**International Journal of Dermatology Sciences**
**Online ISSN: 2664-9780, Print ISSN: 2664-9772**
**Received: 26-01-2020; Accepted: 27-02-2020; Published: 25-03-2020**
**www.dermatologyjournal.net**
**Volume 2; Issue 1; 2020; Page No. 08-21**

# Leprosy: A systematic review

**Mariana Evangelista Gracino[1], Bruna Mendes dos Santos[2], Mariana Verza Garbelini[3], Vanessa Nishi[4], Débora Silva[5], Rogério Nabor Kondo[6]**

[1-5] Medical Doctor graduated from Unicesumar, Maringá, Paraná, Brazil

[6] Assistant Professor of Dermatology Discipline of the Medical School at State University of Londrina, Paraná, Brazil

**Abstract**
Leprosy is a contagious infectious disease caused by the bacillus *Mycobacterium leprae*. Although it is curable with therapy, it is still highly prevalent in underdeveloped areas. This systematic review sought to contemplate the innumerable variables involved in the disease's persistence within certain populations, as in Brazil, despite eradication efforts. Variables verified in its pathogenesis include human leukocyte antigen, toll-like receptor, polymorphism, antibodies, cytokines and cellular immune response. Leprosy reactions are also under the influence of elements that comprise the immune system. On the other hand, lesions emerge due to tropism from the etiological agent through peripheral nerves by means of the linkage of phenolic glycolipid 1 to Schwann cells. The clinical condition of this pathology is related with the host immune condition, originating seven clinical forms that are classified according to clinical, histo pathological, bacteriological, and immune conditions. The diagnosis is based on clinical symptoms and on micro scopical detection of acid-resistant bacilli in skin smears. A multidrug therapy is used as treatment according to the number of lesions the patient presents. Prophylaxis and control depend on early diagnosis and active search of persons under risk of acquiring the disease. Many studies have been carried out but there is much to be explored in researches for the elucidation of the disease's chain of transmission, etiopatho genesis, diagnosis, and treatment.

**Keywords:** mycobacterium leprae; leprosy, lepromatous; leprosy, tuberculoid; leprosy, multibacillary; leprosy, paucibacillary

## 1. Introduction

Leprosy or Hansen's disease is a chronic infectious disease caused by the bacillus *Mycobacterium leprae*, transmitted by direct and long-lasting contact with previously untreated multibacillary patients [1]. In the history of humanity, probably no disease has generated such a strong social stigma as leprosy, always associated with concepts as sin, impurity and punishment [2].

Despite eradication efforts, Brazil is the second country in the world in number of leprosy cases, behind India.[3]Approximately 94% of known cases in the Americas and 94% of registered newly diagnosed cases are in Brazil, with the largest incidence in the North-Eastern, Northern and West-Eastern regions. During the past decades, prevalence rates have presented a yearly decline as a result of the consolidation of polychemotherapy treatment [1]. It is important to note the social determinants of health such as population density, habits in everyday life, and housing and sanitary conditions, which are related with poverty.

There are several clinical forms and histological types that when well documented may help prevention in contacts and early diagnosis in affected patients, thus directing them to appropriate treatment [3]. The disease is characterized by its wide spectrum of clinical manifestations, with stable and opposed poles in its extremes, which are intercalated by unstable forms that may present clinical and immune aspects of both poles, depending on the host's immune response [4]. The predilection for the skin and peripheral nerves gives the disease singular characteristics that makes its diagnosis Simple in most cases. On the other hand, the neurological damage is responsible for possible sequelae [1].

Leprosy is a neglected disease, thus it is necessary to recognize its clinical presentations for an early diagnosis, appropriate treatment and interruption of the chain of transmission [5]. Though a leprosy control program has been implemented in 2004 in Brazil, the disease is persistent and prevalent, which demonstrates the importance of carrying out researches on the theme [6].

## 2. Methodology

A systematic review has been conducted based on the PRISMA method (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), with the synthesis of evidences and critical interpretation of relevant available researches on leprosy.

The criteria of inclusion of the sample according to the type of study were control-case, cohort, and clinical assay, found in the form of scientific article freely and fully available. The electronic database analyzed was the Virtual Health Library (VHL) using the descriptor "Leprosy" and limiting the analysis to articles published in English, Spanish, and Portuguese between 2012 and 2017.

The selection of articles was made from the evaluation of titles and summaries and was independently carried out by researchers, obeying the criteria of inclusion and exclusion. The articles identified according to the initial search strategy were fully evaluated and those approved by the researchers were included in the study. This review has analyzed 75 articles, selected as shown in figure 1.

Relevant data found in the selected articles were then registered on one bibliographic cataloguing form for each study, with the following data: title, author, year of

publication, language, journal and country of publication, type of research, sample, and relevant outcomes. After completion of the above-mentioned stages, the compilation of the main data on the subject was carried out, with the organization of the outcomes in accordance with the discussion of the scientific article proposed in this project.

## 3. Results and Discussion

Graph 1 shows the number of studies published on VHL database according to year of publication in the period 2012 and 2017.

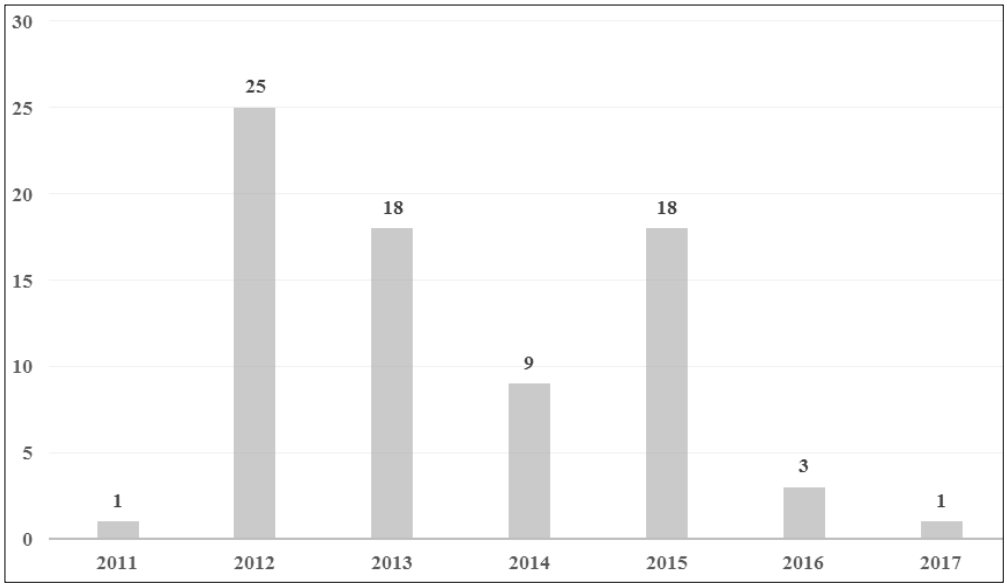


**Fig 1**

With the identification of the selected articles it was possible to subdivide them into 5 categories: epidemiology with 5 (7%) articles; etiopathogenesis 45 (60%); clinical condition 6 (8%); diagnosis 12 (16%); and treatment 7 (9%) studies (graph 2). The greater number of articles on the etiopathogenesis theme is justified by the fact that current studies seek to identify immunogenetic factors related with leprosy, since the criteria for clinical condition, diagnosis, and treatment have already been historically established and accepted.

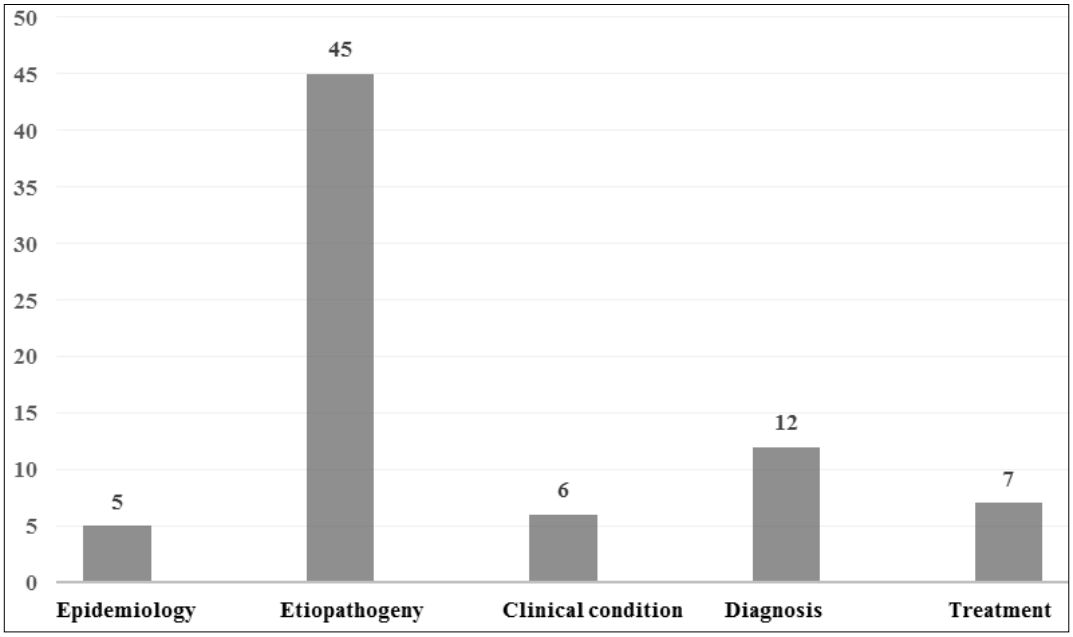


**Fig 2**

Among the selected articles, 72 have been published in English, representing 96%, as shown in graph 3. The countries with higher numbers of published articles on leprosy are Brazil (32%) and the United States of America (31.6%). Brazil is the country with the highest number of studies, with 40 (53.3%), followed by India, with 24 (18.6%); the reason is that these are the countries with the highest prevalence of cases of the disease.

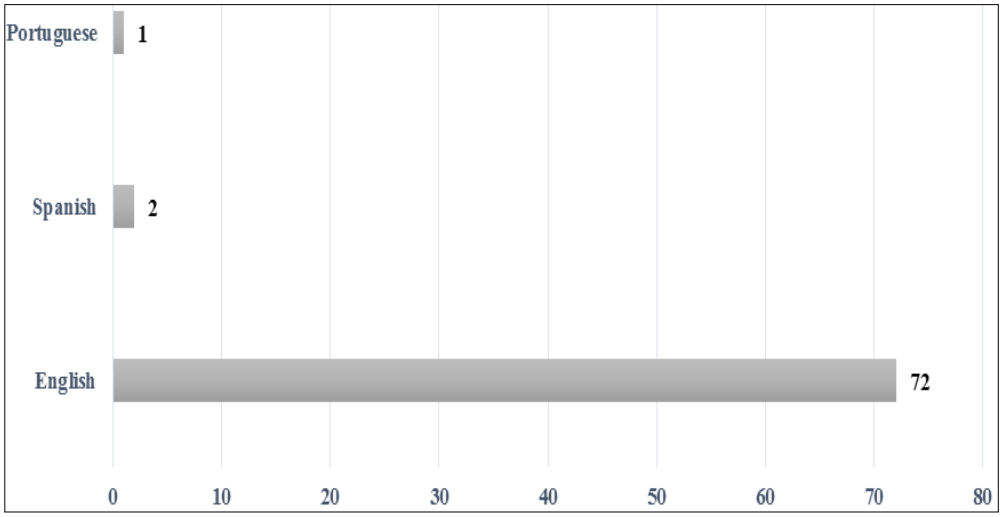


**Fig 3**

The discussion on leprosy is presented next, beginning with the epidemiology of the disease and following with the other thematic axes: etiopathogenesis, clinical condition, diagnosis, and treatment.

### 3.1 Epidemiology

Leprosy is a highly prevalent disease in undeveloped areas, with the largest numbers of cases found in countries of Africa, Southeastern Asia and Latin America [7, 8, 9, 10, 11]. Brazil is one of the countries where leprosy is endemic. The prevalence of the disease has been reduced in 65% in the period 2002-2012, but endemic areas still exist [7, 12, 13].

In the past 20 years, since the use of poly chemotherapy, 14 million individuals have been cured, with the reduction of approximately 90% of the global prevalence of leprosy. However, in 2015 nearly 212,000 new cases of the disease have been reported [7, 14].

The incubation period of leprosy varies from 2 to 5 years and this long timespan hampers the understanding of transmission factors of *M. leprae*, because it is difficult to correlate the circumstances at the moment of the infection and the onset of symptoms years later [15].

Despite this difficulty, the infection from *M. leprae* has been associated with poverty. Assay son the PCR genetic sequencing of this microorganism from nasal cavity smears show greater positivity in individuals with worse socioeconomic condition living in population agglomeration than in those with better economic conditions.[7] Poverty is generally also associated with low per capita income and therefore reduced expenses with food, lower food diversity index and lower body mass index. These factors favor a higher risk of developing leprosy, since the low ingestion of macro and micronutrients lead to an impaired immune system, thus a lower protection against infections [15].

When analyzing the profile of patients with leprosy it is observed that there is a higher incidence among men than women. However, there is a greater number of spontaneous diagnoses performed in women because they seek health care more often and care more about their image, thus in female patients there is an earlier diagnosis. Other data that corroborate the relationship between poverty and leprosy are that the majority of patients have completed at the most primary education and among patients that are employed over 60% receive between 1 and 3 minimum salaries [16, 17].

The delay of several years in having the diagnosis of leprosy is related to the high percentage of individuals who have no knowledge of the disease or think that it is not serious; it is also due to the failure in the health system for not training health professionals for leprosy early diagnosis and treatment. These data demonstrate the need of health promotion measures for the population to increase the level of information on leprosy [16].

Infection from *M. leprae* is commonly found in individuals in the age group between 16 and 60 years, i.e., economically active population [18]. However, there is also a high incidence in children younger than 15 years old. As the disease is endemic in the Northern State of Pará and in the entire Brazilian Amazon region, leprosy cases in this age group is due to active transmission foci within the community. A study conducted with 1592 school children in Pará revealed that 4% presented leprosy and 71.4% of the new cases were paucibacillary leprosy; also, that 50% of the children were seropositive for anti-phenolic glycolipid-1 (PGL-1), i.e., they had the subclinical infection. The prevalence of seropositivity for anti-PGL-1 was higher in the urban area than in the rural area because of poorer conditions in population agglomerates and lower delivery of food in families in the urban area. The high levels of anti-PGL-1 were also associated with children from public school, low income families, families that receive governmental financial assistance, lack of family health care, and having previous contact with at least one person with leprosy. These factors reinforce that leprosy is related with poverty and data reveal the need of active search in endemic regions to identify initial cases without physical disabilities and seek to break the cycle of disease transmission within communities [19].

Migration is another determinant risk factor for *M. leprae* transmission. Migration up to 5 years before the diagnosis of leprosy is observed in a high percentage of patients. The risk of infection rises when migration occurs to endemic regions, when patients present high family density, low education level and reduced family income, as previously discussed. Alcohol consumption during migration is a significant behavioral factor related with the increase of the susceptibility to leprosy transmission among migrants, because even when the individual has been using poly chemotherapy, alcohol consumption reduces medication efficacy, thus raising the risk of infection to contacts [17].

The risk of transmission is related not only to socio

Economical factors; environmental factors can also be related with the infection. Leprosy bacilli survive during several months in adverse environmental conditions. Factors that favor this tolerance are warm and humid climate, humid soil, and water. The exposition to *M. leprae* is thus higher in rainy periods when the climate is more humid and there is the production of nasal secretion, which when dispersed through cough or sneeze from an infected person can disseminate the bacilli through respiratory droplets or dust particles, acting as infection source when falling on the soil or in the water [20].

### 3.2 Etiopathogeny

#### 3.2.1 Genetics

Two characteristics of *M. leprae* explain the strong effects of the host's genetics in the development of the disease, facilitating the genetic analysis of leprosy: the bacillus has a low genetic variability associated with are ducing evolution, thus becoming highly adapted to a specific ecological niche, determining a small variability of clinical responses of the host to the infection [21, 22].

Among the Chinese population, the number of mitochondrial DNA copies in patients with leprosy is higher than the healthy controls, being significant in the MB form (multibacillary, lepromatous), which may correlate with energetic supply favorable for the multiplication of bacilli, as well as the secretion of toxins and oxidative stress [23].

#### 3.2.2 HLA

HLA-A28 and DQB1.06 were observed more frequently in Mexican patients with leprosy; in contrast, frequencies of DQB1.07 were higher in the controls. In total, the findings suggested that HLA-A28 and DQB1.06 are genetic markers for the susceptibility to leprosy and are associated with lepromatous and borderline subtypes [14].

In Brazil, an increased frequency of HLA-B07 in patients with borderline leprosy (BB, BT and BL) was observed. HLA-B53 and HLA-C16were also more frequent in patients with B leprosy than in healthy controls. A reduced frequency of HLA-B49, HLA-B50, HLA-C05, and HLA-DRB1.07was found in patients with leprosy. HLA-C05 and HLA-DRB1.07 suggest a protection against borderline leprosy [24].

The frequency of HLA alleles in patients with leprosy reactions was analyzed only in the group of patients that presented reverse reaction (RR) because only a small number of patients presented erythema nodosum leprosum (ENL). In this analysis, patients who presented reverse reactions were compared with patients without leprosy reactions: HLA-B15 was positively correlated with reverse reactions [24].

The balance between Th1 e Th2 responses suffers control from HLA class II (DRB1), which comprises loci of highly polymorphic genes, with HLA-DR2 and DR3 being associated with TT and HLA-DQ1 with LL [13, 25].

Both in Brazilians and Vietnamese populations, HLA-DRB1.10 is associated with susceptibility and HLA-DRB1.04 with resistance to leprosy. In Argentina, HLA-DRB1.04 has also been highlighted as a protector. In Brazil, allele DRB1.08 is a risk factor. In China, susceptibility has been associated with HLA-DRB1.15 and protection with HLA-DRB1.09 [13, 25].

Brazilian miscegenation may have caused a profile of susceptibility to the disease: 13 (1.33%) among the 975 alleles of risk have been detected. In Brazilians the HLA-DRB1.16 is twice as frequent in LL and HLA-DRB1.14 is three times in TT; the first one has also been described in Suriname, Venezuela, Egypt, China, Japan, Korea, Taiwan, Mexico, and Turkey [13].

The major histocompatibility complex class I gene A (MICA) is highly polymorphic; among Brazilians MICA027 is a protection factor against leprosy and MICA010 against MB form. In China the protection alleles against MB form are HLA-B46 and MICA-A5. Among Indians HLA-DRB1 and MICA-A5.1 alleles are associated with the disease [25].

The KIR activators and inhibitors genes in the presence of their correspondent HLA ligands may have some effect in the development of leprosy and its clinical forms, in the presence of activator genes of TT group. The balance between activator and inhibitory genes may interfere in the progress of the disease in lighter or more aggressive forms or even make it vary between two poles with undetermined forms, highlighting the role of natural killer (NK) cells in the immunopathology of the disease [12].

#### 3.2.3 Toll-like receptor

The Toll-interacting protein (TOLLPI) is an adaptor protein acting as an inhibitory factor that in humans is encoded by the TOLLIP gene. It has been discovered that the TOLLIP expression in Nepal was strongly correlated with IL-1RN (IL-1Ra) and IL-18. The expression TOLLIP was not associated with IL1B or any other gene transcriptions that were present in independent clusters. The expressions of TOLLIP and IL-1Ra are highly correlated in leprosy skin lesions. Three polymorphisms in the TOLLIP gene were associated with susceptibility to leprosy (rs5743942, rs3793964 and rs3829223) in Nepal [26].

It was found that IL-1Ra was highly induced by irradiated *M. leprae* (iMLep) under regulation of TOLLIP, being this induction partially selective because iMLep did not induce several other pro-inflammatory cytokines, including IL-1β, IL-6 and TNF. Finally, the findings suggest that TOLLIP can regulate the first steps in the pathogenesis of leprosy, while other paths regulate polarization [26].

The association of TLRs with susceptibility to leprosy was verified in Indian and African populations, contrary to the observed in Chinese populations [11].

In India it was observed that lower levels of IL-10 in individuals with 'S' allele and high expression of mRNA TLR2 can provide resistance. On the other hand, higher levels of IL-10 in individuals with 'L' allele and low expression of mRNA TLR2 can induce susceptibility [27].

In Brazil, it was verified that *TLR1* SNP N248S contributes to susceptibility to leprosy. It also conducts immune responses to the stimulus of bacillus Calmette-Guerin, which is crucial to understand how to personalize BCG vaccine for hypo-receptor individuals [28].

#### 3.2.4 Polymorphisms

Regarding (GT) n polymorphism, the frequency of allele 2 in patients with leprosy was higher than the control-group, contrary to allele 3. The analysis of 274C/T polymorphism revealed the increase in the frequency of 274T allele and the T/T genotype among Brazilian leprosy patients. Similarly, a higher frequency of 469+14C allele of 469+14G/C polymorphism occurred in patients with leprosy. Regarding 577-18G/A and 1029C/T polymorphisms, all participants presented 'GG' and 'CC' genotypes, respectively [29].

It was revealed that microsatellite (GT) n promoter and 274C/T e 469+14G/C polymorphisms of *Slc11a1 /Nramp1*gene are associated with leprosy. Furthermore, it was also found that two other nucleotide alterations in exon 3 (274C/T) and intron 4 (469+14G/C) of *Slc11a1/Nramp1* gene are also associated. Patients with leprosy have 274T allele and TT homozygosis of274C/T polymorphism and 469+14C allele of 469 + 14G/C polymorphism more frequently than the controls [29].

Among the Chinese, two SNPs (rs7624750 and rs414237) showed an association with leprosy. SNP rs414237 showed an association with LL subtype. SNP rs9838374 showed an association with MB, mostly in LL patients. OPA1 mRNA expression is higher in tissues with high energy consumption. The tissue related to leprosy (skin) has a medium level of OPA1 mRNA expression. Notably, the leprosy risk allele − rs414237 – was correlated with a lower level of OPA1. The closest indirect interaction node with OPA1 was NDUFB10 (NADH dehydrogenase-1beta subcomplex), which plays the role of producing mitochondrial energy. In accordance with the reduced level of OPA1 mRNA expression in patients with leprosy, the NDUFB10 mRNA expression was also negatively regulated in cells infected with M. leprae [30].

In India, the frequency of ff genotype in Fok I position was significantly high in PB patients in comparison with healthy controls. In the same way, in Apa I position, the frequency of genotype AA, Aa and A allele was significantly high in MB patients in relation to healthy controls. The frequency of TFa haplotype, Tfa was significantly high in leprosy, suggesting a positive association, and the frequency of TFA was significantly low, suggesting a negative association [31].

The VDR nuclear hormone receptor mediates many immuno modulator effects of vitamin D active form. Polymorphisms in three loci Taq I, Fok I and Apa I in VDR gene can affect mRNA stability, leading to altered levels of protein and unbalance of cytokines related with Th1 and Th2, which is crucial to determine the clinical results of leprosy. Whereas in India Taq I polymorphism is not associated with leprosy, the contrary was observed in the Mexican population and among Indian Bengalis [31].

In the Chinese population, GG genotype of rs1873613 had a lower frequency in PB group in comparison with the control group. Among the six single nucleotide polymorphisms (SNPs), three seemed to play a protector role against leprosy (rs3761863, GG genotype; rs732374, AA genotype; and rs7298930, CC genotype) and in PB group (rs3761863, GG genotype; rs732374, AA genotype; and rs7298930, CC genotype). However, SNP rs1427267 conferred a risk of leprosy (AA genotype; AG genotype), MB group (AG genotype) and PB group (AA genotype). The AGACA haplotype represented a risk of leprosy per se and for BB group, whereas AGGCA had a significant protector effect against leprosy [32].

In a Chinese study, human genome analysis identified 11 loci of susceptibility. The majority of susceptibility genes encodes proteins involved in immunity, whereas one locus (RAB32 at6q24.3) suggests a role for auto-phagocytose in the pathogenesis of leprosy [33].

In Brazil, it was verified that genes associated to innate immune response, such as TLR1, NOD2 and PARK2, or adaptative immune responses, such as IL10, IFNG and LTA/TNF/HLA were associated with leprosy [34].

In a Brazilian study there was evidence of an association between polymorphisms in *ERBB2* gene of 17q11-q22 chromosome in the Northern state of Pará. However, this association was not replicated in the Northeastern state of Rio Grande do Norte [35].

An Indian study demonstrated that TT genotype of IL-17F 7488T/C polymorphism is associated with susceptibility to leprosy, especially the tuberculoid form, contrary to TC genotype, which confers a reduction of the risk of contracting the disease [10].

In India, PARK2 and ubiquitin ligase were simultaneously involved in the innate immune response that mediates cytokine production, such as IL6, interconnects molecules through a TLR receptor signaling pathway [36].

Another Indian study detected that in TLR4 G896A position, GA genotype may be a protector, while AA genotype represents susceptibility to PB leprosy [37].

There is evidence that MBL2 promoter polymorphisms have been strongly associated with leprosy among the Chinese population. The genetic variants of FCN2, MBL2 and CFH genes of lectin pathway and alternative pathway confer genetic susceptibility to leprosy [38].

An Indian study demonstrated that the transporter associated with antigen processing (TAP) type 1 is associated with sickening, whereas type 2 is not. The HLA class I A1102-B4006-Cw1502 is associated with susceptibility to leprosy in the Indian population. Among class II genes, DRB1.15 e DRB1.16 are associated with susceptibility to leprosy in India, Thailand and Brazil. DRB1.15 has also been associated in China [39].

In studies in Vietnam and Brazil, two single nucleotide polymorphisms (SNPs) in PARK2 e PACRG promoter regions demonstrated association with leprosy: rs9356058 and rs1040079. Among Vietnamese, there is evidence of 56 SNPs in PARK2 e PACRG promoter regions associated with leprosy, whereas among Indians 24 SNPs have been found. Following multivariate analysis only four (rs1333955, rs7744433, rs2023004 and rs6936895) were distinguished among Vietnamese and three (rs1333955, rs9356058 and rs2023004) among Indians. When correlating the analyses between the groups, only two SNPs were established: rs1333955 and rs2023004. Allele T of SNP rs9356058 was consolidated in PARK2 e PACRG promoter regions as an independent risk factor to leprosy in Vietnam and India, as it was observed in a Brazilian study [21].

When stratifying cases based on the age at diagnosis, the outcome was that among Vietnamese the average age was 19.4 years and among Indians it was 33.3 years. Additionally, it was demonstrated that SNPs rs1040079 and rs7451965 were risk factors to the early onset (before 16 years of age) of leprosy in Vietnam; this was not found among Indians, among whom only SNP rs9365460 was significantly associated with the early onset of the disease (before 25 years of age) [21].

Susceptibility to leprosy is related with single nucleotide polymorphisms (SNPs) in IL12B e IL12RB2 genes. Copy-number variations (CNV) polymorphisms involved in contracting leprosy are IL10, IL23R and IL12RB2 genes [40].

In India six SNPs were associated with leprosy: rs2853694, rs2853697, rs3181216, rs3181225, rs1003199 and rs2569253. The rs2853694 was strongly associated with the MB form and with a dominant allele for the development of the disease [40].

The analysis of haplotypes of four SNPs of IL12B

(rs2853694, rs2853697, rs3181216 and rs3181225) demonstrated the CAAC haplotype as protective and ACTT haplotype as a risk to leprosy. There is interaction with risk increase of these SNPs with loci of TNF (rs1800629, rs1800610 and rs769178) and of BTNL2-DRA (rs3135365 and rs7773756). There is also protective association with BAT1 (rs2523504), NFKBIL1 (rs2230365) and LTA (rs13192469 and rs36221459). Regarding CNV polymorphisms, the outcome was a higher number of copies from IL23R gene among PB [40].

Polymorphic variants of TNFa, IFNg, IL10, IL6 and TGFb1 cytokines have implication in leprosy. Fifty per cent of IL10 production is genetically influenced and is higher among Africans than among Caucasians and Asians. In Colombia the genotypes associated with leprosy were CC and CT in SNP -819, CC and CA in SNP -592; and haplotypes were: -819C-519C and -1082A-819C-592C both in heterozygosis and homozygosis. Haplotype -1082A-819C-592C in homozygosis was associated with a low production of IL10 [41].

Among Indians, haplotype -1082A-819C-592C conferred resistance to leprosy and haplotype -1082A-819T-592A conferred susceptibility. Among Brazilians, -819T allele was associated with the development of the disease due to the reduction of IL10 production. Another study in Brazil associated resistance to leprosy with haplotypes 3575A / -2849G / -2763C / -1082G / -819C, and susceptibility with -3575T / -2849A / -2763C / -1082G / -819T [41].

Among Brazilians and Hispanics, genotype+874 A/A was associated with a lower expression of IFNg, with A allele common in the Brazilian population, associated with the high rates of leprosy in the country. In contrast, another Brazilian study evidenced that homozygotes for A allele of +874 gene and carriers of 16CA microsatellites repeats, generally paucibacillary, present greater control of bacilli propagation. In these individuals, the levels of TCD4+ and TCD69+are higher, culminating in higher IFNg production, with a 23% resistance. However, the levels of IFNg production by NK cells are reduced [42].

### 3.2.5 Immunology

In Brazilians, seropositivity of antibodies against LID-1, NDO-LID, NDO-HSA and PGL-1 was higher in MB patients and lower in PB patients (BT and TT forms). Thus, it indicates that the ELISA index (EI) raises with the bacterial index (BI) and in the entire spectrum of the disease [43].

In Brazilians affected by lepromatous leprosy, antigens of *M. leprae* induce a low lymphoproliferative response, but high levels of T regulatory lymphocytes (Treg), such as CD4+, CD25+ e Foxp3+, propitiating the multiplication of the pathogen in an uncontrolled and persistent way, leading to chronic inflammation [44].

Leptin acts in the cell immune response (Th1) in a protective form against intracellular pathogens, with a tendency to lower levels in MB forms and higher levels in PB forms [45].

### 3.2.6 Cytokines

In Brazil it was verified that the differentiation of monocytes (MOs) in dendritic cells (DCs) is not hampered, regardless of the clinical form of leprosy. *M. leprae* induces the maturation of MO-DCs and increases the levels of HLA-DR expression and co-stimulant molecules. It was demonstrated that the sonicated antigen of *M. leprae* is a weak inducer of DC and MO activation in LL patients, which can contribute to the anergy in the cell-mediated immune response [46].

Mutations occurred more frequently in the IL-10 promoter gene than in TNF-a. In India, a higher frequency of 819T allele of IL10was observed, with CC genotype lower and TT higher in leprosy patients. In the case of TNF-a, a higher frequency of 1082GG was detected in patients with leprosy. It was verified that the allele frequency of TNF-a 308 G raised in patients with leprosy, whereas 308 A decreased. High levels of TNF-a, IL-10 and IL-1R1 were observed in leprosy patients. The level of TNF-a was higher in PB leprosy than in MB. A significantly higher level of IL-10 and IL-1R1 was found in MB patients in comparison with PB patients. The TT genotype of IL-10 and the CC genotype of IL-1R1 were found as the largest cytokine producers, thus they are more susceptible to the development of leprosy [47]. In Egypt, it was verified that leprosy is associated with a defect in IL-17 secretion and an overproduction of IL-10 and TGF-β, thus detrimental to the host's defense.[48]Another study revealed high IL-4 in patients with leprosy, being significantly higher among LL, associated with low levels of IFN-γ [49]. In Brazilian patients with leprosy, TNF-α cytokine was detected in skin lesions in the entire clinical spectrum of the disease, being crucial for the development of anti-mycobacterial immunity. Biological activities induced by TNF-α are mediated by two structurally related receptors, but functionally distinct: TNF-R1 and TNF-R2. It was demonstrated that molecules in this inflammatory pathway, for example, TNF-αand sTNFR-2, are markers for the diagnosis and treatment of leprosy. However, they are involved in various inflammatory conditions, thus hampering its specificity [50].

In Brazil, the relation between reactional episodes and infectious processes, such as chronic oral infections, has been analyzed. There was low quantity of IL-2 in patients with leprosy, more accentuated in patients with oral co-infection, suggesting that the infectious process from *M. leprae* may reduce IL-2 response. Oral co-infection may increase pro-inflammatory response mediated by IFN-γ [51].

IL17F is a mediator of retarded hypersensitivity reactions (IV or Th1 type), which is the case of T1R, stimulating the secretion of IL12 and IFNg, that recruit monocytes and neutrophils to destroy pathogens, causing skin lesion. Moreover, this interleukin inhibits Th2 response. A Brazilian study identified that individuals with *M. leprae* in the macrophages and Schwann cells of PB lesions are more prone to experience T1R [52].

The levels of IL17F analyzed in an Indian study are higher in BV and BT (unstable forms) and also in T1R. These factors are explained from the fact that in these clinical conditions there is an increase in Th1 response. The T2R group presented much higher levels of cytokine, demonstrating its influence in this type of reaction. The levels of this interleukin are inversely proportional to BI, with higher levels in the group with BI between 0.1 and 3, indicating that the bacillary charge increase leads to a change in Th1 to Th2 response, inhibiting the production of IL17F [52].

IL6 is the cytokine with the highest concentration in Brazilian patients with T1R and T2R. Its higher levels are associated with LL, linked to T2R occurrence. In non-reactional leprosy, IL6 was detected only in patients with

TT or BT [22].

T1R or reverse reaction is due to the liberation of antigens from bacillary destruction induced by cell response, whereas T2R or erythema nodosum leprosum occurs due to the deposition of immuno complexes from the systemic inflammatory reaction with TNFa liberation [22, 45].

On the TT pole, the strong resistance presented by the cell immune response (Th1 type) by means of TCD4+ lymphocytes infiltration and the liberation of inflammatory cytokines (IFNg, IL2, IL7, IL12, IL15 and IL18) limits the growth and multiplication of *M. leprae* in the granulomatous areas of the skin and the nerves, determining low or null quantity of bacilli (PB), but can degenerated into a severe and rapidly progressing nervous lesion. On the LL pole, immune response barriers are crossed by bacilli excess (MB), since the humoral immune response (Th2 type) unleashes an harmful immunosuppressive response to the host, characterized by a specific anergy to the antigen. In BT, BB and BV spectra there is also humoral immune response, with high bacilli charge (MB) [13, 25, 35, 36, 44, 52, 53, 54]. IL23 is essential to the combat against infection from *M. leprae*. In opposition, IL23 generalized expression causes a severe multiorgan inflammatory syndrome and high expression of TNF and IL1, suggesting direct and indirect effects on T and NK cells. MB form is associated with the lower expression of IL12B gene that reduces IL12 and 23 secretion. In this form of the disease there is higher IL4 concentration that induces the activation of Th2 lymphocytes [25, 40, 42, 45].

Hepcidin is produced by hepatocytes in response to inflammation, causing the accumulation of intracellular iron through ferroportin destruction. Since iron is a substrate for the bacilli multiplication, hepcidin is harmful in *M. leprae* infection. High levels of hepcidin are found in the urine and in skin lesions of MB, having a positive correlation with the increase of IL1b and BI, whereas in PB there is a higher concentration of ferroportin. The SLC11A1 gene is associated with the resistance to intracellular pathogenic Agents, such as *Mycobacterium*, since it influences iron content and pathogen survival within macrophages [54].

An estimate of immuno marker of cells with anti-Slc11a1 or anti-iNOS antibodies revealed that both were detected in all biopsies of Mexican patients with leprosy. The number of foamy macrophages marked with Slc11a1 was very high in most LL samples. However, Slc11a1 immuno marker was predominantly moderate and low in LT samples. The immuno marker of iNOS also followed a similar pattern. There was a direct relation between the intensity of Slc11a1 and iNOS immuno marker and the type of leprosy [55].

### 3.2.7 Costimulatory molecules

In a Brazilian study, in patients with TT and contacts 80% of monocytes (CD14+) express CD86 and in patients with LL only 50% express it, which probably contributes to anergy in this spectrum. The Treg cells, IL10 and the presence of the bacillus in monocytes reduce the CD86 expression, explaining the deficiency of this molecule in patients with LL, who present a higher number of Treg cells, IL10 and bacilli. In consonance, there is reduced expression of CD28 and CD86 in TCD3+ cells in patients with LL. In TT the levels of CD28 are higher, indicating the exacerbated cellular immune response [53].

Brazilian patients with TT presented increased expression of CD152 e PD1 negative signaling molecules in TCD3+ cells and patients with LL had an increase only of PD1 and this proves the different modulation of the immune response; CD152 acts in the inductive phase and PD1 maintains the anergic state and inhibits the effector T cells. As both clinical forms express PD1, this molecule is a marker of the disease [53].

### 3.2.8 Leprosy reactions

The reverse reaction (RR) is due to the liberation of antigens from bacillary destruction induced by cellular response, whereas the erythema nodosum leprosum (ENL) results from the deposition of immuno complexes from the systemic inflammatory reaction with the liberation of TNFa [22, 45].

It was observed in Brazilians with RR the increased transcription of CXCL10, transglutaminase 2 (TGM2) and C-type lectin family of pattern recognition receptor 7, member A (CLEC7A [dectin-1]), and macrophages receptor with collagen structure (MARCO). There were reduced transcripts of molecules CD79, CD19, CD27 and CD27, and molecules associated with B cells modulators of CCR7 signaling. The analysis of IPA biomarkers identified chemokine (CXC motif) ligand (CXCL) 10 and Fc fragment of IgE, low affinity II (FCER2) as specific of RR. Transcripts that increased in exclusive form in ENL were receptors of C3AR1 and C5AR1 complement; 3 ribo nucleases (RNASE1, RNASE2 and RNASE3) and CCL3L3 and CXCL8. Reduction restricted to ENL included IL10 and cytotoxic T lymphocytes-associated antigen 4 (CTLA4), which can contribute to the inflammatory cascade or reflect a high level of inhibitor protein in patients with LL without ENL. The components of the innate immune response were increased in RR and ENL, including C1q (C1QA, C1QB and C1QC). Notably, both RR and ENL had an increase in the expression of hepcidin antimicrobial peptide (HAMP) and cathelicidin (CAMP). The expression of CAMP is increased by a process mediated by Toll-like receptor 1 and 2, and the polymorphisms of receptor 2 were associated with an increased risk of RR. Defenses were increased in ENL (DEFA1, DEFA1B, DEFA3 and DEFA4) and in RR (DEFA4). Potential biomarkers for RR or ENL were CCL2, CCL3 and SOD2. IFN-γwas identified as a regulator of differential transcription in RR and ENL, which can be a stimulus for the immune reaction cascade [56].

Among the factors of susceptibility to leprosy reactions are variants in Toll-like receptors 1 and 2 (TLR1, TLR2) genes, mycobacteriare cognition mediators during the innate immune response. SNP rs3804099 and an intragenic microsatellite for TLR2 were associated with T1R in an Ethiopian population. SNP rs5743618 of TLR1 was associated with the protection against T1R in Nepal and to leprosy in Indian and Turkish populations. Another TLR1 polymorphism found in patients with T2R is N248S. There is association between T2R and three SNPs in the entire IL6 locus (rs2069832, rs2069840 and rs2069845), as well as the functional variant IL6 rs1800795 [22].

### 3.2.9 Vascularization

In a Brazilian study, micro vessels (CD31+) were identified in TT and BT lesions, both in peripheral and granulomas, with a prevalence in peripheral. Vessels (CD105+) were also detected inside and outside granulomas. The data also show that the involution of skin lesions after treatment results in regression of angiogenesis and in particular the

regression of neovascularization. Neovascularization is present in a greater proportion in the lepromatous spectrum and in reactional states, indicating that anti-angiogenic drugs, including anti-CD105, can be useful in the treatment. Regarding the lymphatic vascular system (LVS), it was demonstrated that there is lymphangiogenesis in the tuberculoid pole, especially in the TT form. Angiogenesis is more closely related with the extension of the inflammatory infiltrate than with the forms of the spectrum or the bacilloscopic index [57].

### 3.2.10 Nervous lesion

Leprosy is a neurologic condition affecting the peripheral nerves, from dermal terminations to nerve trunks, as a clinically mixed neuropathy; as a result, the sensitive nervous fibers, both motor and autonomic, are impaired, leading to altered sensitivity of thermic, pain and tactile stimuli. The nervous lesions occur because *M. leprae* has tropism for the peripheral nerves by means of the binding of glycolipid PGL-1 with Schwann cells [25, 58, 59].

Electroneuromyography was used for the evaluation of bilateral conduction of the ulnar/median nerve in Brazilian patients with leprosy who presented severe impairment (latency, amplitude and speed of conduction) both of the motor and the sensory fibers of the ulnar nerve in one of the limbs, defined as "the most affected hand" (MAH). The findings showed smaller adherence and prehension strength for MAH. The evaluation of corticospinal excitability presented lower motor threshold for the hand flexor muscle also in the less affected hand. The motor threshold of the contralateral hemisphere of MAH was lower than in the ipsilateral hemisphere [60].

The microscopic characteristics of neural lesions include Schwann cells degeneration, loss of the myelin sheath, axonal retraction, and periaxonal fibrosis. The PB forms demonstrate an intense inflammatory infiltrate that leads to tissue destruction and Wallerian degeneration. Without the neurotrophic factors – neural growth factor (NGF), Neurotrophins (NT3 and NT4/5), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) – neural regeneration is impaired because such factors regulate neural development, promote neuroprotection and reduce neural degeneration. In Brazilian LL patients, the deficiency of NGF factor and 75 neurotrophic receptor (p75NTR) in Schwann cells was associated with the early loss of nociception and neuropathic pain. In BB and LL patients, the reduction of NGF, PGP 9.5 and NF-L was observed, suggesting advanced degeneration of nerve terminations [58].

### 3.2.11 Transmission

There is an increasing recognition that soil and water are important vehicles of dissemination of *M. leprae* and may be responsible for the continued transmission of the disease. *M. leprae* can survive in the soil for 40 days. Among the environmental samples collected from areas of residence of Indian patients, samples of soil and water were positive for 16S ribosomal RNA gene [61].

Studies have verified that the progression of the exposition to leprosy is determined in a multifactorial way, causing the installation of the disease in only 1% to 3% of those infected. Therefore, the onset of the disease includes immunological, genetic and nutritional aspects, environmental factors, BCG vaccination and bacillary load [13, 21, 40, 45, 52].

The risk of leprosy from social contact occurs in closed environments, either in extended or short periods, but anyhow frequent. In Bangladesh, the development of leprosy has been associated with intense social contact in the home and in the nearby neighborhood. It was advised that disease control should not be limited to the house holds but include the neighborhood of patients. It was verified that there is a higher risk of infection from *M. leprae* for males due to multiple social contacts. The male/female ratio of newly detected cases for the study area was 1.35, similar to sex ratios observed in other Asian countries. In Africa and South America, the new case detection rate is the same for both sexes [62].

## 3.3 Clinical condition

Leprosy is a chronic infectious disease that affects mainly the skin, mucosa and peripheral nerves. The average period of incubation of *M. leprae* is 2 to 5 years, but it may last 20 years. Clinical manifestations may include patches and nodules on the skin of face and limbs, ulcers on the soles of feet and on the hands, alopecia, madarosis, xerosis, and anhidrosis [13, 45, 52, 53, 54, 62].

The time span between symptoms appearance and diagnosis is directly related to the emergence of physical impairment. Therefore, the longer this interval is, the worse will the neurologic prognosis be. Other factors that are associated to physical incapacity are: age $\geq$ 30 years; MB leprosy; and initial BI $\geq$ 2. On the other hand, female gender and level of education are factors of protection against leprosy [18].

Depending on the host's immune state, the disease can manifest in seven clinical forms, which are classified according to the clinical, histopathologic, bacteriologic and immunologic state. There are three unstable forms between the tuberculoid (TT) and the lepromatous (LL) poles: borderline tuberculoid (BT), borderline (BB), and borderline lepromatous (BL). In addition, there is the pure neuritic form of leprosy (PNL), characterized by neuropathy without skin lesions [22, 45, 52, 53, 54, 63].

The TT spectrum is characterized by a lower number of lesions, while the LL form is disseminated with innumerable lesions. Indeterminate leprosy (I) is characterized by one or several hypochromic macules, emergence of areas with sensitivity disturbance and inducing undifferentiated immune response that can evolve into spontaneous cure or PB and MB forms [45, 54].

Acute inflammatory episodes that may occur before, during or after treatment are named leprosy reactions, which are the main causes of nerve lesion and permanent impairment [22, 45, 52].

Type 1 reaction (T1R) occurs predominantly in borderline cases (BT, BB, and BL) and TT; it is characterized by acute inflammation of preexisting lesions or the emergence of new lesions and/or neuritis. Skin lesions become erythematous and/or edematous and may ulcerate. It may also present malaise, edema on hands, feet and face. Acute neuritis leads to neurologic deficit that requires urgency treatment to avoid the permanent loss of function. Type 2 reaction (T2R) generally occurs within the lepromatous spectrum (LL, BL, and BB), with skin erythematous nodules and papules, diffuse and/or deep. Ulcerated, necrotic, pustular and bullous forms also occur. Some nodules may persist as chronic painful panniculitis, leading to fibrosis and scars. Neuritis and systemic symptoms such as fever

and malaise may also occur [9, 22, 52].

The main characteristics of leprous rhinitis are chronicity, absence of acute phenomena, and incomplete obstruction of nasal mucosa presenting dryness and scab formation. The most reported symptom is nasal obstruction and the less reported is anosmia, followed by epistaxis, nasal secretions and coryza. The most important differential aspects are the location of lesions (inferoanterior part of the septum) and the nasal deformities. Sometimes it is possible to observe endonasal hansenoma. With the progression of the condition, there is often the production of septum perforation and the destruction of the nasal septum cartilage. General manifestations may include lymphadenopathy, anemia or fever. It occurs mainly in the lepromatous (worse and progressive) and tuberculoid types [64].

Oral lesions develop insidiously and in general are asymptomatic and secondary to nasal involvement. Currently, oral lesions are not often observed due to the early diagnosis and use of multidrug therapy (MDT) [65].

In the majority of patients, neural lesions and manifestations precede skin lesions. The most affected cranial nerve is the trigeminal nerve (V), responsible for tactile and thermic sensitivity of the face, at the anterior two-thirds of the tongue and hard and soft palates. The second most affected nerve is the facial nerve (VII), responsible for the innervation of the facial muscles and taste sensitivity at the anterior two-thirds of the tongue [65].

The average pain level in leprosy patients were 50% in the VAPS scale, in a range between 0 (absence of pain) and 10 (most severe pain). The pain may result from chronic inflammation in which there is significant thickening [66].

Reactions and neuritis are the main causes of peripheral nerve damage. Although the main underlying mechanisms and causes of these complications are not well understood, some studies suggest that MB forms of the disease, high bacilloscopic index (BI), vaccination, and emotional stress may be considered as risk factors [67]. There is a strong association between leprosy and HIV infection, especially in PB. During this phase the lesions are not apparent yet and the emergence of clinical signs of *M. leprae* in HIV patients would not represent a manifestation of immunological suppression, but rather an immune reconstitution that follows the occurrence of highly active antiretroviral therapy. A higher occurrence of reactions in leprosy patients without HIV is observed when there is the prevalence of borderline clinical forms since these are immunologically unstable [68].

In individuals with or without viral co-infection, patients with co-infection present higher rates of T1R, T2R or neuritis. It was verified that hepatitis B co-infection was associated with a higher rate of neuritis and damage of nervous function. It was found that HTLV-1 co-infection is associated with a high rate of unfavorable leprosy outcomes, because all patients co-infected with this virus presented complications and acute inflammatory episodes. Therefore, the determination of the serologic status of leprosy patients for hepatitis B and C, HIV and HTLV-1 should be considered as an important standard of care and implemented in areas where these viruses are endemic [67].

The clinical condition of leprosy engenders discrimination and stigma regarding patients. This idea is verified from the fact that over 90% of patients have reported the perception of prejudice against the disease. Moreover, patients report the fear of developing physical disabilities and being socially rejected. The vast majority of patients have low self-esteem and feel depressed or sad. It was also observed that there is a direct relation between patients with leprosy reactions and low quality of life; patients who do not present those reactions have much less complaints about this aspect. Therefore, psychosocial support should be delivered to those individuals and their families. There is also the need of disseminating the information to the entire population to reduce the prejudice that historically affects these individuals [16].

### 3.4 Diagnosis

The diagnosis of leprosy continues to be based on clinical symptoms. An early diagnosis and treatment are crucial to avoid physical impairment and transmission. Diagnosis often depends on microscopic detection of acid-alcohol resistant bacillus (AARB) on skin smear. The combination of different methodologies may increase the success rate of leprosy diagnosis [65, 69, 70].

According to WHO, leprosy patients are classified as paucibacillary (PB) or multibacillary (MB). PB is characterized by the presence of up to five skin lesions and MB over five lesions. The classification of Ridley-Jopling (1996) is based on clinic, bacilloscopic and histologic criteria. The forms of presentation of PB leprosy are indeterminate (I), tuberculoid (TT) or borderline tuberculoid (BT); and MB leprosy presents in the borderline-borderline (BB), borderline-lepromatous (BL) or lepromatous (LL) forms [25, 42].

The disease may be classified according to the bacilloscopic index (BI): MB with index higher than zero; PB index zero. Leprosy is difficult to be diagnosed in its early stage and bacilloscopic sensitivity is considerably low. Specific serologic assays against *M. leprae* antigens do not detect all clinical cases because most patients in the PB stage of infection do not develop significant levels of antibodies [25, 42, 69].

The presence of antibodies against phenolic glycopid-1 (PGL-1) is related with a higher BI in leprosy patients; high titers are found in MB patients and low titers in PB patients. The combination of clinic examination and anti-PGL-1 assay may lead to a significant reduction of errors in the classification of leprosy patients, in comparison with the isolated counting of lesions. Therefore, despite its limited application for the diagnosis of leprosy, the use of an expressanti-PGL-1 assay may be an auxiliary tool for the correct classification of patients, thus facilitating the prescription of the appropriate multichemotherapy and the prevention of transmission [70].

Three endemic areas in Brazil – in the states of Goiás (GO), Mato Grosso (MT) and Pará (PA) – were evaluated for serologic detection of anti-PGL-1 and new *M. leprae* antigens. The ELISA method was used to detect IgG for proteins (92f, 46f, leprosy IDRI diagnostic-1, ML0405, ML1213) and IgM for PGL-I [71].

Patients with MB leprosy presented positive rates for PGL-1, similar to those found for proteins LID-1, ML0405 and 46f; however, some individuals anti-PGL-1-negative were positive for antibodies against proteins of *M. leprae*, suggesting that the addition of protein antigen to the serologic assay that detects PGL-1 may increase the sensitivity for the detection of multibacillary leprosy. Although *M. leprae* fusion proteins 46 f and 92f have homology with proteins of other mycobacterial species, they

were not recognized by most patients with tuberculosis [71].
The development of rapid serologic tests based on proteins to detect specific IgG should complement the PGL-1 test to detect IgM antibodies and this combination should be used in non-specialized environments as initial test [71].
Other rapid diagnosis tests to detect antibodies against *M. Leprae* have been studied in the Philippines: the On Site Leprosy Ab Rapid Test and Leprosy Detect™ fast ELISA test presented high specificity on MB patients (96.4 and 93.7%, respectively) and NDO-LID® RDT presented low specificity (only 25%). Given its high level of specificity, a positive outcome of the On Site Leprosy Ab Rapid Test is virtually conclusive for a MB leprosy diagnosis. However, a negative outcome does not exclude PB patients or the initial stage of MB leprosy [72].
Due to its applicability and speed, the On Site Leprosy Ab Rapid Test can be used as an initial test for diagnosis protocols, with the confirmation of outcomes being reached in a highly quantitative form by the Leprosy Detect™ fast ELISA after serum transfer to a reference laboratory [72].
Recently, several PCR-based molecular techniques have become available for leprosy diagnosis for *M. Leprae* DNA detection [65, 69, 73].
A Chinese study evaluated the repetitive sequence of *M. lepra*-specific (RLEP) PCR. The sensitivity of RLEP Real-Time PCR assay detected up to 8 fg of *M. leprae* DNA or 240 bacteria in infected tissues. RLEP Real-Time PCR was highly specific for the 60 confirmed leprosy diagnosis patients and did not show cross-reactivity with other mycobacterial or bacterial DNA; only one Xanthomata presented cross-reactivity [69].
The rate of RLEP Real-Time PCR detection for the 51 PB samples was 74.5% (38 out of 51). It was verified that the bacilloscopy index was positive in 31.4% (16 out of 51) and conventional histopathology in 52.9% (27 out of 51). The outcomes of the Real-Time PCR assay may be obtained Within two-and-a-half hours. It can be used as a rapid confirmation, sensitive and specific test to identify the presence of *M. leprae* in biopsy samples for the diagnosis of PB leprosy. The performance of the test suggests that it can be useful not only for the early diagnosis of leprosy but also to exclude some skin lesions not caused by leprosy [69].
The PCR-Pra method has shown to be potentially useful for *M. leprae* DNA detection in urine samples as an additional resource for the diagnosis of leprosy, especially in TT form (75% positivity in patients under treatment and untreated patients), where the microscopic detection by skin smear bacilloscopy is normally negative due to the low quantity of bacilli, or in inconclusive cases with negative bacilloscopy. The PCR-Pra should be assessed in a larger numberof patients in endemic and non-endemic regions in order to study its limitations [73].
The outcomes of a Brazilian study show that 15 out of 45 (33.3%) individuals with leprosy presented reduced oral sensitivity, indicating that the evaluation of the oral cavity sensitivity could be useful as a complementary test in the physical examination of suspect cases of leprosy [65].
*M. leprae* DNA has been detected in the saliva of 16 out of 45 leprosy patients. The outcomes of the qPCR method to detect *M. leprae* DNAin the saliva revealed that 10 out of 26 MB patients and 6 out of 19 PB patients were positive. The qPCR made on skin smear revealed that 10 out of 26 MB patients and 3 out of 19 PB patients were positive. The positive outcomes of the saliva qPCR, particularly in PB patients, suggest that the saliva may be considered a new locus to collect samples. Besides allowing DNA detection in MB and PB individuals, it is noteworthy that the saliva collection is less invasive than that of skin smear [65].
This study did not manage to make a linkage between alterations in the sensitivity of the oral cavity and the presence of *M. leprae* in the saliva. However, one third of leprosy patients presented alterations in the oral sensitivity. Thus, additional studies should be carried out to investigate the viability of bacilli in the saliva of PB patients and this should consider a larger number of patients to further understand the physiopathology of leprosy in the oral cavity [65].
Leprosy reactions are one of the main causes of nerve damage related with leprosy. An Indian cohort study showed that these reactions are more frequent than what is clinically evident. Leprosy reactions are often underdiagnosed by clinical doctors; therefore, increasing the use of biopsy could contribute for the diagnosis, particularly when leprosy reaction is suspected [9, 74, 75].
Since there are no laboratory tests for the early diagnosis of these episodes, a study conducted in Nepal revealed for the first time that urine metabolic profilesrelated with the early onset of leprosy reactions may be identified [74].
Pure neuritic leprosy (PNL) is characterized by nerve damage without the emergence of skin lesions. Its diagnosis confirmation requires the presence of *M. leprae* in the biopsy of any affected sensorial nerve. However, nerves do not always contain acid resistant bacilli; often they only present morphologic alterations developed during disease progression. When the bacillus is not detected in the nerve sample, the value of other unspecific histologic alterations should be considered together with clinical, electro neuromyo graphic, and laboratory data (detection of *M. leprae* DNA through PCR method and serologic detection of anti-phenolic glycolipid-1 antibodies) to support a possible or probable PNL diagnosis. A study conducted in Brazil analyzed histopathological examination of nerve samples from PNL patients and patients with non-lepromatous peripheral neuropathies. Samples from PNL patients with both negative and positive bacilloscopy presented higher frequency of histopathological alterations (epithelioid granuloma, mononuclear infiltrates, fibrosis, perineurial and sub-perineurial edema, and loss ofmyelinated fibers) than samples from the group of patients with non-lepromatous peripheral neuropathies [63].

### 3.5 Treatment

The World Health Organization (WHO) recommended the standardization of leprosy therapy in 1981 and proposed a treatment regimen with a multidrug therapy (MDT). The treatment consists of medicines dapsone and rifampicin during 6 months for paucibacillary patients; and dapsone, rifampicin and clofazimine during 12 months for multibacillary patients.[74] For leprosy reactions WHO recommends the use of corticoids for type 1reaction (T1R) and corticoids and/or thalidomide for type 2 reactions (T2R) [22].
Despite MDT efficacy, this treatment regimen has limitations, such as the high rate of abandonment due to the long duration, difficulty for health professionals to classify the clinical types, and diagnoses mistakes in making the distinction between paucibacillary and multibacillary types due to being based only on the number of lesions. In the

face of such data, several studies seek uniform and short-term MDTs [76].

A study with a group of paucibacillary patients, treating half of the patients with paucibacillary MDT (PB MDT) and the other half with multibacillary MDT (MB MDT); another group of multibacillary patients was treated with multibacillary MDT. The groups were treated for 6 months and then the patients were evaluated to assess if the pharmacokinetic differences between the treatment regimens had an influence on the emergence and severity of adverse effects in both clinical types [76].

It was observed that the most frequent adverse effect was hemolytic anemia, often associated with MB MDT, presenting a significant difference between paucibacillary patients with MB MDT and paucibacillary patients with PB MDT, suggesting the influence of clofazimine in hemolytic anemia. However, the types of leprosy do not have an influence on the severity of the anemia, because there was no significant difference between the severity of hemolytic anemia in patients with MB and PB under MB MDT [76].

The administration of folic acid is suggested as a preventive and/or therapeutic measure for patients under treatment with MB MDT. Furthermore, the fact that there is low incidenceof hemolytic anemia in both types of leprosy reinforces the argument in favor of the possibility of using MB MDT on PB patients, thus uniformizing the therapy [76].

After completion of the multidrug regimen, whether multi- or paucibacillary, if the patient develops new lesions or there is an increase in the number of lesions and/or a new thickening of the nerveit will be considered that the patient had a relapse. The global relapse rate may reach 1.97/100 patients and it is directly associated with the absence of nerve damage and borderline-borderline patients due to their immunologic instability. The main cause of early relapse is inefficient treatment, whereas late relapses are due to persistent mycobacteria in immunologically favorable loci, such as skin nerves and lymphonodes. Therefore, a complete and appropriate treatment must be emphasized, as well as the surveillance after the cure, so relapses and agent transmission are avoided [77].

The completion of the recommended leprosy therapy is important also due to its influence on the degree of impairment of patients. There are records showing that upon completion of the treatment regimen there is regression of the neurologic damage caused by the disease or maintenance of the initial disability degree. However, 40% of patients present worsening of the neurologic condition after 10 years of the conclusion of the treatment. This worsening is closely related with the severity of the disease; other important factors directly involved are the late diagnosis and the presence of neuritis. Therefore, athorough follow-up is recommended for after-treatment patients with high probability of disability development, so that damage can be identified or prevented at an early stage. Patients who are discharged with no neurologic damage should be informed of the possibility of its development and oriented about signs and symptoms of such alterations so that an early diagnosis is carried out and patients are referred to centers specialized in peripheral neuropathies [78].

With the aim of reducing such peripheral neuropathies, leprosy multidrug treatment can be complemented with neural mobilization, which consists in the performance of flexion/inflexion of ankle, foot and toes, followed by leg raising, rip extension and knee extension, plantar flexion and inflexion, and leg elevation until the patient mentions discomfort such as pain and numbness. This treatment significantly reduces the degree of disability and pain, besides providing improvement in the neural function and electromyographic levels of muscle strength. Therefore, leprosy treatment centers should improve the delivery of complementary therapeutic measures in order to avoid disabilities, alterations in daily life activities, and social exclusion of these patients [59].

Other measures for leprosy control are early diagnosis of new cases and active search of individuals considered as contacts whoare under risk of acquiring the disease, with the performance of physical examination and BCG vaccination. Another method to identify the risk of leprosy development is the phenolic glycolipid-1 (PGL-1) antigen test in leprosy patients' contacts. Studies demonstrate that the risk of infection from *M. leprae* decreases for contacts who received BCG intradermal vaccination and the index patient started treatment [79]. Leprosy rate is higher in patients who are PGL-1 positive; therefore, individuals in this group need closer surveillance and are chemoprophylaxis candidates. In this group, individuals who are PGL-1 positive and vaccinated tend to develop more clinical manifestations of the disease than those non-vaccinated and PGL-1 positive contacts. However, all PGL-1 positive and vaccinated contacts developed paucibacillary leprosy, whereas most multibacillary cases occurred in non-vaccinated and PGL-1 positive contacts.[80] This demonstrates the importance of vaccination of contacts regardless of their serology for PGL-1. Patients who are early diagnosed by surveillance present less severe clinical forms, are usually classified as paucibacillary and present lower risk of developing physical impairments, whereas index cases are mostly multibacillary. This demonstrates that bacillary charge in index cases is a risk factor for the transmission of the disease to their contacts [79]. These data highlight the importance of surveillance of contacts for early diagnosis of leprosy and therefore the reduction of cases of impairments, which create stigma to patients, besides providing treatment opportunity and the decrease of new cases [79].

## 4. Conclusions

There is evidence that leprosy is a prevalent disease mainly in undeveloped countries, like Brazil, despite WHO recommendations for early diagnosis and treatment. Furthermore, the bacillus of leprosy is resistant in adverse environmental conditions and is capable of surviving in the soil and/or in water, thus facilitating its transmission mechanism.

The clinical condition of leprosy is extremely varied from one population to another and within individuals of the same population. This demonstrates that there are numerous etio patho genetic factors involved in the entire disease spectrum. Genetic and immunological characteristics of each population also determine how leprosy will manifest. Early diagnosis, besides propitiating immediate treatment to the patient, helps in the vaccination of contacts, thus decreasing the risk of infection from *M. leprae* Multidrug treatment must be concluded to avoid relapse and bacillus transmission. This therapeutic approach includes psychosocial support to patient and family because of the stigmatizing prejudice that may arise from deformities caused by leprosy.

The need of more in-depth research was highlightedin most

articles selected for this analysis, based on genetics and immunology studies involved in the etiopathogeny and the development of clinical forms of leprosy. In the face of the epidemiological data presented and the degree of severity that the disease may develop, emerges the importance of investing in further studies, from the understanding of its development in various populations until the best form of diagnosis, therapeutics and prophylaxis.

## 5. References


1. Araújo M. Hanseníase no Brasil. Rev Soc Bras Med Trop. 2013; 36(3):373-382.
2. Prevedello FC, Mira MT. Hanseníase: uma doença genética? an Bras Dermatol. 2007; 82(5):451-9.
3. Gracie R, Peixoto J, Soares F, Hacker M. Análise da distribuição geográfica dos casos de hanseníase. Rio de Janeiro, 2001 a 2012. Ciênc. saúde coletiva. 2017; 22(5):1695-1704.
4. Foss NT. Hanseníase: aspectos clínicos, imunológicos e terapêuticos. An Bras de Imunol. 1999, 74(2).
5. Andrade P, Ferreira P, Machado A, Messias S, Sales A, Nery J, *et al*. Histoid leprosy: a rare exuberant case. An Bras Dermatol. 2015; 90(5):756-757.
6. Leal D, Cazarin G, Bezerra L, Albuquerque A, Felisberto E. Programa de Controle da Hanseníase: uma avaliação da implantação no nível distrital. Saúde Debate. 2017; 41(1):209-228.
7. Lima L, Frota C, Mota R, Almeida R, Pontes M, Gonçalves H, *et al*. Widespread nasal carriage of Mycobacterium leprae among a healthy population in a hyperendemic region of northeastern Brazil. Mem Inst Oswaldo Cruz. 2015; 110(7):898-905.
8. Wagenaar I, van Muiden L, Alam K, Bowers R, Hossain M, Kispotta K, *et al*. Diet-Related Risk Factors for Leprosy: A Case-Control Study. Plos Negl Trop Dis. 2015; 9(5):e0003766.
9. Patnaik N, Agarwal S, Sharma S, Sharma S, Pandhi D. Evaluation of key histologic variables in skin biopsies of patients of borderline leprosy with type 1 lepra reaction. Indian J Dermatol Venereol Leprol. 2014; 80(5):402-408.
10. Chaitanya V, Jadhav R, Lavania M, Singh M, Valluri V, Sengupta U, *et al*. Interleukin-17F single-nucleotide polymorphism (7488T>C) and its association with susceptibility to leprosy. Int J Immunogenet. 2013; 41(2):131-137.
11. Zhang D, Huang X, Wang D, Li Y, Yao Y. Genetic variants of complement genes Ficolin-2, Mannose-binding lectin and Complement factor H are associated with leprosy in Han Chinese from Southwest China. Hum Genet. 2013; 132(6):629-640.
12. Jarduli L, Alves H, de Souza-Santana F, Marcos E, Pereira A, Dias-Baptista I, *et al*. Influence of KIR genes and their HLA ligands in the pathogenesis of leprosy in a hyperendemic population of Rondonópolis, Southern Brazil. BMC Infect Dis, 2014, 14(1).
13. Corrêa R, Aquino D, Caldas A, Serra H, Silva F, Ferreira M, *et al*. Association analysis of human leukocyte antigen class II (DRB1) alleles with leprosy in individuals from São Luís, state of Maranhão, Brazil. Mem Inst Oswaldo Cruz. 2012; 107(1):150-155.
14. Aguilar-Medina M, Escamilla-Tilch M, Frías-Castro L, Romero-Quintana G, Estrada-García I, Estrada-Parra S, *et al*. HLA Alleles are Genetic Markers for Susceptibility and Resistance towards Leprosy in a Mexican Mestizo Population. Ann Hum Genet. 2016; 81(1):35-40.
15. Wagenaar I, van Muiden L, Alam K, Bowers R, Hossain M, Kispotta K, *et al*. Diet-Related Risk Factors for Leprosy: A Case-Control Study. Plos Negl Trop Dis. 2015; 9(5):e0003766.
16. Garbin C, Garbin A, Carloni M, Rodavida T, Martins R. The stigma and prejudice of leprosy: influence on the human condition. Rev Soc Bras Med Trop. 2015; 48(2):194-201.
17. Murto C, Chammartin F, Schwarz K, da Costa L, Kaplan C, Heukelbach J, *et al*. Patterns of Migration and Risks Associated with Leprosy among Migrants in Maranhão, Brazil. Plos Negl Trop Dis. 2013; 7(9):e2422.
18. Guerrero M, Muvdi S, León C. Retraso en el diagnóstico de lepra como factor pronóstico de discapacidad en una cohorte de pacientes en Colombia, 2000–2010. Rev Panam Salud Pública. 2013; 33(2):137-143.
19. Barreto J, Guimarães L, Frade M, Rosa P, Salgado C. High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region. Mem Inst Oswaldo Cruz. 2012; 107(1):60-67.
20. Lavania M, Turankar R, Karri S, Chaitanya V, Sen gupta U, Jadhav R, *et al*. Cohort study of the seasonal effect on nasal carriage and the presence of Mycobacterium leprae in an endemic area in the general population. Clin Microbiol Infect. 2013; 19(10):970-974.
21. Alter A, Fava V, Huong N, Singh M, Orlova M, Van Thuc N, *et al*. Linkage disequilibrium pattern and age-at-diagnosis are critical for replicating genetic associations across ethnic groups in leprosy. Hum Genet. 2012; 132(1):107-116.
22. Sousa A, Fava V, Sampaio L, Martelli C, Costa M, Mira M', *et al*. Genetic and Immunological Evidence Implicates Interleukin 6 as a Susceptibility Gene for Leprosy Type 2 Reaction. J Infect Dis. 2012; 205(9):1417-1424.
23. Wang D, Su L, Zhang A, Li Y, Li X, Chen L, *et al*. Mitochondrial DNA Copy Number, but Not Haplogroup, Confers a Genetic Susceptibility to Leprosy in Han Chinese from Southwest China. PLoS ONE. 2012; 7(6):e38848.
24. de Souza-Santana F, Marcos E, Nogueira M, Ura S, Tomimori J. Human leukocyte antigen class I and class II alleles are associated with susceptibility and resistance in borderline leprosy patients from Southeast Brazil. BMC Infect Dis, 2015, 15(1).
25. Sacramento W, Mazini P, Franceschi D, de Melo F, Braga M, Sell A, *et al*. Frequencies of MICA alleles in patients from southern Brazil with multibacillary and paucibacillary leprosy. Int J Immunogenet. 2011; 39(3):210-215.
26. Shah J, Berrington W, Vary J, Wells R, Peterson G, Kunwar C, *et al*. Genetic Variation in Toll-Interacting Protein Is Associated With Leprosy Susceptibility and Cutaneous Expression of Interleukin 1 Receptor Antagonist. J Infect Dis. 2015; 213(7):1189-1197.
27. Suryadevara N, Neela V, Devalraju K, Jain S, SivaSai K, Valluri V, *et al*. Influence of Intron II microsatellite polymorphism in human toll-like receptor 2 gene in

leprosy. Hum Immunol. 2013; 74(8):1034-1040.
28. Cezar-de-Mello P, Toledo-Pinto T, Marques C, Arnez L, Cardoso C, Guerreiro L, *et al*. Pre-miR-146a (rs2910164 G>C) Single Nucleotide Polymorphism Is Genetically and Functionally Associated with Leprosy. Plos Negl Trop Dis. 2014; 8(9):e3099.
29. Brochado M, Gatti M, Zago M, Roselino A. Association of the solute carrier family 11 member 1 gene polymorphisms with susceptibility to leprosy in a Brazilian sample. Mem Inst Oswaldo Cruz. 2016; 111(2):101-105.
30. Xiang Y, Zhang D, Wang D, Li Y, Yao Y. Common variants of OPA1 conferring genetic susceptibility to leprosy in Han Chinese from Southwest China. J Dermatol Sci. 2015;80(2):133-141.
31. Neela V, Suryadevara N, Shinde V, Pydi S, Jain S, Jonnalagada S, *et al*. Association of Taq I, Fok I and Apa I polymorphisms in Vitamin D Receptor (VDR) gene with leprosy. Hum Immunol. 2015; 76(6):402-405.
32. Wang D, Xu L, Lv L, Su L, Fan Y, Zhang D, *et al*. Association of the LRRK2 genetic polymorphisms with leprosy in Han Chinese from Southwest China. Genes Immun. 2014; 16(2):112-119.
33. Liu H, Irwanto A, Fu X, Yu G, Yu Y, Sun Y, *et al*. Discovery of six new susceptibility loci and analysis of pleiotropic effects in leprosy. Nat Genet. 2015; 47(3):267-271.
34. Cezar-de-Mello P, Toledo-Pinto T, Marques C, Arnez L, Cardoso C, Guerreiro L, *et al*. Pre-miR-146a (rs2910164 G>C) Single Nucleotide Polymorphism Is Genetically and Functionally Associated with Leprosy. Plos Negl Trop Dis. 2014; 8(9):e3099.
35. Araújo S, Jamieson S, Dupnik K, Monteiro G, Nobre M, Dias M, *et al*. Examining ERBB2 as a candidate gene for susceptibility to leprosy (Hansen's disease) in Brazil. Mem Inst Oswaldo Cruz. 2014; 109(2):182-188.
36. Chopra R, Kalaiarasan P, Ali S, Srivastava A, Aggarwal S, Garg V, *et al*. PARK2 and proinflammatory/anti-inflammatory cytokine gene interactions contribute to the susceptibility to leprosy: a case–control study of North Indian population. BMJ Open. 2014; 4(2):e004239.
37. Suryadevara N, Neela V, Kovvali S, Pydi S, Jain S, Siva Sai K, *et al*. Genetic association of G896A polymorphism of TLR4 gene in leprosy through family-based and case-control study designs. Trans R Soc Trop Med Hyg. 2013; 107(12):777-782.
38. Zhang D, Huang X, Wang D, Li Y, Yao Y. Genetic variants of complement genes Ficolin-2, Mannose-binding lectin and Complement factor H are associated with leprosy in Han Chinese from Southwest China. Hum Genet. 2013; 132(6):629-640.
39. Shinde V, Marcinek P, Rani D, Sunder S, Arun S, Jain S, *et al*. Genetic evidence of TAP1 gene variant as a susceptibility factor in Indian leprosy patients. Hum Immunol. 2013; 74(6):803-807.
40. Ali S, Srivastava A, Chopra R, Aggarwal S, Garg V, Bhattacharya S *et al*. IL12BSNPs and copy number variation in IL23R gene associated with susceptibility to leprosy. J Medical Genet. 2012; 50(1):34-42.
41. Cardona-Castro N, Sánchez-Jiménez M, Rojas W, Bedoya-Berrío G. IL-10 gene promoter polymorphisms and leprosy in a Colombian population sample. Bio médica (Bogotá). 2012; 32(1):71-76.
42. Silva G, Santos M, Mota-Passos I, Boechat A, Malheiro A, Naveca F, *et al*. IFN-γ +875 microsatellite polymorphism as a potential protection marker for leprosy patients from Amazonas state, Brazil. Cytokine. 2012; 60(2):493-497.
43. Fabri A, Carvalho A, Araujo S, Goulart L, de Mattos A, Teixeira H, *et al*. Antigen-specific assessment of the immunological status of various groups in a leprosy endemic region. BMC Infect Dis, 2015, 15(1).
44. Palermo M, Pagliari C, Trindade M, Yamashitafuji T, Duarte A, Cacere C, *et al*. Increased Expression of Regulatory T Cells and Down-Regulatory Molecules in Lepromatous Leprosy. Am J Trop Med Hyg. 2012; 86(5):878-883.
45. Macedo A, Gigliotti P, Gameiro J, Souza V. Quantification of serum leptin levels in leprosy. Hansen Int. 2012; 37(2):40-46.
46. Braga A, Moretto D, Gigliotti P, Peruchi M, Vilani-Moreno F, Campanelli A, *et al*. Activation and cytokine profile of monocyte derived dendritic cells in leprosy: *in vitro* stimulation by sonicated Mycobacterium leprae induces decreased level of IL-12p70 in lepromatous leprosy. Mem Inst Oswaldo Cruz. 2015; 110(5):655-661.
47. Tarique M, Naqvi R, Santosh K, Kamal V, Khanna N, Rao D. Association of TNF-α-308(GG), IL-10−819(TT), IL-10−1082(GG) and IL-1R1+1970(CC) genotypes with the susceptibility and progression of leprosy in North Indian population. Cytokine. 2015; 73(1):61-65.
48. Attia E, Abdallah M, El-Khateeb E, Saad A, Lotfi R, Abdallah M, *et al*. Serum Th17 cytokines in leprosy: correlation with circulating CD4+ CD25highFoxP3+ T-regs cells, as well as down regulatory cytokines. Arch Dermatol Res. 2014; 306(9):793-801.
49. Abdallah M, Attia E, Saad A, El-Khateeb E, Lotfi R, Abdallah M, *et al*. Serum Th1/Th2 and macrophage lineage cytokines in leprosy; correlation with circulating CD4+CD25highFoxP3+T-regs cells. Exp Dermatol. 2014; 23(10):742-747.
50. Costa R, Mendonca V, Soriani F, Lyon S, Penido R, Costa A, *et al*. Serial measurement of the circulating levels of tumour necrosis factor and its soluble receptors 1 and 2 for monitoring leprosy patients during multidrug treatment. Mem. Inst. Oswaldo Cruz. 2013; 108(8):1051-1056.
51. Motta A, Simão J, Furini R, Ferreira M, Palma P, Komesu M, *et al*. Oral coinfection can stress peripheral lymphocyte to inflammatory activity in leprosy. Soc Bras Med Trop. 2013; 46(1):73-78.
52. Chaitanya S, Lavania M, Turankar R, Karri S, Sengupta U. Increased Serum Circulatory Levels of Interleukin 17F in Type 1 Reactions of Leprosy. J Clin Immunol. 2012; 32(6):1415-1420.
53. Palermo M, Trindade M, Duarte A, Cacere C, Benard G. Differential expression of the costimulatory molecules CD86, CD28, CD152 and PD-1 correlates with the host-parasite outcome in leprosy. Mem Inst Oswaldo Cruz. 2012; 107(1):167-173.
54. Souza V, Malaspina T, Campanelli A, Ghidella C, Ura S, Dalpino D, *et al*. Increased hepcidin expression in multibacillary leprosy. Mem Inst Oswaldo Cruz. 2012; 107(1):183-189.

55. Fafutis-Morris M, Pereira-Suárez A, Alvarado-Navarro A, Barrietos-García J, Estrada-Chávez C, Muñoz-Valle J, *et al*. Differential expression of solute carrier family 11a member 1 and inducible nitric oxide synthase 2 in skin biopsies from leprosy patients. Indian J Dermatol Venereol Leprol. 2015; 81(6):594.
56. Dupnik K, Bair T, Maia A, Amorim F, Costa M, Keesen T, *et al*. Transcriptional Changes That Characterize the Immune Reactions of Leprosy. J Infect Dis. 2014; 211(10):1658-1676.
57. Soares C, Rosa P, Trombone A, Fachin L, Ghidella C, Ura S, *et al*. Angiogenesis and Lym phangio genesis in the Spectrum of Leprosy and Its Reactional Forms. PLoS ONE. 2013; 8(9):e74651.
58. Michellin L, Barreto J, Marciano L, Lara F, Nogueira M, Souza V, *et al*. Leprosy patients: neurotrophic factors and axonal markers in skin lesions. Arquivos de Neuro-Psiquiatria. 2012; 70(4):281-286.
59. Véras L, Vale R, Mello D, Castro J, Lima V, Trott A *et al*. Electromyography
60. Batista e Sá V, Gomes M, Rangel M, Sanchez T, Moreira F, Hoefle S, *et al*. Primary Motor Cortex Representation of Handgrip Muscles in Patients with Leprosy. Plos Negl Trop Dis. 2015; 9(7):e0003944.
61. Mohanty P, Naaz F, Katara D, Misba L, Kumar D, Dwivedi D, *et al*. Viability of Mycobacterium leprae in the environment and its role in leprosy dissemination. Indian J Dermatol Venereol Leprol. 2016; 82(1):23.
62. Feenstra S, Nahar Q, Pahan D, Oskam L, Richardus J. Social contact patterns and leprosy disease: a case-control study in Bangladesh. Epidemiol Infect. 2012; 141(3):573-581.
63. Antunes S, Chimelli L, Jardim M, Vital R, Nery J, Corte-Real S, *et al*. Histopathological examination of nerve samples from pure neural leprosy patients: obtaining maximum information to improve diagnostic efficiency. Mem Inst Oswaldo Cruz. 2012; 107(2):246-253.
64. Torre J. Manifestaciones nasales de la lepra. Rev Cuba Med Gen Integr. 2015; 31(1):52-60.
65. Rosa F, Souza V, Almeida T, Nascimento V, Vásquez F, Cunha M, *et al*. Detection of Mycobacterium leprae in saliva and the evaluation of oral sensitivity in patients with leprosy. Mem Inst Oswaldo Cruz. 2013; 108(5):572-577.
66. Véras L, Vale R, Mello D, Castro J, Lima V, Silva K, *et al*. Degree of disability, pain levels, muscle strength, and electromyographic function in patients with Hansen's disease with common peroneal nerve damage. Rev Soc Bras Med Trop. 2012; 45(3):375-379.
67. Machado P, Machado L, Shibuya M, Rego J, Johnson W, Glesby M, *et al*. Viral Co-infection and Leprosy Outcomes: A Cohort Study. Plos Negl Trop Dis. 2015;9(8):e0003865.
68. Pires C, Jucá Neto F, de Albuquerque N, Macedo G, Batista K, Xavier M, *et al*. Leprosy Reactions in Patients Coinfected with HIV: Clinical Aspects and Outcomes in Two Comparative Cohorts in the Amazon Region, Brazil. Plos Negl Trop Dis. 2015; 9(6):e0003818.
69. Yan W, Xing Y, Yuan L, Yang R, Tan F, Zhang Y, *et al*. Application of RLEP Real-Time PCR for Detection of M. leprae DNA in Paraffin-Embedded Skin Biopsy Specimens for Diagnosis of Paucibacillary Leprosy. Am J Trop Med Hyg. 2014; 90(3):524-529.
70. Stefani M, Grassi A, Sampaio L, Sousa A, Costa M, Scheelbeek P, *et al*. Comparison of two rapid tests for anti-phenolic glycolipid-I serology in Brazil and Nepal. Mem Inst Oswaldo Cruz. 2012; 107(1):124-131.
71. Hungria E, Oliveira R, Souza A, Costa M, Souza V, Silva E, *et al*. Seroreactivity to new Mycobacterium leprae protein antigens in different leprosy-endemic regions in Brazil. Mem Inst Oswaldo Cruz. 2012; 107(1):104-111.
72. Duthie M, Orcullo F, Abbelana J, Maghanoy A, Balagon M. Comparative evaluation of antibody detection tests to facilitate the diagnosis of multibacillary leprosy. Appl Microbiol Biotechnol. 2016; 100(7):3267-3275.
73. Caleffi K, Hirata R, Hirata M, Caleffi E, Siqueira V, Cardoso R, *et al*. Use of the polymerase chain reaction to detect Mycobacterium leprae in urine. Braz J Med Biol Res. 2012; 45(2):153-157.
74. Mayboroda O, van Hooij A, Derks R, van den Eeden S, Dijkman K, Khadge S *et al*. Exploratory urinary metabolomics of type 1 leprosy reactions. Int J Infect Dis. 2016; 45:46-52.
75. Lockwood D, Nicholls P, Smith W, Das L, Barkataki P, van Brakel W, *et al*. Comparing the Clinical and Histological Diagnosis of Leprosy and Leprosy Reactions in the INFIR Cohort of Indian Patients with Multibacillary Leprosy. Plos Negl Trop Dis. 2012; 6(6):e1702.
76. Gonçalves H, Pontes M, Bührer-Sékula S, Cruz R, Almeida P, de Moraes M *et al*. Brazilian clinical trial of uniform multidrug therapy for leprosy patients - the correlation between clinical disease types and adverse effects. Mem Inst Oswaldo Cruz. 2012; 107(1):74-78.
77. Kumar A, Girdhar A, Girdhar B. Twelve months fixed duration WHO multidrug therapy for multibacillary leprosy: incidence of relapses in Agra field based cohort study. Indian J Med Res. 2013; 138(4):536-540.
78. Sales A, Campos D, Hacker M, da Costa Nery J, Düppre N, Rangel E, *et al*. Progression of leprosy disability after discharge: is multidrug therapy enough? Trop Med Int Health. 2013; 18(9):1145-1153.
79. Hacker M, Duppre N, Nery J, Sales A, Sarno E. Characteristics of leprosy diagnosed through the surveillance of contacts: a comparison with index cases in Rio de Janeiro, 1987-2010. Mem Inst Oswaldo Cruz. 2012; 107(1):49-54.
80. Düppre N, Camacho L, Sales A, Illarramendi X, Nery J, Sampaio E, *et al*. Impact of PGL-I Seropositivity on the Protective Effect of BCG Vaccination among Leprosy Contacts: A Cohort Study. Plos Negl Trop Dis. 2012; 6(6):e1711.