patients that eventually failed, standard antimicrobial therapy was provided [79]. The treatment was well tolerated and accepted by patients and their guardians. The authors argue that their treatment has efficacy that is comparable to reported results from clinical trials with antimicrobial therapy, but their phase II study was not a randomized comparison with antimicrobial treatment.

45.3.3 Dressings; Topical Substances; Traditional Treatments

Wound care is essential for patients with BU; in Africa and in Australia, the vast majority of patients have ulcerated lesions at any point in time; see, e.g., Fig. 45.3. Wound treatment guidelines that apply to a wide range of skin lesions have been provided by the WHO [80]. Sufficient general care for patients, addressing nutritional status, and preventing anemia and uncontrolled hyperglycemia in patients with diabetes are all essential; see Fig. 45.1. Next, rinsing and cleaning of wound service using saline, if necessary, adding careful limited wound debridement using analgesics, and regular dressing changes (Figs. 45.2, 45.3, 45.4, 45.5); prevention and treatment of lymphoedema by appropriate compression therapy (Fig. 45.6); using absorptive dressing materials in discharging wounds; and applying non-adhesive wound cover especially when the wound service is not discharging are all considered part of standard wound care (see Fig. 45.6). In rural Africa, knowledge and practice around wound care differ widely in clinical practice across treatment centers for BU [81]. Although BU typically presents as a painless ulcer in early stages, many patients experience considerable pain and anxiety related to wound care, especially dressing changes [82]. Knowledge and practice to relieve and prevent procedural pain need improvement [83], and former patients indicated that this aspect needs more attention [84].

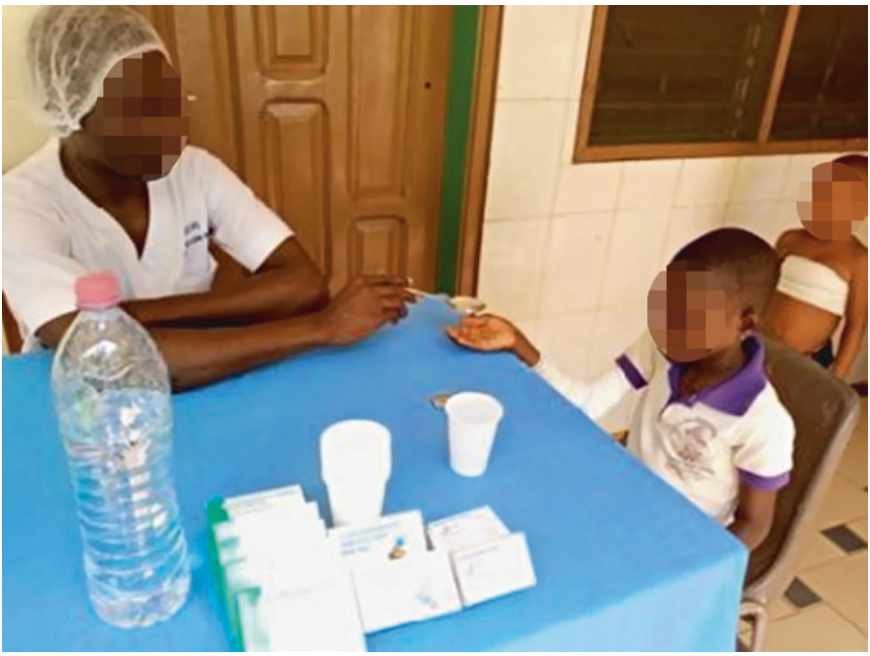

Fig. 45.1 'Look at the whole before looking at the hole': right hand side showing pallor reflecting anemia in a patient with long-standing Buruli ulcer; optimized nutritional support, deworming and malaria treatment are all essential for adequate wound healing

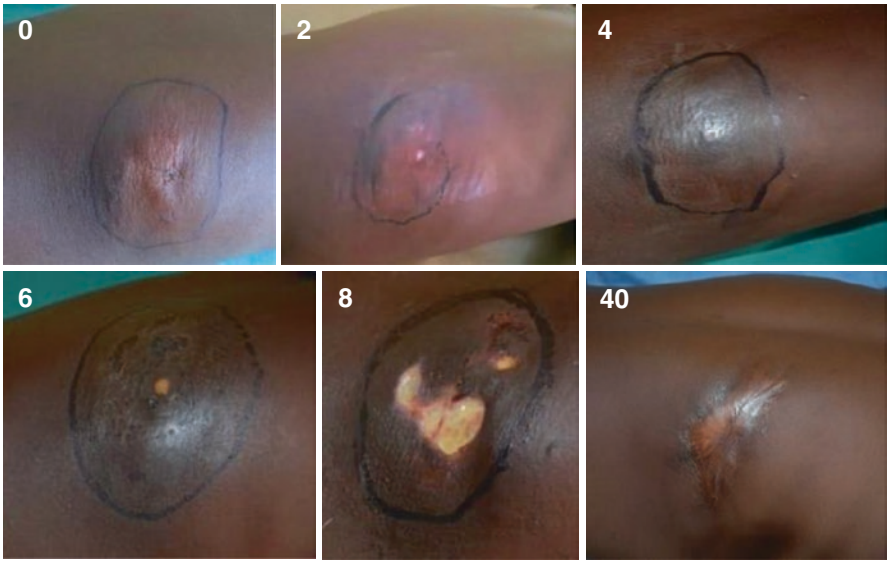

Fig. 45.2 Fully oral treatment with clarithromycin-rifampicin for 8 weeks has become the new standard treatment in Africa

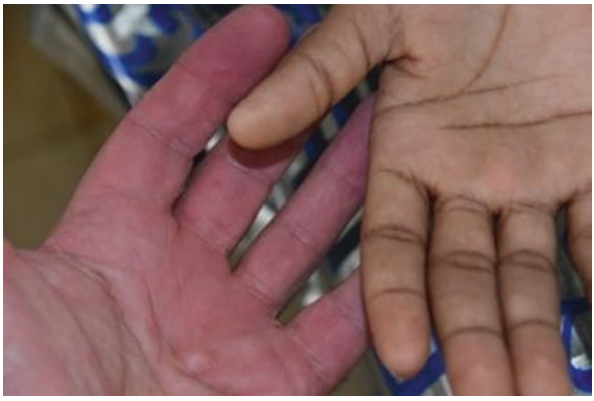

Fig. 45.3 Patient enrolled in a drug trial; plaque, PCR-confirmed as *M. ulcerans* infection located at the right lateral aspect of the trunk. Patient was followed from week 0; ulcer developing at week 6, clearly larger at week 8, but eventually healed at week 28; stable scar recorded at week 40. Without repetitive trauma, these lesions heal, even if dressing changes are irregular or sub-optimal

Many patients seek relief by traditional treatments including herbal topical dressings [85]. No randomized studies have been published to date, and most authors believe that it merely delays starting effective treatment [19].

Wound microbiota in BU are different from non-BU lesions [86]. Moreover, BU may be secondarily contaminated and/or infected by a diversity of organisms like *P. aeruginosa* and *S. aureus* [87] some of which carry resistance and virulence factors [88]. Whether these secondary infections cause delayed wound healing or not is currently uncertain; the practice around assumed secondary infection varies widely across treatment centers and is more often than not irrational and ineffective [89].

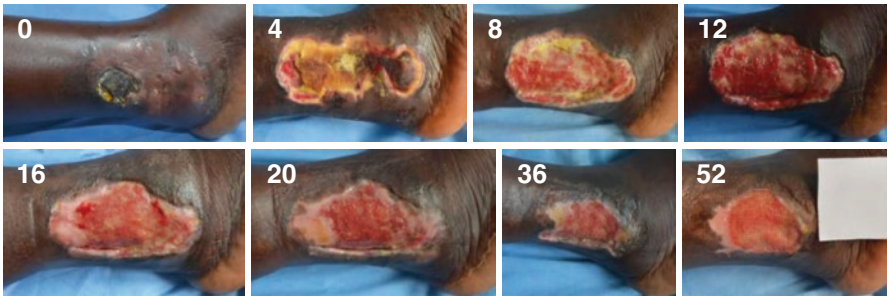

Fig. 45.4 Patient enrolled in drug trial, with PCR-confirmed *M. ulcerans* infection at the lateral malleolus of right foot; necrotic slough, purulent aspect at week 8; granulating surface at week 12. Partial healing with shallow ulceration still present at week 16; non-epithelialized shallow lesion at week 52, classified as failure, according to strict definitions used in the trial; the study team suspected that the lesion did not represent residual *M. ulcerans* infection, but rather non-healing as a result of limited compliance with topical dressings treatment and recurrent trauma

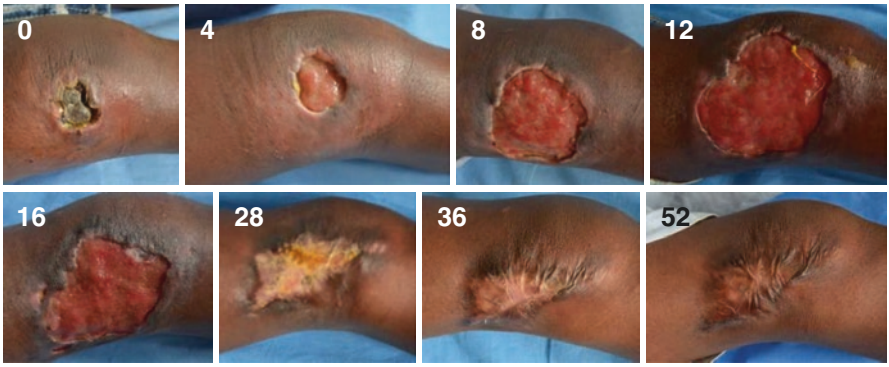

Fig. 45.5 Patient with *M. ulcerans* infection located at the lateral aspect of the right knee, enrolled in a drug trial. Although lesions located at joints run an increased risk for development of contractures, resulting in functional limitations, Prevention of Disability activities under trial conditions appeared effective, with negligible functional disabilities among study participants. At week 0, necrotic slough is clearly present; paradoxical enlargement of the lesion appears at week 4, with edema present; lesion stabilizes at around week 26, while at week 28, the lesion is almost complete epithelialized; stable scar with excellent functional results at completion of follow-up at week 52

45.4 Supportive Measures

Early detection and prompt treatment with currently available interventions, such as those described above, are critically important to achieve prompt and uncomplicated healing. Health-seeking behavior is complex and generally poorly understood; many drivers for delay in health seeking in health institutions have been identified including stigma, fear for mutilating surgery, and traditional beliefs and attitudes favoring traditional treatment [19, 90, 91].

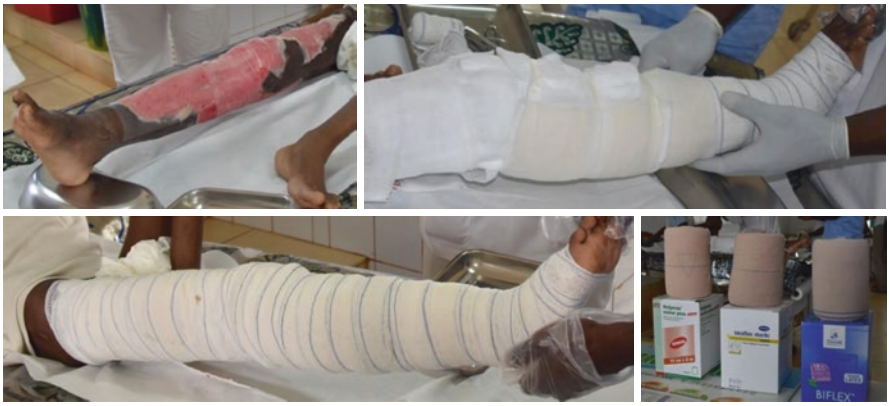

Fig. 45.6 Large Buruli ulcer lesion involving almost the entire right leg. Pain management during wound care is essential; topical treatment includes careful saline rinsing, application of non-adhesive cover with paraffine or vaseline gauze; absorptive dressing material, with gentle compression bandage, preferably using short-stretch bandage. Prevention of Disabilities that may also require careful pain management, following the WHO pain ladder, includes physiotherapy, in order to prevent contracture and functional limitation. In lesions of this size, split skin grafting helps to speed up healing

Active case finding in highly endemic regions appears to reduce the number of individuals with advanced disease [92]. Indeed, antimicrobial treatment started early on has tremendous potential to prevent disabilities, resulting in excellent quality of life [93] and societal participation and inclusion [94]. Patient delay may ultimately be the hurdle to take, to optimize outcome. Fear for disfiguring surgery kept some patients from reporting timely [19, 90], but the good news from recent work is that disfiguring resection surgery is no longer necessary to achieve relapse-free cure, without severe sequelae.

45.5 Comorbidities and Coinfections

The large trials providing the evidence for the effect of drug treatment for BU excluded potential study participants with many comorbid conditions. Drug exposure—the result of resorption, distribution, and elimination—is described by the pharmacokinetics (PK) of each individual drug. PK may change with comorbid conditions like diabetes and obesity [42]. Drug-drug interactions are common with rifampin and clarithromycin, and comedications are common with these comorbid conditions, especially with HIV [95]. Like in HIV co-infected patients with tuberculosis, protease inhibitors cannot be combined with rifampin. Otherwise, the same principles for treatment apply to patients with BU and comorbid conditions [96], although the evidence base to guide treatment for co-infected patients is small.

45.6 Conclusions

The case for early detection and prompt treatment with a combination of antimicrobial drugs, primarily, rifampin and clarithromycin, offers an excellent chance of relapse-free healing, with minimal chance of residual functional limitations; median time to healing even with small category I–II lesions remains a concern, even if supportive treatment with appropriate wound care and measures to prevent contractures are optimized. Treatment duration less than 8 weeks has been anecdotally reported, but formal studies have not addressed the question of optimal treatment duration for different categories or presentations of BU disease. In advanced lesions, there may be a place for additional surgery; the role of surgery to obtain cure has become obsolete, apart from special circumstances, e.g., in pregnant patients, who might also benefit from topical heat treatment.

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Rehabilitation in Buruli Ulcer

46

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This chapter describes rehabilitation and shows the importance of including rehabilitation interventions early within Buruli ulcer (BU) disease control activities. When the term rehabilitation is used in this chapter, it is inclusive of prevention of disability (POD) interventions. WHO "Rehabilitation 2030" considers rehabilitation as a core health service for individuals with health conditions throughout their life and across their continuum of care [1]. Rehabilitation uses biological, psychological, social, and technological interventions to maximize function and inclusion. The best results are seen when rehabilitation interventions are started early and done in the community and at all levels of healthcare.

This chapter is divided into three sections. The first part explains the terms, *disability* and *rehabilitation*. The second part presents common causes of disability in BU. The final part discusses common problems and actions required to prevent or minimize the disabling effects of BU.

46.1 What is Disability and Rehabilitation?

The WHO International Classification of Functioning, Disability and Health (ICF) is a framework applicable to all health conditions and provides a standard language to understand the effects of disease on functioning and disability [2]. Rehabilitation

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addresses the impact of a health condition on a person's everyday life as it seeks to optimize function and reduce the experience of disability [3]. Rehabilitation enables individuals of all ages to maintain or return to their daily life activities, fulfill meaningful life roles, and maximize their well-being [3].

46.1.1 Disability

Disability is a universal human experience that can sometimes be permanent or sometimes transient. The WHO ICF uses disability as an "umbrella term" for impairments, activity limitations, and participation restrictions as a result of a health condition [4]. Personal and environmental factors can further affect disability by creating barriers or facilitating function and inclusion [4].

The ICF defines *impairments* as changes or losses in body functions and structures (physical or mental) [4]. In BU, skin changes (papules, nodule, plaque, edema, or ulcer) and bone and joint involvement are considered impairments. Difficulties experienced by the individual in doing or executing a task or an activity are referred to as an *activity limitation* [4]. In BU, these limitations are noted in difficulties doing personal care, problems with dexterity needed for dressing and writing, and difficulty with mobility affecting walking, squatting, and lifting. *Participation restrictions* refer to the difficulties the person has to participate or be included in family, school, work, and community life [4]. In BU, these restrictions may result in poor access to school, unemployment, and restrictions with domestic activities and agricultural work, playing games, or participating in community life (social and civic) as well as affect relationships.

Personal and environmental factors can either create barriers or facilitate function and inclusion [4]. Environmental factors include terrain, climate, and building design as well as societal attitudes, practices, and laws that can lead to social exclusion, stigma, and discrimination. Personal factors include age, gender, social status, cultural role expectations, spiritual life, intelligence, educational level, coping skills, and self-esteem.

46.1.2 Rehabilitation

WHO defines health-related rehabilitation as a set of measures or interventions that assist individuals, who experience or are likely to experience disability, to achieve and maintain optimum functioning in interaction with their environments [5]. Rehabilitation in BU starts in the early phase of recognition of the disease and continues alongside other health interventions. Rehabilitation is cross-sectoral and may be carried out by both non-specialized and specialized health professionals in conjunction with specialists in education, employment, social welfare, and others within the community in collaboration with the person affected by BU and their family.

46.2 What Causes Disability in BU?

46.2.1 Late Disease Diagnosis and Treatment

BU is caused by an environmental bacterium called *Mycobacterium ulcerans* that mainly affects skin, soft tissues, and sometimes bone. The organism produces a toxin, mycolactone, that causes tissue damage [6–9]. Early disease diagnosis and treatment with a combination of antibiotics are crucial to preventing impairments and long-term disability (see Chap. 45). https://www.who.int/health-topics/buruli-ulcer#tab=tab_1. Late diagnosis of BU results in larger ulcers and more complications such as edema, bone involvement, greater movement limitations, cancerous lesions, and disability [6–8].

46.2.2 BU Lesions at or Near a Joint or Critical Area

The risk of movement limitations and disability is greater if the wound is large or at or near a joint or critical area, even with smaller category I or II BU lesions (see Chap. 42).

46.2.3 Beliefs and Practices During Wound and Scar Management

The beliefs that the affected part should not be moved or exercised till the wound is healed combined with the fear of pain with movement further restricts movements and using the affected part in daily activities. Tight bandages and incorrect bandaging technique can cause edema and impede movement [6–8]. Inappropriate application of traditional treatments, iatrogenic damage from surgical debridement, and repeated or incorrect skin grafts can also contribute to the furthering of disabilities [8]. After wound healing, the thicker, less flexible scars can be easily injured during daily activities, burned by the sunshine, and restrict full movement. Poor care of the immature scar (first 1–2 years after the wound is healed) can result in skin cracks, repeated injury, sunburn, and contractures [6–8].

46.2.4 Lack of Early Integration of Rehabilitation Interventions With BU Treatment

Disability can result by not starting rehabilitation interventions early during BU treatment. In addition, limited or no access to specialized rehabilitation services and assistive technology (AT), such as crutches, wheelchairs, and upper and lower limb prosthesis, can limit the possibility of maximizing function and participation. These limitations and restrictions impact mental well-being, causing depression, anxiety, and low self-esteem. Initiating rehabilitation early and assuring access to specialized rehabilitation interventions and AT help the person affected by BU to experience less disability during and after his or her clinical care for BU. [6]

46.2.5 Stigma and Discrimination About BU Disease and Disability

Cultural beliefs, attitudes, and stigma about BU disease and disability can also limit the participation and inclusion of the person affected by BU within family, school, work, and community life [6]. Rehabilitation interventions may include community and educational interventions that address family and community issues which limit and restrict function and inclusion of persons affected by BU.

In summary, disability can be prevented or minimized in BU by doing the following [6–8].

- Detect and treat BU disease early before disabilities are visible.
- Detect problems early and take action for movement limitations, edema, scars, activity limitations, participation restrictions, and mental well-being.
- Include rehabilitation interventions within wound management practices.
- Improve knowledge and skills of persons affected by BU and their families to do daily self-care and rehabilitation interventions.
- Include early access to AT to improve function and independence.
- Facilitate timely access to specialized rehabilitation and cross-sectoral services.
- Implement community health education strategies and interventions to address structural barriers, social stigma, and discrimination issues related to BU and disabilities.

46.3 What Interventions can be Implemented Early to Prevent or Minimize the Disabling Effects of BU?

The best outcomes are when rehabilitation interventions start early and are person-centered involving the person affected by BU and their family in collaboration with health workers and specialist. When needed, it is important to facilitate timely referral and access to specialized rehabilitation services that may include surgery, physical therapy, occupational therapy, psychosocial support, AT, and others. Frequently rehabilitation is cross-sectoral involving educators, employers, religious leaders, social welfare, and others in the community. Rehabilitation interventions may be short term or periodic throughout one's life span.

More detailed information on BU rehabilitation interventions can be found in WHO publications, *Buruli Ulcer: Prevention of disability* by Lehman et al. [6] *and Prevention of disability I Buruli ulcer: basic rehabilitation—Practical Field Guide* by Simonet [7]. ANESVAD also published an excellent BU rehabilitation work, *Guide for the prevention and rehabilitation of functional limitations* by Bonifacio [8]. Other useful resources are *Ten steps: A guide for health promotion and empowerment of people affected by Neglected Tropical Diseases* by Lehman et al. [10] https://www.leprosy.org/ten-steps/ and the NTD Information portal sections on cross-cutting issues https://www.infontd.org/cross-cutting-issues and toolkits https://www.infontd.org/toolkits.

46.3.1 Detect Problems Early and Take Action

This section will focus on rehabilitation interventions that can be used during wound care, during BU treatment, and after BU treatment is completed. Detecting problems early and taking action minimize the disabling effects of BU. Limitation of movement (LOM), edema, scars, and pain can limit function, restrict participation, and affect mental well-being. Developing self-care skills and participating in self-help groups help sustain health and rehabilitation results and in assuring social inclusion.

46.3.1.1 Limitations of Movement (LOM)

The risk of movement limitations is greater when the lesion is at or near a joint. Edema, pain, scars, adhesions, pain, and the fear to move can further limit mobility. LOM is required to be documented on the WHO BU 01 form (Annex 3: Buruli Ulcer Clinical and Treatment Form—New Case (BU 01) [9], p.62. Standardized methods to assess LOM are not specified. Several field comparisons, done by Lehman, demonstrated that between 20% and 30% of people with LOM were not identified [11]. This results in delays in recognition of LOM and taking action to improve mobility. When health workers and BU patients had guidelines, they more easily and consistently identified LOM but were unable to quantify the degree of limitation [11]. Another important factor contributing to LOM is the belief by many healthcare workers, BU patients, and their families that movement cannot be started until after the wound is healed. Permission to move is needed.

The only time movement of the affected part is not allowed is following a skin graft or other surgeries. The surgeon authorizes when movement can be started. Special care is needed post-skin graft.

Guideline to identify LOM: [11] Observe movements of both the affected and non-affected sides together, and compare whether the movements are the same or different. LOM is identified when the BU affected side demonstrates less movement than the non-affected side. See Table 46.1 for procedures to observe LOM in lower and upper extremities.

Actions to Prevent or Minimize LOM in Affected Areas Without Recent Skin Grafts

Give permission to person affected, family, and health worker to move and stretch affected part frequently during the day.

- Move and stretch affected part at wound dressing change, after bandages are removed.
- Check that bandages are not too tight or restricting movement.
- Position the affected part during rest and at night to stretch opposite the direction of the strong contracting forces of wound healing.
- Encourage the person affected by BU to participate in doing daily self-care and activities of daily living (ADLs).
- Use AT to help facilitate mobility and independence (e.g., crutches, wheelchair, others).
- Refer to rehabilitation specialist if LOM does not improve.

Table 46.1 Procedures to check for limitations of movement (LOM) in BU

Movements for lower extremity: [11] Sit in a chair with legs extended. Curl toes down and straighten. Sit with knees slightly bent with soles of the feet on the ground. Keep heels on the ground while raising feet. Press toes down while lifting the heels off the ground. Lay on stomach with feet off the edge of table/bed. Slowly bend knees to touch heels as close as possible to the buttocks then straighten the legs. Observe the hip, does it stay flat or lift up? If it lifts up, there is a limitation at the hip		
Check if:	Yes	No
• Toe movement is less? (curl/straighten)		
• Ankle movement is less? (sit with knees bent, foot up/down)		
• Knee movement is less? (lay on stomach, bend and straighten knees)		
• Hip movement is less? (lay on stomach, hip lifts up when knee bends)		
Movements for upper extremity: [11] Raise arms up to shoulder height with elbows extended. Make a fist with both hands, and move wrist up and down. Open hands and show the palm of the hands, spreading fingers out, and then bring together. Turn hands over (supinate), and bend elbows so that the hands can touch the back shoulder (scapula). Extend arms out to each side (abduction) with thumbs up. Raise arms up above head until hands touch		
Check if:	Yes	No
• Thumb movement is less?		
• Hand/finger movement is less?		
• Wrist movement is less?		
• Elbow movement is less? (flexion/extension, pronation/supination)		
• Shoulder movement is less?		

46.3.1.2 Edema

There is an edematous form of BU (see Chap. 42), but edema can also be seen in the BU affected area of all BU forms and when the wound has a secondary infection. Edema appears when affected limbs are positioned downward or when the BU affected area is not moved frequently. Tight, restrictive bandages can also cause edema [6–8]. Forceful manipulation to soft tissues and joints by physiotherapy or surgery frequently results in increased edema. Scars can adhere (adhesions) to underlying structures, restricting movement and interfering with lymphatic drainage of edematous limbs. Swollen limbs are heavy, painful, and difficult to move.

Detect edema by comparing one side of the body with the other side. A tape measure can be used to measure the diameter of the edematous limb. Care is needed to measure the same way each time. A simple method to show how extensive the edema is to count the number of areas with edema. In the lower limb, observe if there is edema in the toes or foot, ankle, lower leg, knee, and thigh. In the upper limb, observe if there is edema in the fingers or hand, wrist, forearm, elbow, and upper arm. Frequently it is easier to see edema looking at boney prominences, such as the knuckles or ankle bones. Take action to reduce edema as soon as possible.

Actions to Prevent or Minimize Edema

- Treat secondary infection with antibiotics.
- Elevate affected part higher than the heart.
- Give permission to person affected and family to move affected part.

- Encourage the person affected by BU to participate in doing daily self-care and ADLs.
- Check that bandages are not too tight or restricting movement.
- Apply light compressive bandages, wrapping the bandage upward on the limb, distal to proximal.
- Rest affected part in a functional position.
- Refer to rehabilitation specialist if edema does not improve.

46.3.1.3 Scars

A scar forms when a wound heals. It is never as strong or flexible as the original skin and can restrict movement and cause soft tissue and joint contractures [6–8]. After wound healing, the thicker, less flexible scars can be painful. Scars require good daily self-care practices to prevent skin breakdown, sunburn, and movement limitations. Sun-damaged scars and those that chronically break down and re-heal over time have a higher risk of developing cancer.

If scarring and contractures are extensive, specialized physiotherapy and occupational therapy rehabilitation services and surgery are required to regain mobility, optimize function, and improve esthetic appearance. This also improves social inclusion and mental well-being.

Actions to Prevent or Minimize Complications with Scars

Common scar problems and actions to care for scars are summarized in Table 46.2 [6–8].

Table 46.2 BU scar issues and interventions used to prevent or minimize complications

Scar problems	Rehabilitation interventions
• Dry and itchy	• Moisturize daily with shea butter, coconut oil, or others to keep scar soft and flexible
• Strong contracting forces of scar is at or near a joint	• Move and stretch frequently in the opposite direction of the scar's strong contracting forces • At rest, position with pillows, weighted bags, or splints to stretch opposite the direction of the strong contracting forces of the scar
• Scar sticking to underlying structures (adhesions)	• Moisturize and gently mobilize scar to loosen from underlying structures. Be careful not to rub superficially creating blisters
• Fragile, easily injured	• Protect from sunburn and injury during ADLs
• Thick scars less than 1 year old	• Flatten and soften scar by applying constant light pressure with thin foam rubber padding with bandages
• Thick scars limiting movement	• If no improvements are noted with 1–2 months from community-based interventions, refer to rehabilitation specialist for progressive splinting, mobilization, surgical correction, and possible fabrication of compression garments [6]

46.3.1.4 Pain

Initially, BU lesions are not painful due to the mycolactone produced by *M. ulcerans*. However, they can be very painful when there are secondary infections. Velink et al. (2016) found that former patients felt research should prioritize better accessibility to care and pain management [12]. Patients stated they experienced pain during treatment procedures, in particular wound care, the streptomycin injections, and physiotherapy [12]. Specifically pain is noted when bandages, sticking to the wound bed, are removed, when movement is attempted with edematous limbs, when forceful passive mobilizations are used in physiotherapy, and when movement is attempted in areas where scars are adhering to underlying structures.

A self-assessment of pain can be done using the *Wong-Baker Face Pain rating scale* (1983). https://wongbakerfaces.org/wp-content/uploads/2016/05/FACES_English_Blue_w-instructions.pdf

Actions to Minimize Pain: [6–8]

- Recognize when there is pain and what makes it worse or better.
- Discuss fears and anxiety about treatment, wound care, and exercises.
- Inform and involve the person affected in procedures to lessen their fears.
- Treat secondary wound infections with appropriate systemic antibiotics.
- Use analgesic, as needed, before wound care procedures and physiotherapy treatments.
- Use appropriate wound care procedures and materials which reduce trauma and pain to the wound during bandage removal.
- If pain increases after physiotherapy, review and modify physiotherapy techniques for mobilization, stretching, and strengthening.
- Reduce edema with elevation and frequent movement.
- Use appropriate massage over scar area to reduce sensitivity and it adhering to underlying structures.
- Use light constant pressure over scars that are less than 1 year old to flatten and soften the scar.
- Refer to clinician or rehabilitation specialist if pain does not improve.

46.3.1.5 Mental Well-Being

Mental health is an integral part of health and is determined by a range of socioeconomic, biological, and environmental factors [13, 14]. Eaton et al. [14] note a high rate of mental health problems among people living with neglected tropical diseases (NTDs) [13, 14]. The combination of the direct physical consequences of BU (pain, LOM, scars), social attitudes (BU and disability), and stigma can result in the person not participating fully in family, school, work, or community life. When treatment and care do not address mental well-being, more profound depression, anxiety, or other mental health conditions can result [14].

Actions to Improve Mental Well-being

Health workers and peer counselors can be trained to recognize problems and provide basic counseling or refer to a specialist when needed [14]. Community

education campaigns and peer support groups can help fight the damaging effects of social exclusion. Self-care groups provide psychosocial support and improve the sense of well-being (physically, socially, and psychologically).

46.3.1.6 Empowerment to Sustained Health and Rehabilitation Results Through Self-Care Practice and Self-Help Groups

Developing the capacities of the person affected by BU to do daily self-care is one of the most important rehabilitation interventions to prevent and minimize the disabling effects of BU. WHO (2020) defines self-care as "the ability of individuals, families and communities to promote health, prevent disease, maintain health, and to cope with illness and disability with or without the support of a healthcare provider." [15] Self-help groups bring people together to solve problems as well as provide an important psychosocial support. Unified, they are empowered with a stronger voice to address barriers limiting inclusion. The individual and their family's belief in their capability to exercise control over their disease, health condition, and disability is called *self-efficacy* [16]. This belief improves the person's treatment compliance, their practice of daily self-care, and their sense of well-being.

46.4 Conclusion

To conclude, the disabling effects of BU can be prevented or minimized when rehabilitation interventions are integrated early within BU disease control programs. Rehabilitation interventions improved quality of life with better physical function, improved mental well-being, and social inclusion. Rehabilitation requires task sharing and task shifting to include the person affected, their family, health workers at all levels, rehabilitation specialist, and others within the community. Referral pathways to specialized rehabilitation services, assistive technology, and social welfare support need to be planned, available, and accessible. Some persons affected by BU may require lifelong access to specialized rehabilitation services and social-welfare support. Monitoring and evaluation of health programs can assure early rehabilitation interventions are available, accessible, and acceptable across the continuum of care for persons with BU.

Self-care practices and self-help groups empower the persons affected by BU and their family to obtain and sustain improved function and social inclusion.

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Part XII

Buruli Ulcer and Community

Epidemiology of Buruli Ulcer

47

Katharina Röltgen, Paul D. R. Johnson, and Gerd Pluschke

47.1 Characteristics of BU Patients

BU is commonly regarded as a disease that mainly affects children, and this holds true for BU endemic areas of Africa and Papua New Guinea [1]. In contrast, the average age of BU patients in Victoria (south-eastern Australia) is high [2], which may be partly related to the high average age of the general populations living in this BU endemic area. However, a bi-modal distribution of the age-related risk of developing BU is also observed in Africa with young teenagers and the elderly carrying the highest risk [3]. The increasing susceptibility in the elderly may be related to immunosenescence. In Africa, children below 4 years are underrepresented, as exposure to environmental reservoirs of *M. ulcerans* may be relatively limited [4]. With increasing movement radius of the children, the risk of older children to develop BU is increasing until they are 12–14 years old. A subsequent decline in susceptibility in older children and young adults may be related to the development of protection by the adaptive immune system. In larger BU case series, a balanced male/female ratio was found. However, differences in the gender ratio have been

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reported, when study populations were stratified by age, with boys being overrepresented in children below the age of 15 years and females being overrepresented in the older age groups [1]. These differences may be related to differences in environmental contact patterns associated with movement radius of children and occupational exposure to environmental reservoirs in adults.

Studies on the protective efficacy of *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccination against BU have yielded controversial results. Randomized controlled clinical trials in Uganda have provided evidence for some short-lived protection from developing BU [5]. Although the prevalence of HIV infection is high in many BU endemic African settings, information about the consequences of BU-HIV co-infection is fragmentary. HIV/AIDS seems to increase the susceptibility for *M. ulcerans* infection, as the prevalence of HIV in BU patients tends to be higher than the prevalence in the general local population [6]. Furthermore, HIV co-infected BU patients tend to have often multiple and more severe lesions. The marked clinical variability of BU suggests an important role of host factors in determining the outcome of infection [7]. Case control studies aiming at the identification of genetic factors that influence susceptibility to develop BU have identified several relevant polymorphisms among a limited number of genes tested. Several of these polymorphisms are relevant for macrophage activation, which supports the hypothesis that macrophages play an important role in early host defense against *M. ulcerans* [8].

Among *M. ulcerans* isolates from human lesions, two principal lineages have been identified. While classical lineage disease strains are isolated from BU patients from Africa, Papua New Guinea, and Australia, where BU occurs with high local prevalence in geographical hot spot areas, ancestral lineage clinical strains have been isolated from patients from Asia and the Americas, where BU cases are much more rare and scattered [9]. In spite of differences in the composition of the secreted mycolactones, the unique macrolide toxin produced by *M. ulcerans*, there is no clear evidence for a marked difference in the pathogenic potential of the lineages isolated from human BU lesions.

47.2 Transmission of *M. ulcerans*

M. ulcerans endemic areas are generally associated with stagnant or slow-flowing water bodies and are found in both river basins and coastal lowlands. It is suspected that environmental changes may lead to the emergence of new BU geographical foci and changes in BU incidence [1]. Lack of reliable historical prevalence data is making it often difficult to prove that changes in agricultural activities, damming of water bodies, mining activities, sand mining, and deforestation indeed affect BU incidence. The exact source of *M. ulcerans* causing infection in humans is not clear. Because of massive loss of gene function by genome reduction and pseudogene development in the course of the divergence of *M. ulcerans* from *M. marinum* [10], it has been suggested that *M. ulcerans* survives in the environment as a commensal associated with protective organisms. The mode(s) of transmission from environmental reservoirs to humans and

other mammalian hosts is not clear, and possibly *M. ulcerans* is transmitted by several distinct mechanisms into susceptible layers of subcutaneous tissue. Direct human-to-human transmission seems to be rare, if ever. Wearing protective clothing and appropriate wound management seem to reduce the infection risk [1]. Insect bites but also cuts and scratches near or in water bodies have been linked to an increased risk. Progression to disseminated disease is rare, and as about 95% of BU patients present with a single lesion, it is assumed that the site of inoculation is usually also the site of infection. Most BU lesions are found on the lower limbs, followed by the upper limbs. While overall patterns in African and Australian patients are similar [3, 11], the anatomical site of the lesions may differ with both age and gender. BU lesions are most commonly located on parts of the body which are not protected by clothing, and protective clothing has been described to reduce the risk of acquiring BU [1]. The location of BU lesions may speak for a role of insect bites in transmission, but this does not rule out that traumatic skin injuries play an important role. The incubation period determined for short-term visitors to BU endemic areas in south-eastern Australia was widely variable with a mean of about 4.5 months [12], and in a study in the Kinyara refugee camp in Uganda, the incubation period was estimated to be between 1 and 3 months [13]. Incubation periods may depend on the mode of transmission and the inoculation dose of *M. ulcerans*. Seasonality of *M. ulcerans* transmission may be related to changes in the presence of the pathogen or of potential vectors in the environment and to behavior-associated variations in exposure, for example, associated with agricultural activities. In particular, seasonal changes in the aquatic ecosystems, such as flooding, may be of crucial importance. A well-documented example is a BU outbreak in the Daintree region of northern Australia, which occurred 7—8 months after a flooding event in February 2011 [14]. Recognition of seasonal patterns of *M. ulcerans* transmission is difficult, because both incubation periods and intervals between the occurrence of first symptoms and reporting to the health system may vary substantially.

Environmental investigations in BU endemic areas by environmental PCR targeting the *M. ulcerans* insertion sequence IS*2404*, have revealed that *M. ulcerans*-specific DNA can be detected in aquatic ecosystems, including plants, snails, fish, or insects [15]. However, for unknown reason, the reported PCR positivity rates differ substantially in different environmental studies in African BU endemic areas. In south-eastern Australia, possums have been found to be highly susceptible to *M. ulcerans* infection [16]. They may represent an animal reservoir, as comparative whole genome analyses revealed that classical lineage isolates from possums and humans resemble each other. In African BU endemic areas, a potential role of an animal reservoir is less clear, and it has been suggested that the frequently observed large chronic human BU lesions may contribute to the dissemination of *M. ulcerans* in the environment.

47.3 BU in Specific Epidemic Scenarios

In the following four subchapters, epidemiological features of BU are described for four specific endemic areas.

47.3.1 Emergence of BU in Uganda

The expatriate physician Sir Albert Cook (1897) was perhaps the first to record a description of chronic necrotizing ulcers with undermined edges, but it was only in 1961 that the cause in the Ugandan patients could definitely be attributed to infection with acid-fast mycobacteria [17]. A high prevalence of cases was recorded in a geographically limited area of Uganda in close proximity to the River Nile and the Lake Kyoga in an area then known as Buruli—hence the name for the disease. A second BU focus was discovered in 1962 with patients living along the Nile in a remote area of the Madi region, where the disease had been known among the local population for many years as "*juwe okoro*"—*the sore that heals in vain* [18]. The marked increase in the incidence of the disease and the increasingly more widespread distribution of cases had been associated with the unprecedented flooding of rivers and lakes after heavy rainfall in the early 1960s [19]. The unfortunate circumstance that a community of ~2500 Rwandan refugees had settled in late 1964 close to the Nile at Kinyara in what turned out to be a hotspot for *M. ulcerans* transmission provided valuable insights into the epidemiology of BU. The refugees developed BU lesions shortly after (and never before) they arrived at Kinyara and continued to contract the disease until they moved 5 years later to a different locality (Kyangwali), indicating an involvement of environmental factors in transmission and a low likelihood of contagiousness. The incidence of BU in the refugees did not decrease during the 5-year period. Proximity to the Nile appeared to be pivotal for pathogen transmission, although the Rwandans only rarely had actual contact with the Nile, pointing toward a role of environmental factors related to but not necessarily found within the river.

Of the 220 cases of BU described by the "*Uganda Buruli Group*" [13], the highest incidence was seen in children aged between 5 and 14 years. Ninety-five percent of all patients had a single lesion at presentation, suggesting local introduction as opposed to systemic spread of the pathogen with body parts not commonly protected by clothing being the most affected. The distribution of lesions in children below 5 years of age, who wore few clothes, appeared random, whereas lesions on the extremities as opposed to the head and trunk became more frequent with increasing age. In male patients, lesions became increasingly confined to the lower extremities, while arms and legs, but not the chest and abdomen, were common sites for BU in older girls and women. These findings together with the observations that more women living close to the river than those living farther away from it were affected by the disease, while men had a less variable spatial and more variable seasonal incidence, indicated that certain behavioral factors, such as domestic or agricultural activities, in which males and females have different roles, are responsible for a difference in exposure to *M. ulcerans*. The timing of the development of BU in individuals living in BU non-endemic areas after brief visits to Kinyara and in refugees with disease onset after moving to Kyangwali led to an estimated incubation period of 4–10 weeks [13]. Relying on this estimate, seasonal fluctuations seemed to coincide with the second rainy season and with the time when men did most of their work in the fields.

47.3.2 Association of BU with the Mbam River in Cameroon

In Cameroon, BU was for the first time described in 1969 in patients coming from the Nyong River valley, in the Centre region. Between 2007 and 2009, first suspected BU cases were also reported from the Mapé Dam region, which consists primarily of the Bankim Health District (HD) but includes also parts of neighboring HDs. This region subsequently turned out to be one of the BU endemic foci of Cameroon [3]. Main environmental features of the area are the Mapé Dam, an artificial lake created by damming the Mapé River in 1988 and the Mbam River, which delimits the natural border of the Bankim HD. From 2010 to 2014, 274 suspected BU cases were detected, and diagnosis of 148 was confirmed by diagnostic IS*2404* qPCR. For 136 of the BU patients, the location of their house was mapped, and a clustering of patients was observed in association with the Mbam River [3]. Patients had their farms mostly close to the Mbam River, and only a few cases were living in the immediate proximity of the Mapé Dam reservoir, speaking against a direct importance of this man-made lake for the spread of BU. However, this finding does not exclude that environmental changes caused by the damming of the Mapé River may have indirect effects on environmental reservoirs of *M. ulcerans* in the wider area. In fact, bacterial population genomic and molecular dating analyses of a set of *M. ulcerans* case isolates indicated a strong temporal association between construction of the dam and the emergence of BU in the region [20].

The population age-adjusted prevalence of BU in the Mapé basin showed a biphasic pattern with the lowest prevalence in the 2–4-year-olds and peak prevalence in the 12–14-year-olds and in the <50-year-olds [3]. When antibody responses against the immunodominant 18 kDa small heat-shock protein overexpressed by *M. ulcerans* were used as marker for exposure to *M. ulcerans*, sero-conversion was found to set in at an age of about 5 years [4]. In contrast, serological responses to the mosquito-transmitted malaria parasites emerged already at around 1 year. This indicates that *M. ulcerans* is rarely transmitted at the homes of the children and that exposure to these environmental bacteria intensifies at an age when children are developing more intense contact with the environment (including water bodies), outside the movement range of very young children. The serological data indicate that only a small proportion of individuals exposed to *M. ulcerans* develop clinical disease.

As proximity to water bodies undoubtedly is a risk factor for BU, samples from village and farm water sources of the BU patients, including water, soil, plant material, and animal feces, were collected and tested by IS*2404* qPCR for the presence of *M. ulcerans* DNA. While only a few sites tested positive, a longitudinal analysis of one of the positive permanent small village water bodies yielded positive results over a period of more than 2 years [21]. While an area with compacted and sandy ground was consistently negative, an adjacent area covered with decaying organic matter was consistently positive. Additional analyses using IS*2606* and ketoreductase specific qPCR tests indicated that the detected mycobacterial DNA was derived from the lineage of *M. ulcerans*, which is found in human lesions. These data

indicate that underwater detritus represents a microenvironment to which *M. ulcerans* has adapted in the course of its evolution from the more generalist *M. marinum* and that stagnant and slow-flowing water bodies may represent an environmental reservoir of human pathogenic *M. ulcerans*. A comparative whole genome sequencing analysis of clinical isolates from BU patients from the Mapé basin and the Nyong basin revealed the existence of two phylogenetically distinct clonal complexes in Cameroon [22]. These results showed that *M. ulcerans* clones diversify locally by the accumulation of a very limited number of SNPs, that clinical variability of BU observed in a given area is most likely not accounted for by microbial diversity, and that there is no highly mobile reservoir, which would carry strains from one endemic area to another.

47.3.3 Rising Incidence of BU in Victoria

"Bairnsdale ulcer" (synonymous with BU) was first recognized in the late 1930s in Bairnsdale—a regional town in east Gippsland, Victoria, Australia [23]. Cases were from separate households, suggesting from the outset chance exposure to a widely dispersed environmental pathogen rather than to a single point source or person-to-person transmission. Also noted at the time was the wide age distribution with the disease affecting children, adults, and both genders equally, which has remained a consistent pattern in Victoria since then. During the 1980s, *M. ulcerans* infection was identified in koalas from a population close to Bairnsdale [24], the first recognition of naturally occurring BU in any species other than humans. Starting in 1990 there was a notable change from low-level endemicity in a fixed rural geographic region to higher-level transmission in completely new semi-urban regions. Initially, new cases were identified on the northern shores of Western Port, 240 km to the west of Bairnsdale, and next on Phillip Island from 1992 to 1995, almost all within a very small region surrounding a golf course and a newly formed shallow lake. Improved drainage of the lake and separation of recycled from ground water at the golf course were followed by a sustained reduction in new cases, and BU is now rare on Phillip Island. In the first ever example of environmental detection of *M. ulcerans*, water samples from the Cowes golf course tested positive by IS*2404* PCR [25]. The sudden appearance of a new focus of transmission, the high attack rate (up to 6% of the permanent population of east Cowes), and the recognition of infection in visitors who sometimes spent only brief periods in the outbreak area have become hallmarks of the new epidemiology of BU in Victoria since 1990.

Between 1995 and the early 2000s, there were very few BU cases in Victoria, but the situation changed again with large new outbreaks to the southeast of Melbourne on the Bellarine and most recently the Mornington Peninsula. Possible explanations to explain the outbreak at the time included much higher than usual rainfall and local reports of high mosquito numbers. A small proportion of mosquitoes trapped in CO_2 traps at St. Leonards were subsequently shown to harbor *M. ulcerans* DNA [26]. In 2002, new cases of BU abruptly appeared at Point Lonsdale, heralding the onset of an intense, sustained outbreak that peaked in 2011 and is slowly abating

now. The crude incidence of BU in 2011 was estimated as 770/100,000 of the permanent population (C. Lavender *personal communication*). Many inhabitants of the small seaside resort town of Point Lonsdale are retirees, and a feature of the outbreak was the high attack rate in older people with up to 3.7% of all residents aged over 75 requiring treatment for BU. In 2005, a new focus appeared at Barwon Heads, a further 10 km around the coast from Point Lonsdale, and from 2012 onward new endemic foci have also appeared along the Mornington Peninsula.

Studies from the Bellarine Peninsula found that being bitten by mosquitoes increased the risk of BU, and use of insect repellent reduced the risk [27]. When 11,500 mosquitoes were trapped, 4/1000 were PCR-positive for *IS2404*, and in a subset of samples, there was molecular evidence identifying the human outbreak strain of *M. ulcerans* [28]. In the late 1990s, several sick adult ringtail possums were detected at Phillip Island with ulcerative disease, and subsequently, *M. ulcerans* DNA was detected in samples of naturally occurring possum excreta. In a systematic study at Point Lonsdale, 42% of possum excreta samples were strongly PCR-positive for *M. ulcerans* compared with <1% in non-endemic areas. Further, both ringtail possums and brushtail possums had clinical BU lesions, and some of the trapped animals were found to be excreting *M. ulcerans* DNA in their feces despite the absence of clinical lesions [16].

Possums have been found to carry and excrete *M. ulcerans* DNA in high concentration in feces at places where human BU has become endemic since 2002. Similar surveys outside endemic areas yield negative results [16]. The frequent acquisition of BU by visitors to endemic areas in Victoria as well as local people in almost equal proportion suggests that transmission of *M. ulcerans* is a chance event and that risk may not be present all year. The progressive increase in BU incidence and its encroachment on the suburban fringes of Melbourne is unprecedented; all other regions of the world where BU is of public health significance are in tropical zones suggesting a unique set of local circumstances. In temperate Victoria, BU appears to be a zoonosis with opportunistic transmission to humans from biting insects, particularly mosquitoes. Currently there is no evidence that mosquitoes are a biological vector and may just passively shuttle *M. ulcerans* from active lesions on possums to humans, or pick *M. ulcerans* up as adults from drains, which collect contaminated possum excreta [16].

47.3.4 Sporadic Cases of BU in Japan

The causative agent of BU lesions in Japan is often described as *M. shinshuense* or *M. ulcerans subspecies shinshuense* [29], a sub-lineage belonging to the ancestral lineage, whereas cases in Africa and Australia are caused by classical lineage strains. Intriguingly, the epidemiology of BU in Japan is markedly different from that in Africa and Australia, where the incidence of BU in geographically confined areas is often high. In contrast, in Japan, BU cases have been reported only sporadically in the past decades, with a scattered occurrence in 17 of the 47 prefectures in Japan. The first case of BU was reported in 1980 and the second, a full 23 years

later, followed by occasional case reports in the years thereafter. Detailed study of 60 BU patients diagnosed between 1980 and 2016 revealed that middle-aged adults were the most affected age group and that infections were more frequent in women [29]. The clinical presentation was similar to that of patients in other BU endemic areas, but lesions were more often reported as being painful. Lesions commonly developed on body parts that are often not protected by clothing, particularly on the extremities and in the face. Multiple lesions were more frequent than in patients from other BU endemic areas.

For most of the cases, no obvious connection between aquatic environments and potential infection events could be established. An exception was a familial case cluster affecting three members of a family, whose residence was surrounded by farmland and extensive irrigation channels [30]. Environmental samples collected from a water channel slowly running through the family's property were IS*2404* PCR positive. Another familial cluster associated with exposure to aquatic environments was reported in two brothers, who developed BU lesions 3 months after camping at water sites during summer [31]. The vast majority of BU cases from Japan were diagnosed in autumn and winter, implying that patients were likely infected during or shortly after the hot and rainy season in summer, assuming an incubation period of about 2 months and a common delay of both seeking medical care and final diagnosis of BU. The climate in Honshu is subtropical in the South, temperate in the central regions, and cold in the North with Akita, where temperatures can fall below 0°C during the winter season, being the northernmost prefecture reporting BU cases. Most of the BU cases to date have been recorded in the Okayama Prefecture, located in the central-western region of Honshu, where the average annual precipitation is extremely high.

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Buruli Ulcer Control

48

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48.1 Introduction

Buruli ulcer (BU) control strategy recommended by the World Health Organization (WHO) focuses on early diagnosis and antibiotic treatment to minimize morbidity, disability, and socioeconomic impact [1]. There is currently no specific vaccine against BU although BCG has a demonstrated short-term protection [2]. Epidemiological evidence suggests the disease to be water-related, especially stagnant water in swampy areas [3]. Nevertheless, the modes of transmission of the causal agent from the environment to humans have not yet been clearly established [4].

The incubation period is estimated to be about 1–3 months. In Australia, a recent study in Victoria reported an average incubation period of 19.2 weeks (minimum 4.5 weeks, maximum 37.7 weeks) [5].

To date, there are no rapid point-of-care diagnostic methods to confirm BU, and research is underway to develop one.

Thus, control efforts are currently focused on promoting early case detection, through active case detection, health information and education, and appropriate prompt treatment. Studies have shown that early detection and antibiotic treatment can prevent the serious consequences of BU at an affordable cost (see Chap. 45).

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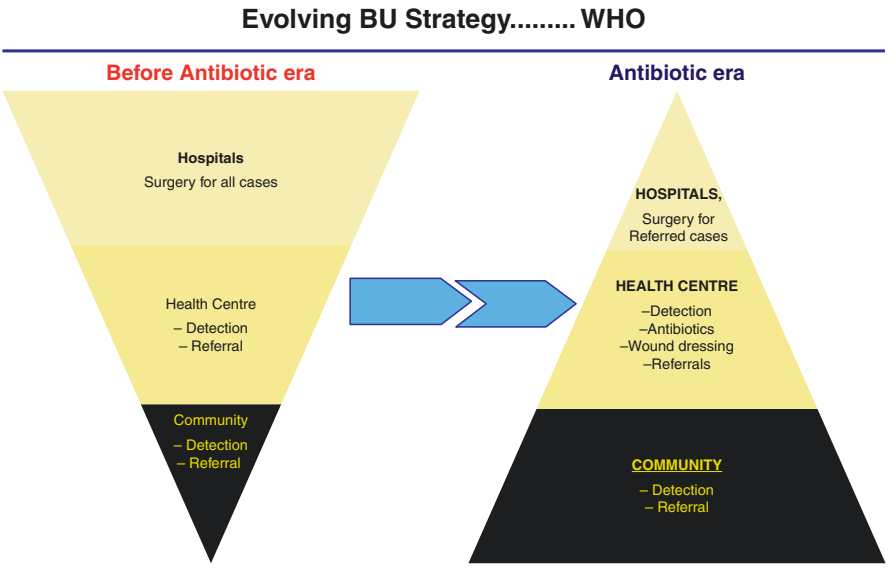

Fig. 48.1 Evolving BU strategy

48.2 The Control Strategy

Over time, the strategy has evolved with the introduction of effective antibiotic treatment, and the care of the disease has been gradually decentralized to lower-level health facilities (e.g., health centers). Only severe cases are admitted to hospitals for further management.

The strategy hinges around four pillars and is implemented through the three-tier levels of the health system as shown in Fig. 48.1.

The four pillars are as follows.

48.2.1 Pillar 1: Community-Level Activities

The community level is the first and critical level where efforts to educate the population about BU and other skin diseases should be implemented. Years of experience has shown that health education and early detection of suspected cases can be carried out by trained community health workers (sometimes referred to as community volunteers or village volunteers) [6, 7]. These community health workers are also trained on a number of health problems and serve as the critical link between their communities and primary healthcare level. In addition to community health workers, teachers are trained to report any suspected cases among schoolchildren who are often affected by NTDs. Many countries have developed innovative approaches to health education using radio and night video shows on BU, videos to help the

Fig. 48.2 Community health volunteer in action. (Credit: Y. T. Barogui)

communities understand the disease, importance of early reporting, and the consequences of late reporting (Fig. 48.2).

48.2.2 Pillar 2: Strengthening of the Health System

The three-tier health system needs to be equipped (infrastructure, equipment, transport, and logistics) to allow the appropriate management and referral of BU patients. At the community and health center level, basic facilities for wound dressing should be available so that ambulatory patients can get their dressings done while taking their antibiotics.

At the district hospital level, the capacity to do debridement and skin grafting is essential in managing patients with extensive lesions to facilitate early healing. Basic physiotherapy facilities should also be available as part of the interventions to prevent disability. It is important to have basic laboratory services to allow Ziehl-Neelsen staining, collection, and storage samples for PCR at the national reference laboratory.

At the regional and tertiary levels, the capacity for plastic and reconstructive surgery is needed to treat complicated cases. National reference laboratories capable of carrying out PCR are essential for the confirmation of cases. In 2019, WHO together with partners supported the establishment of the BU Laboratory Network for Africa to enhance the confirmation of cases in all endemic countries. A number of laboratories of the BU laboratory network (BU LABNET) in Africa provide confirmation of cases in their respective countries. Other logistic and equipment (e.g., computers, audiovisual, public address system) are needed for field activities and referral of patients.

Capacity building of health workers at all levels is also crucial. Indeed, it is essential to organize training and re-training for health workers (doctors, nurses, laboratory technicians, and physiotherapists/physiotherapists assistants) in the

diagnosis and management of the disease—antibiotics, surgery, and prevention of disabilities. In addition, a short-term training should be organized for general doctors and surgeons to improve competence in basic plastic surgery to deal with complicated cases at the district and regional levels. The training should be provided for the regional and district health managers in the organization and management of a BU control program [8].

Finally, to ensure proper recording and report of cases, WHO has produced standard forms (BU 01, BU 02, and BU 03) [9] to be used at all levels of the health system. The mapping of the cases is important in the control of BU so that interventions can be better targeted.

48.2.3 Pillar 3: Standardized Case Management

The first step in the identification of patient care is the correct taking of the history and clinical examination to arrive a reasonable diagnosis (see Chap. 42). This requires the training of health workers on the cardinal features of BU and common differential diagnosis (see Chap. 43). WHO has published documents to guide health workers in making the diagnosis. More recently, WHO has also provided a ten-point clinical and epidemiological scoring criteria to help improve the accuracy of clinical diagnosis (see Table 44.2; Chap. 44). These criteria are now included in the revised BU 01 [9], and operational research about the reliability of these criteria should be conducted prospectively to allow any adaptation in the future.

Once the clinical diagnosis is made, the clinician should collect samples from the lesion (swabs or fine-needle aspiration) for PCR confirmation following the guidance [10] provided on the collection, storage, and transport of specimens to the reference laboratory (see Chap. 41). At the treatment facility level, direct smear examination by Ziehl-Neelsen staining technique can initially be done to have preliminary confirmation, but the sensitivity of this test is less than 60%. Other tests like the fluorescent thin layer chromatography to detect mycolactone are being evaluated in selected health facilities [11] (see Chap. 41).

The treatment of BU has been improved over time [12, 13]. The current recommended specific antibiotics are a combination of rifampicin (10 mg/kg once daily) and clarithromycin (7.5 mg/kg twice a day) for 8 weeks for all forms of the disease (Table 48.1). Depending on the severity of the disease (Categories I and II) and patient's circumstances (e.g., distance from nearest health facility), the treatment may be given for the patient to take at the home or at the nearest health center where dressing will also be done. In severe cases (Category III), it may be appropriate to admit the patient and administer the treatment alongside other complementary treatments such as wound care (see Chap. 42 for definition of Categories). The role of surgery in BU has significantly evolved over the past 20 years. The current evidence supports a more conservative approach combined with antibiotics. In general, surgery in BU is not urgent, and experience emerging over the years has shown there is a benefit for the patient to complete the full 8 weeks of antibiotics before decision about surgery is made. Furthermore, a clinical trial has shown that patients benefited

Table 48.1 Dosage guide

	Weight (kg)	Maximum dose (mg) per day	Actual tablets to be administered per day
Rifampicin			
Children (150 mg) Rifampicin	5–10	75	0.5 × 150 mg
	11–20	150	1.0 × 150 mg
	21–39	300	2.0 × 150 mg or 1.0 x 300 mg
Adults (300 mg) Rifampicin	40–50	450	(1.0 × 300 mg + 1.0 × 150 mg) or 3 × 150 mg
	>50	600	2.0 × 300 mg
Clarithromycin			
Children (250 mg) Clarithromycin	5–10	125	(0.25 × 250 mg) × 2 per day
	11–20	250	(0.5 × 250 mg) × 2 per day
	21–39	500	(1.0 × 250 mg) × 2 per day
Adults (500 mg) Clarithromycin	40–50	750	(1.0 × 250mg + 0.5 × 250 mg) × 2 per day
	>50	1000	(1.0 × 500 mg) × 2 per day

from delaying the decision to operate [14]. Most Category I and II lesions will heal without surgery. Physiotherapy to prevent limitation of movement should be initiated early in cases where lesions are located at joints or critical sites (see Chap. 46). Like in all patient care issues, the clinical judgment is an important factor, and the clinician should make the decision based on the patient condition and available evidence. Social support includes the provision of nutritious food supplements for in-patients, education of children during hospitalization and after treatment, and social and economic rehabilitation of those deformed to restore them to position of dignity in society.

48.2.3.1 The Organization of Case Management

On the basis of the three-tier system in Fig. 48.1, case management can also be organized following the same system.

- *Community level:* community health workers can directly supervise patients on treatment, assist in the prevention of disability, report any complications to the nearest health facility, and provide social support.
- *Health center level:* the nurse can administer antibiotic treatment, dress the wound, provide basic prevention of disability, and offer social and mental health support.
- *District hospital level*: at this level often with availability of doctors and operating theaters, antibiotic administration, surgery (mainly debridement and skin grafting), wound care, prevention of disability, and social and mental health support can be provided.
- *Regional and tertiary levels:* mainly for the management of complicated referred cases where plastic and reconstructive surgery may be offered.

48.2.4 Pillar 4: Supervision, Monitoring, and Evaluation of Control Activities

The successful implementation of the BU control strategy requires very close supportive supervision and monitoring. Data should be analyzed at local and national levels to monitor trends and guide any adjustments in the implementation of activities. Data reporting should follow the three-tier system, and national programs will have to report annually to WHO. Mainly, it is important to [8]:

- Monitor trends of the disease at national and local levels; and reporting surveillance data to WHO and other partners.
- Monitor the quality of patient care, sequelae, and long-term treatment outcomes at the district level.
- Evaluate the impact of control program activities, such as training and early detection.
- Organize regular meetings (quarterly or 6-monthly) with all local actors to review progress of implementation of activities.
- Organize an evaluation of the program by an external team at least once every 5 years.

The BU control indicators are very important for proper monitoring of control.

Advocacy, social mobilization, partnerships, and operational research should be promoted at all levels to support the strategy. To share experiences with the world community, publication of data and experiences is encouraged.

48.3 The BU Control Indicators

The BU key indicators and targets used to monitor program performance are presented in Table 48.2.

Table 48.2 The BU key indicators

Indicator	2020
% new Buruli ulcer cases confirmed by a WHO-recommended method[a]	>70%
% new Buruli ulcer cases under 15 years[b]	*~50%*
% female among new Buruli ulcer cases[b]	*~52%*
% new Buruli ulcer cases with ulcerative lesions[a]	<80%
% new Buruli ulcer cases with limitation of movement[a]	<18%
% new Buruli ulcer cases with lesions on lower limbs[b]	*~60%*
% new Buruli ulcer cases in category III[a]	<22%
% new Buruli ulcer cases who completed antibiotic treatment[a]	100%

[a]Mandatory indicators
[b]Additional indicators

Fig. 48.3 Awareness session on BU clinical manifestation in an endemic village of Songololo, Democratic Republic of Congo. (Credit: D.M. Phanzu)

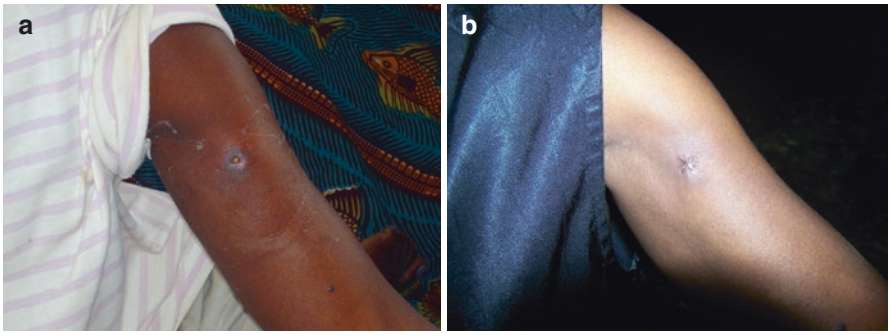

Fig. 48.4 (**a**) Early ulcerated BU lesion and (**b**) healed lesion after antibiotic treatment alone. (Credit: D.M. Phanzu)

48.4 Integrated Approach to Skin NTDs

Since 2016, WHO has been promoting the integrated approach to the control of skin NTDs [15]. This is also reflected in the WHO-NTD-Roadmap 2021–2030 which focuses on the shift from disease-specific to integrated platforms to deal with multiple diseases at the same time [16]. The rationale for the skin NTDs approach includes the co-endemicity of certain diseases, the similarity of the clinical signs of BU and other skin NTDs, as well as the scarcity of resources called for an integrated management of skin NTDs. Two resolutions were adopted to support the integration (WHA 66.12 AFR/RC63. R6). Integration experiences have been made in several countries such as Benin [17, 18]. Integration with other programs and sectors is encouraged at all levels to maximize the reach and impact of control interventions (Figs. 48.3 and 48.4).

48.5 Conclusion

BU control has evolved over the past 20 years with the development of oral antibiotics and activities to improve early detection of cases. The mode of transmission is still unknown, so there is limited measure to prevent the acquisition of the disease. However, the integration of BU activities within the district healthcare system and with other NTDs particularly the skin NTDs, decentralization of care to peripheral health facilities, and the availability of mobile phone educational platforms provide opportunities to further enhance BU control to achieve greater coverage. Late detection of cases still poses a problem in some countries, and efforts should be made to reduce this through the implementation of innovative control activities.

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