Journal of Infection and Public Health 13 (2020) 1078–1088

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Review

Diagnostic accuracy of tests using recombinant protein antigens of *Mycobacterium leprae* for leprosy: A systematic review

Ana Laura Grossi de Oliveira[a], Vanêssa Gomes Fraga[b], Nathalia Sernizon-Guimarães[c], Mariana Santos Cardoso[b], Agostinho Gonçalves Viana[b], Lilian Lacerda Bueno[b], Daniella Castanheira Bartholomeu[b], Cristiane Alves da Silva Menezes[d], Ricardo Toshio Fujiwara[a,b,*]

[a] *Post-Graduate Program in Health Sciences: Infectious Diseases and Tropical Medicine, School of Medicine, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[b] *Department of Parasitology, Institute of Biological Sciences (ICB), UFMG, Brazil*
[c] *Federal University of Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil*
[d] *Department of Clinical and Toxicological Analyses, School of Pharmacy, Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*

ARTICLE INFO

Article history:
Received 10 October 2019
Received in revised form
13 December 2019
Accepted 15 December 2019

Keywords:
Leprosy
Mycobacterium leprae
Serological tests
Recombinant protein antigens
Sensitivity
Specificity

SUMMARY

The aim of this systematic review was to investigate the studies that evaluated the sensitivity and specificity of serologic tests using recombinant protein antigens from *Mycobacterium leprae* for leprosy diagnosis. We included 13 studies that were available in PubMed, Brazilian Virtual Library of Health, Web of Science, ScienceDirect and Scopus. From these studies, we found that the recombinant serine-rich 45-kDa protein of *M. leprae* (ML0411) demonstrated high performance for multibacillary (MB) also to paucibacillary (PB) patients, although this study was tested only for Indian population. Despite that, studies using the ND-O-LID antigen have been able to more accurately identify new cases of leprosy among people living in endemic or non-endemic areas and household contacts in Brazil, Colombia, and the Philippines, especially when combined with other biomarkers. Finally, low sensitivity values for PB patients' antibodies response remain challenging for tests intended to diagnose clinical forms that comprise this classification in leprosy.

Contents

* Corresponding author at: Parasitology Department, Biology Science Institute, Federal University of Minas Gerais/Brazil, Av. Antônio Carlos, 6627, Pampulha, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil.
E-mail addresses: analauragrossi@gmail.com (A.L.G. de Oliveira), vanessagomesfraga@yahoo.com.br (V.G. Fraga), nasernizon@gmail.com (N. Sernizon-Guimarães), marianascardoso@yahoo.com.br (M.S. Cardoso), agostinhogv@yahoo.com.br (A.G. Viana), lilacerdabueno@gmail.com (L.L. Bueno), daniellaufmg@gmail.com (D.C. Bartholomeu), menezescristiane1@gmail.com (C.A. da Silva Menezes), fujiwara@icb.ufmg.br (R.T. Fujiwara).

https://doi.org/10.1016/j.jiph.2019.12.011

Introduction

Leprosy remains one of the most disabling infectious diseases in the world. During 2018, 208,619 new cases were reported from around the world, and of these, about 10,000 had grade-2 disabilities (G2D). South-East Asia had 7.49 new cases detected per 100,000 inhabitants, however, India and Brazil have been reported the highest leprosy burden over decades [1]. One of the targets of the Global Leprosy Strategy 2016–2020 is to stop leprosy and its complications to improve early diagnosis and to reduce the disability burden [2].

Early diagnosis of leprosy is particularly important for providing early treatment and reducing the infection. In most countries with high leprosy rates, the diagnosis of the disease is still mainly based on clinical observations as the appearance of the hypopigmented or reddish lesion with hypoesthesia, presence of acid-fast bacilli lymph node smears and compatible skin lesion histopathology. Although the histopathology of a cutaneous lesion may support the clinical diagnosis, the problem is that this technique is not always easy to access in patient care services, or it may not have good sensitivity in indeterminate and tuberculoid clinical forms. Therefore, the urgent focus should be given to the early detection of cases before visible skin deficiencies arise [2].

At present, to promote effective diagnosis, many serological tests have been developed to detect circulating antibodies that should be responsive to native or recombinant protein antigens from *Mycobacterium leprae*, as phenolic glycolipid (PGL-I), leprosy Infectious Disease Research Institute (IDRI) diagnostic-1 (LID-1), protein advances diagnostic of leprosy (PADL), recombinant culture filtrate protein-10 (rCFP-10), and many others. These antigens have been used in several immunoassays to detect responses of both paucibacillary (PB) and multibacillary (MB) leprosy patients, especially in the early stages of the disease. Most of these tests have shown satisfactory results in the detection of MB, but an inadequate capacity for detecting PB, because of the specific immune response with reduced production of antibodies against *M. leprae* [3–7].

In the present study, a systematic review was conducted to examine previous publications that evaluated the sensitivity and specificity of the recombinant protein antigens derived from *M. leprae* for the leprosy diagnosis.

Methods and materials

Literature search and selection criteria

This systematic review was fully based on The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 guideline [8] and the protocol was registered in PROSPERO database (CRD42018098711).

Two reviewers (ALGO and VGF) independently extracted all trial data; and when necessary, a third reviewer (NSG) interceded whether any disagreements remained. Inclusion criteria were: (1) clinical trials, case–control, cohort and diagnostic studies that had been conducted in humans, and, (2) articles provided sufficient data, including the total number of subjects in both patients and healthy controls and providing sufficient data about sensitivity, specificity, and accuracy of *M. leprae* recombinant protein antigens used for leprosy diagnosis. No restrictions were imposed on the country, language or date of publications, even for sex and age of the participants; however, review articles and letters to the editor were excluded.

Until June 2019, we sought for five independent databases to perform the relevant literature search: PubMed, Brazilian Virtual Library of Health (BVS), Web of Science, ScienceDirect and Scopus databases. We used a comprehensive list of indexed key terms (medical subject headings terms – MESH) that were adapted to each database: "Leprosy" (Mesh) AND [("sensitivity" (Mesh) OR "specificity" (Mesh) OR "data accuracy" (Mesh)] AND ("serologic tests" OR "diagnostic tests" (Mesh) OR "*Mycobacterium leprae*" (Mesh) OR "recombinant protein antigens"). Furthermore, a manual search was conducted in all references of the included studies for further studies (gray literature: Google Scholar, Digital Library of theses and Dissertations and Open Access Thesis and Dissertation). The detailed search strategies are described according to PRISMA flowchart (Fig. 1).

Data extraction and study characteristics

All studies that matched the inclusion criteria were reviewed by the authors (ALGO and VGF), and disagreements on inclusion were resolved by consensus with the third reviewer (NSG) and trying to answer the questions: (1) What are the recombinant protein antigens derived from *Mycobacterium leprae* relevant to the serological diagnosis of leprosy?; (2) What are the accuracy and reliability of the serologic tests using recombinant antigens in leprosy?; (3) What are the sensitivity, specificity and predictive values of the serological tests using recombinant antigens in leprosy?, and (4) What is the prognostic validity of the serological tests for leprosy diagnosis?

After analysis of the title and abstract, the selected studies were read in full to confirm their eligibility and to extract the relevant data in an attempt to produce a better analysis and discussion of the main results. The data extracted were related to the publication (authors, year of publication, journal and its impact factor, country, sample size, leprosy clinical forms, comparative groups, recombinant protein antigens, molecular weight, obtaining method, laboratory tests, diagnostic reference method, sensitivity, specificity, and authors conclusions).

We appraised the quality of the included studies using a tool for the quality assessment of diagnostic accuracy studies 2 (QUADAS-2) and Standards for Reporting Studies of Diagnostic Accuracy (STARD). These tools have been broadly adopted and applied in reviews to assess the risk of bias, and/or the applicability to the general population of patient selection criteria, the index test, the reference standard used, and the flow of patients through the study or timing of the index test and reference standard. We used a scale with possible scores ranging from 0 to 10, and the assessment was done following: studies with scores from 7–10 marks have the lowest risk of bias and represent the highest quality (>75%), while studies with scores lower than 4 have the highest risk of bias and the lowest quality (<25%). Studies with scores of 5–7 have a moderate risk of bias and quality (25–75%) [9–11].

Results

Initially, a total of 135 studies were identified for this review through electronic database searching, as previously described. Of these, 73 duplicates or reviews were excluded. After that, we read its title, abstract and full-text, excluding 49 publications due to the following reasons: 28 articles were excluded because they did not present enough data for the discussion of the results, 19 did not associate antigenic protein with a serological diagnosis, and 2 studies evaluated vaccine candidate peptides. Finally, we included 13 articles that met the inclusion criteria.

After that, we summarized relevant aspects of included studies as author and year of publication, title, journal and impact factor, country, number of participants: leprosy patients and comparative groups, gender, and age range (Table 1).

Furthermore, in Table 2, we specified relevant characteristics of the antigens as molecular weight, antibody detection method, gold standard comparative method, sensitivity and specificity data, statistical analysis and the conclusion of the research.

Table 1
The main characteristics of the included studies in the systematic review.

N	Authors, year	Title	Journal/impact factor (year)	Country	Population	Gender	Age range	Comparative groups (*n*)	Healthy control group (*n*)	Household contacts (*n*)
1	Roche et al., 1992	Antibody responses to the 18-kDa protein of *Mycobacterium leprae* in leprosy and tuberculosis patients'	Int. Journal of Leprosy/0.247 (2001)	Nepal	Leprosy = 146, PB = (primary neurite = 8, TT = 10, BT = 61), MB = 67 (BB = 4, BL = 42, LL = 21)	Male: 101, female: 45	9–80 years	44 tuberculosis	50	NI
2	Triccas et al., 1998	Specific serological diagnosis of leprosy with a recombinant *Mycobacterium leprae* protein purified from a rapidly growing mycobacterial host	Journal of Clinical Microbiology/4.054 (2017)	Nepal	Leprosy = 90 PB = 30, MB = 60	NI	NI	Tuberculosis 15 endemic area, pulmonary tuberculosis 15 non-endemic area.	50	NI
3	Parkash et al., 2006a	Detection of antibodies against *Mycobacterium leprae* culture filtrate protein-10 in leprosy patients	Journal of Medical Microbiology/2.112 (2017)	India	Leprosy = 56 IB+ (18), IB- (38)	NI	NI	Pulmonary TB = 15, other skin pathology = 14	20	NI
4	Parkash et al., 2006b	Evaluation of recombinant serine-rich 45-kDa antigen (ML0411) for detection of antibodies in leprosy patients	Scandinavian Journal of Immunology/2.314 (2017)	India	Leprosy = 56 PB = 38, MB = 18	NI	NI	Pulmonary TB = 15, other skin pathology = 14	20	NI
5	Parkash et al., 2007	Performance of recombinant ESAT-6 antigen (ML0049) for detection of leprosy patients	Letters in Applied Microbiology/1.471 (2018)	India	Leprosy = 48 Smear-positive = 17 Smear-negative = 31	NI	NI	TB: 13 Other skin diseases: 14	21	NI
6	Bezerra, 2012 (thesis)	Desempenho de antígenos nativo, recombinante e sintético, em testes imunoenzimáticos, para diagnóstico e prognóstico de pacientes com diferentes formas clínicas de hanseníase	Doctorate Thesis from School of Medicine, São Paulo University	Brazil	Leprosy = 61 PB = 28 (Indeterminate = 8, TT = 11, BT- BI- = 9), MB = 33 (BT BI + = 6, BB = 5, BL = 10, LL = 12)	Male: 41, female: 20	Mean = 40, range = 8–90 years	Pulmonary TB = 20	21 non-endemic area, 30 endemic area	30
7	Duthie et al., 2014	Rapid quantitative serological test for detection of infection with *Mycobacterium leprae,* the causative agent of leprosy	Journal of Clinical Microbiology/4.054 (2017)	Philippines	*N* = 270 (MB = 208, PB = 62)	NI	NI	NI	63 Endemic control	51

Table 1 (*Continued*)

N	Authors, year	Title	Journal/impact factor (year)	Country	Population	Gender	Age range	Comparative groups (*n*)	Healthy control group (*n*)	Household contacts (*n*)
8	Souza et al., 2014	Utility of recombinant proteins LID-1 and PADL in screening for *Mycobacterium leprae* infection and leprosy (population-based study)	Transactions of the Royal Society of Tropical Medicine and Hygiene/3.130 (2018)	Brazil	834 (LID-1); 573 (PADL) - sera-evaluation + clinical evaluation (Clinically confirmed cases: LID-1 = 19; PADL = 15)	Male: 35.9%; Female: 64.1%	Mean = 38.4 years, range = 10–100	NI	(LID-1 *N* = 344, PADL *N* = 212) based on negative diagnosis	NI
9	Amorim et al., 2016	Identifying leprosy and those at Risk of developing leprosy by detection of antibodies against LID-1 and LID-NDO	PLOS Neglected Tropical Diseases/4.367 (2017)	Brazil	561 (Leprosy = 98 PB = 32, MB = 66)	Male: 48%, female: 52%	Mean: 45.6 years ± 16	NI	98 endemic control	365 (followed for 7–10 years = 332) 32.7years ± 20
10	Marçal et al., 2018	Utility of immunoglobulin isotypes against LID-1 and NDO-LID for, particularly IgG1, confirming the diagnosis of multibacillary leprosy	Memórias do Instituto Oswaldo Cruz/1.592 (2014)	Brazil	106 (PB = 20, MB = 18)	Male: 40, female: 66	Mean: 40.6 years, range: 13–80 years	NI	20 endemic area	*N* = 48 (PB = 20, MB = 28)
11	Muñoz et al., 2018	Comparison of enzyme-linked immunosorbent assay using either natural octyl disaccharide-leprosy IDRI diagnostic or phenolic glycolipid-I antigens for the detection of leprosy patients in Colombia	The American Journal of Tropical Medicine and Hygiene/2.564 (2017)	Colombia	396 (MB = 338, PB = 58)	Group 1: MB = male: 260, female:78, PB = male: 37, female: 21, Group 2: male: 75%, female: 25%	Group 1: leprosy patients: mean: 52.6 years, range: 5–95 years	Group 2: pulmonary TB: 15; Leishmaniasis = 21	Group 2: 100	NI
12	Leturiondo et al., 2019	Performance of serological tests PGL1 and NDO-LID in the diagnosis of leprosy in a reference Center in Brazil	BMC Infectious Diseases/2.062 (2016)	Brazil	171 (PB = 50, MB = 121)	Leprosy patients: female: 34%, male:66%)	Global mean: 41.24 years; range: 10–77 years	NI	530	NI
13	Gama et al., 2019	A novel integrated molecular and serological analysis method to predict new cases of leprosy amongst household contacts	PLOS Neglected Tropical Diseases/4.367 (2017)	Brazil	38 (PB/MB)	Leprosy patients/endemic control: male: 38 female: 40	Leprosy patients/endemic control: range: 8–92 years	NI	40	2011: 113 2012: 80 2016: 44 Total:237

MB, multibacillary leprosy; PB, paucibacillary leprosy; TB, tuberculosis; TT, tuberculoid-tuberculoid; BT, borderline-tuberculoid; BB, borderline-borderline; BL, borderline-lepromatous; LL, lepromatous–lepromatous; BI, bacilloscopic index; *N*, number of enrolled individuals; NI, no informed data.

Table 2
Characteristics of *Mycobacterium leprae* protein antigens and performance indexes of the tests for serodiagnosis of leprosy patients.

N	Authors, year	Protein antigen/(molecular weight)	Comparative test (gold standard)	Detection of antibodies/methods	SEN (%) rAg.	SPE (%) rAg	Statistical analysis	Authors conclusions
1	Roche et al., 1992	18-k-Da expressed from *Escherichia coli*	Disaccharide-bovine serum albumin (dBSA) conjugate for the detection of IgM anti-PGL-I antibodies was measured by ELISA	Enzyme-linked immunosorbent assay – **ELISA** to detect IgG anti-18-kDa antibodies.	**PB = 10%** **MB = 42%** TB = 47% HC = 2%	PB = 90% MB = 58% TB = 53% HC = 98%	Chi-square and Student's *t* test	The sensitivity and specificity of the anti-M. *leprae 18-kDa* ELISA **are insufficient** for the assay to be of clinical utility in leprosy patients.
2	Triccas et al., 1998	35-kDa purified from recombinant *Mycobacterium smegmatis*	Detection of IgM-antiphenolic glycolipid-I antibodies (PGL-I) was measured by **direct ELISA,** with the disaccharide bovine serum albumin glycoconjugate	1. **Inhibition ELISA** using monoclonal antibodies and a sonicate of recombinant *M. smegmatis* (MLS-MIA) 2. **Inhibition ELISA** using monoclonal antibodies and a purified 35-kDa (r35-kDa-MIA), 3. **35-kDa direct ELISA**	**MLS-MIA**: (PB: 17%, MB:90%, Endemic-TB: 6.7%, Nonendemic TB: 0, Endemic control: 6%); **r35-kDa-MIA**: (PB: 17%, MB: 90%, Endemic-TB: 6.7%, Nonendemic TB: 0, Endemic control: 2%); **Direct ELISA**: (PB: 17%, MB: 83%, Endemic-TB: 13.4%, Nonendemic TB: 0, Endemic control: 4%);	**Total specificity using r35kDa-MIA** (Endemic-TB + Nonendemic TB + Endemic control: 97.5%) **Total specificity using r35kDa and Direct ELISA**: Endemic-TB + Nonendemic TB + Endemic control: 95%)	Chi-square	The 35-kDa antigen **can be utilized** in a sensitivity and specificity assay of the humoral response to *M. leprae*, independent if the antigen was purified or not.
3	**Parkash et al., 2006**	10-kDa protein (rCFP-10) obtained from *Escherichia coli*	Detection of IgM antibodies anti-PGL-I were measured by **ELISA**	IgG anti-rCFP10 antibodies detection - **ELISA**	BI + (83.3%), BI- (18.4%), Total: 39.3%; TB: 0%, Other skin diseases: 0%, HC: 5%, Total: 2.04%	HC = 98%	ROC-curve	rCFP-10 seems to be a **suitable antigen** for classification of leprosy patients into BI + and BI- groups.
4	Parkash et al., 2006	Serine-rich 45-kDa antigen (ML0411) expressed using *Escherichia coli*	Detection of antibodies anti-PGL-I conjugated to human serum albumin (ND-O-HSA) (IgM) were measured by ELISA	IgG anti-45-kDa (ML0411) antibodies detection – **ELISA**	**PB (47.4%**), **MB (100%)** Total: 64.3%; TB: 0%, Other skin diseases: 0%, HC: 0%, Total: 0%	**HC = 100%** TB: 100% Other skin diseases: 100%	Student's t-test, ROC curve, Chi-square test	The results indicate that the *M. leprae* 45-kDa protein is a potent B-cell antigen and **may be a useful** serodiagnostic reagent.
5	Parkash et al., 2007	Recombinant ESAT-6 antigen (ML0049)	Detection of IgG antibodies against *M. leprae* rESAT-6 measured by ELISA	IgG anti-rESAT-6 – **ELISA**	Smear-positive leprosy patients = 82.4% Smear-negative leprosy patients = 19.4% Total: 41.7%	**HC: 100%** TB: 100% Other skin diseases: 100%	Chi-square	Recombinant ESAT-6 seems to be a **potential serological reagent** for detection of *M. leprae* infection

Table 2 (*Continued*)

N	Authors, year	Protein antigen/(molecular weight)	Comparative test (gold standard)	Detection of antibodies/methods	SEN (%) rAg.	SPE (%) rAg	Statistical analysis	Authors conclusions
6	Bezerra, 2012 (thesis)	1. LID-1, 2. Raw extract fractions (38-kDa, 3.5-kDa)	Detection of antibodies anti-LID-1 and ND-O-BSA (IgM) were measured by ELISA	IgG anti LID-1, anti 38-kDa, anti 3.5-kDa - **ELISA**	1. LID (PB = 45% MB = 91% TB = 15% HC endemic area = 5%, HC non-endemic area = 10%), 2. 38-kDa (PB = 7.14% MB = 86% TB = 10% HC endemic area = 0%, HC non-endemic area = 0%, HHC = 3.3); 3. **3.5-kDa (PB = 3.57%** MB = 37%, TB = 10% HC endemic area = 0%, HC non-endemic area = 0%, HHC = 0%)	LID: HC endemic area: 95%, HC non-endemic area = 90%, TB = 85%.	ROC-curve, Pearson correlation, McNemar's method	1. **LID can be used for diagnosis** by having good indexes of sensitivity, specificity, and predictive values. 2. The fractions **38-kDa and 3.5-kDa** of the crude extract can be considered **good immunological markers** for diagnosis, confirmation of serology and prognosis of the disease.
7	Duthie et al., 2014	1. ND-O-LID, 2. Standard Diagnostic leprosy test (SD)	IgM antibodies to NDO-BSA (PGL-I conjugated to BSA)	**Immunochromatographic lateral flow tests**, NDO-LID detects IgM antibodies to PGL-I and IgG antibodies specific to LID-1	1. MB (NDO-LID = 87%), 2. PB (NDO-LID = 32.3%, HHC = 3.9%, EC = 1.6%), Leprosy = 74.4%, Not leprosy = 2.6%	NDO-LID = 97.4%	Unpaired t-Student test	The highly quantifiable nature of the NDO-LID test/Smart Reader platform **appears to have utility for the detection and monitoring** of MB leprosy.
8	Souza et al., 2014	1. LID-1, 2. PADL	A clinical evaluation to confirmed leprosy cases	anti-LID-1 and anti PADL antibodies - **ELISA**	1. LID = 89% (17/19), 2. PADL = 87% (13/15)	1. LID = 89%, 2. PADL = 87%	Chi-square test	The results indicate the utility of LID-1 and PADL as **primary screening tools** for the detection of *M. leprae* infection and the identification of leprosy patients.
9	Amorim et al., 2016	1. LID-1, 2. LID-NDO	–	Anti-LID-1 and anti ND-O-LID antibodies - **ELISA**	1. MB (LID = 89%, LID-NDO = 95%), 2. PB (LID = 16%, LID-NDO = 6%)	1. MB cases (LID = 96%, LID-NDO = 88%), EC = (LID = 99%, LID-NDO = 98%)	ROC-curve and area under curve (AUC)	The findings support a role for the LID-1 and LID-NDO antigens in **diagnosing** MB leprosy and **identifying** people at greater risk of developing clinical **disease**.

Table 2 (*Continued*)

N	Authors, year	Protein antigen/(molecular weight)	Comparative test (gold standard)	Detection of antibodies/methods	SEN (%) rAg.	SPE (%) rAg	Statistical analysis	Authors conclusions
10	Marçal et al., 2018	1. LID-1, 2. ND-O-LID	ND-O-HSA (IgM, IgG)	Anti-LID-1 and anti ND-O-LID IgG/IgM antibodies **- ELISA**	**MB:** **NDO-HSA**: IgM = 70%, IgG = 85%, IgG1 = 75%, IgG2 = 65%, IgG3 = 70%, **LID-1**: IgM = 80%, IgG = 90%, IgG1 = 90%, IgG2 = 80%, IgG3 = 85%, **NDO-LID:** IgM = 85%, IgG = 95%, IgG1 = 90%, IgG2 = 85%, IgG3 = 90%	**MB:** **NDO-HSA**: IgM = 98%, IgG = 78%, IgG1 = 88%, IgG2 = 78%, IgG3 = 84%, **LID-1**: IgM = 82%, IgG = 100%, **IgG1 = 100%,** IgG2 = 88%, IgG3 = 74%, **NDO-LID**: IgM = 86%, IgG = 98%, IgG1 = 90%, IgG2 = 82%, IgG3 = 88%	ROC curve	The results indicate that serological tests based on the detection of **antigen-specific IgG1 antibodies are a useful tool to differentiate MB from PB patients** and indicate **the enhanced performance of the LID-1 and NDO-LID** antigens in the serodiagnosis of leprosy.
11	Muñoz et al., 2018	1. ND-O-LID	PGL-I (IgM)	Anti-ND-O-LID IgG/IgM antibodies; Protein A (IgG/IgM simultaneous) **- ELISA**	**NDO-LID** (IgM: Leprosy per se: 78%; PB: 20.3%; MB: 89.6%) IgG: Leprosy per se: 81.6%; IgG/IgM/Protein A: 86.3%	**NDO-LID** (IgM: Leprosy per se: 97%; PB: 97%; MB: 97%) IgG: Leprosy per se: 95.6%; IgG/IgM/Protein A: 93.6%	Chi-square test	The data indicate that the detection of both IgG and IgM antibodies against **NDO-LID with protein A** provided the **best overall ability to detect** Colombian leprosy patients.
12	Leturiondo et al., 2019	1.NDO-LID	PGL-I rapid test (IgM)	anti-ND-O-LID IgG/IgM antibodies simultaneous **- ELISA**	**NDO-LID** (IgM/IgG: PB: 34%; MB: 73.6%) **PGL-I** (PB: 32%; MB: 81%)	HC: **NDO-LID: 81.7%** PGL-I: 75.9%	Chi-square test	The tests showed **limited capacity in the diagnosis of the disease**
13	Gama et al., 2019	1. ND-O-LID, 2. LID-1	Clinical and histopathologic assessment	IgG anti-LID-1 and IgG/IgM anti-ND-O-LID – **ELISA**	**Serological test: ND-O-LID** 57.9%; **LID-1:** 63.2% (with Random Forest: PB: 70.6% and MB: 90.5%)	**Serological test: ND-O-LID:** 97.5%; **LID-1:** 92.5% (with Random Forest: 92.5%)	ROC curve	The proposed model **using Random Forest allows for the diagnosis of leprosy** with high sensitivity and specificity and the early identification of new cases among household contacts.

MB, multibacillary leprosy; PB, paucibacillary leprosy; TB, tuberculosis; HC, health control; BI, bacilloscopic index; IgM, immunoglobulin M; IgG, immunoglobulin G; PGL-I, phenolic glycolipid-I; rCFP-10, recombinant culture filtrate protein-10; rESAT-6, recombinant early secreted antigenic target (ML0049); LID-1, recombinant fusion of ML0405 and ML2331 *M. leprae* proteins; ND-O-LID, semi-synthetic disaccharide (ND) attached to the octyl radical, which mimics PGL-1 conjugated with two fusion proteins – ML0304 and ML0331 forming LID, ND-O-BSA/HSA native phenolic glycolipid-1 (PGL-I) conjugated to bovine or human serum albumin; PADL, protein advances diagnostic of leprosy (including reactive portions of ML0405, ML2331, ML2055, ML0091, and ML0411).

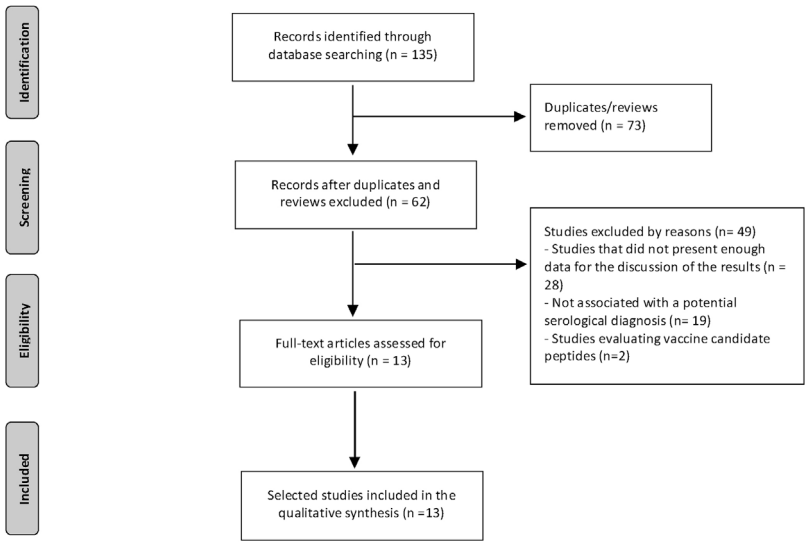

Fig. 1. PRISMA flowchart describing our search strategy by identifying the studies to be included in this systematic review.

The majority of studies investigated Brazilian leprosy patients [12–17], followed by Indians [18–20], Nepalese [21,22], Philippines [23], and the Colombians [7]. They were all conducted over 27 years (between 1991 and 2018) and were published in important academic journals that presented a range of impact factors from 0.247 to 4.367, besides one thesis from the University of São Paulo, Brazil.

The population of all studies included in this systematic review was human (no animal researches), and the total number of leprosy patients (paucibacillary and multibacillary) was 1502 (range: 34–396 cases) and age range from 8 to 100 years, also 1619 healthy people were evaluated. In addition, 9 (69.2%) studies examined both men and women. As comparative groups, 7 (53.8%) studies considered tuberculosis and other diseases and, 6 (46.1%) studies had household contacts.

The main criterion for previously exclusions was the availability of measurement data as sensitivity and specificity. Therefore, no data were inferred or even calculated to predict the efficacy of the recombinant protein antigen used in the leprosy diagnosis, as well as, to determine cross-linked reactivity. Thus, we analyzed the range value for sensitivity and specificity of assays that used *M. leprae* proteins expressed in bacteria – Group 1 [18,19,21,22] and proteins predict after the sequencing of the *M. leprae* genome – Group 2 [7,12–17,20,23].

The methodological quality of studies showed that the records included in this systematic review had a high score, with most satisfying the following criteria: (1) Was the patients' spectrum representative of the patients who will receive the test in practice? (13/13); (2) Were selection criteria clearly described? (10/13); (3) Is the time period between the reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? (11/13); (4) Was the whole sample or a randomly selected sample verified using a diagnostic reference standard? (11/13); (5) Did patients receive the same reference standard regardless of the index test result? (13/13); (6) Was the execution of the index test described in sufficient detail to permit replication of the test? (12/13); (7) Was the execution of the reference standard described in sufficient detail to permit its replication? (12/13); (8) Were the index test results interpreted without knowledge of the results of the reference standard? (9/13); (9) Were the reference standard results interpreted without knowledge of the results of the index test? (9/13); and (10) Are the sensitivity and specificity results presented with their respective confidence intervals? (10/13).

Table 2 presents the results found regarding the included studies that showed 12 (92.3%) of them used ELISA and 1 (7.7%) performed an immunochromatographic lateral flow test. Different types of *M. leprae* antigens were implemented to diagnose leprosy (paucibacillary and multibacillary) from infected individuals and their household contacts in 5 (38.4%) studies. Besides that, seven studies compared the results with tuberculosis patients, two used other skin diseases and, one analyzed leishmaniasis patients to compare with leprosy.

In addition, 4 (36.4%) studies used a monoclonal antibody inhibition enzyme-linked immunosorbent assay based on the recombinant *M. leprae* protein, expressed from *M. smegmatis* 1 (8.3%) or *Escherichia coli* 3 (25%) – Group 1. On the other hand, the other 9 (69.2%) studies detected circulating IgM and IgG antibodies using the following antigens: native phenolic glycolipid (PGL-I) or its semisynthetic bioproducts as IDRI diagnostic-1 (LID-1) developed by fusing ML0405 and ML2331 genes of *M. leprae*; also glycoconjugate NDO-LID (ND-O is the synthetic natural disaccharide epitope of PGL-I that is covalently coupled to the LID fusion protein – LID-1); besides ND-O-BSA or ND-O-HSA antigens, natural disaccharide octyl bovine/human serum albumin – Group 2, for serological examination of leprosy patients (PB and MB) compared to tuberculosis, healthy control, and household contacts.

Regarding the leprosy classification, it was observed that most of the authors, 9 (69.2%), considered the operational classification of paucibacillary (PB) and multibacillary (MB) to compare the sensitivity and specificity results, however; some studies [18–20], correlated their results considering the bacilloscopic index and the smear positivity, respectively. Differently, Souza et al. [13] described the positivity of the LID-1 and PADL antigens to identify leprosy *per se*.

We also observed that the gold standard for the leprosy diagnosis used in some studies was the clinical evaluation criterion for the diagnosis. However, most of the same studies used as a comparative test, the detection of IgG or IgM antibodies against LID-1, PGL- 1 and ND-O-HSA or ND-O-BSA.

Concerning the specific characteristics, we observed a wide range of values to PB sensitivity from 3.57% of 3.5 kDa [12] to 47.4% of serine-rich 45-kDa protein of *M. leprae* (ML0411) [19], and to MB sensitivity varying from 42% of 18 kDa [21] to 100% of serine-rich 45-kDa protein of *M. leprae* (ML0411) [19].

Despite the heterogeneity related to sensitivity and specificity results, some tests were performed only in a restricted population. For example, the test that used serine-rich 45-kDa antigen

(ML0411) in the Indian population, whose sensitivity values were 47.4% to PB and 100% to MB, however, presenting discrete representativeness within the universe of people with leprosy.

Moreover, we observed that studies using the ND-O-LID conjugate have been able to accurately identify new cases of leprosy among people living in endemic or non-endemic areas and household contacts in Brazil [14–17], Colombia [7] and the Philippines [23]. This conjugated antigen, when combined with other biomarkers, can further improve sensitivity and specificity, encouraging conclusive decisions in leprosy diagnosis. Finally, low sensitivity values for PB patients remain challenging for tests intended to diagnose the clinical forms that comprise this classification in leprosy.

Discussion

Since the 1980s, with the prospect of identifying components isolated from *M. leprae* and *M. tuberculosis* [3,24,25], there has been a growing number of experimental trials using the immunogenic effects of these compounds to readily react with antibodies from leprosy patients [26–29].

A variety of antigens have been included such as the *M. leprae* surface phenolic glycolipid-I (PGL-I), and especially a number of recombinant proteins that detect antibodies against the pathogen. For some time, the focus has been the recognition of specific antibodies in patients with leprosy, household contacts, changes in the production of antibodies before and after multidrug therapy [30], leprosy reactions [31–33] and the early detection of the disease [34]. Some detection methods have found specific barriers especially in the recognition of antibodies to paucibacillary patients whose immune response against *M. leprae* is primarily cellular, and therefore does not present satisfactory sensitivity for such individuals [35,36]. On the other hand, these diagnostic serological tests are useful for the detection of MB leprosy because these individuals respond better by producing humoral immunity than PB patients [37,38].

Nowadays, only a few rapid tests for leprosy diagnosis have been developing and this review includes only one study in this way [23]. It is worth considering that rapid tests are able to offer more efficiency and better cost-benefit in public health systems. The ASSURED criteria, proposed by WHO, describes an ideal test for tropical diseases as being affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered, beyond to keep focusing on point-of-care approaches [39].

In this systematic review, as expected, the magnitude of the antibody responses varied as much for operational classification, paucibacillary or multibacillary, as for the way of obtaining the antigens, proteins expressed in bacteria or predicted by the *M. leprae* genome. MB leprosy patients presented higher levels of antibodies compared to PB and comparative groups as tuberculosis, leishmaniasis, other skin diseases, healthy control, and household contacts, as reported by other authors [23,40–42]. Consequently, the highest sensitivity and specificity values were observed for MB patients regardless of the antigens, and a broad range of accuracy results in the PB leprosy patients were found (Table 2).

Importantly, very sensitive/specific tests can correctly detect sick individuals, but also, can detect healthy patients who have previously been exposed to the bacteria, or who have been vaccinated with BCG. Therefore, in infectious diseases such as leprosy, tests whose sensitivity and specificity have very high values may not be so adequate, because the test may be positive, but the individual has not manifested the disease, and it may remain silent for many years before to exteriorize the specific cutaneous manifestations.

In this sense, the existence of heterogeneity in the recognition of antigens between individuals indicates that the immune response in front of the antigens from *M. leprae* could be distinctively defined. At the same time, it is also known that the genetic difference presented by individuals around the world may affect the pathogen response pattern, generating different sensitivity and specificity values, as previously reported [23,43].

Unfortunately, in the studies that compose this systematic review, there was no unanimity to define a protein antigen to be used in leprosy ELISA tests, maybe because of factors such as the experimental design of the research, the genetic variation of the populations that were investigated, a wide variation in the number of sample, the methodological standard used for the ELISA, the performance and recovery of antibodies after the test, and mainly, the cut-off points for the antigens in the ELISA optical density reading. These conditions imply variations in the values of sensitivity and specificity, and therefore in the diagnostic accuracy, resulting in numbers of individuals that may have been included as true or false positives and negatives from each of the studies included in this systematic review.

Some studies have indicated the specificity of approximately 100%, however, a few have included comparison groups for this calculation such as tuberculosis, healthy control, household contacts, and other infectious diseases with neuro-dermatologic manifestations similar to leprosy. Heterogeneity of the experimental design, the selection of the samples and the knowledge about whether it was a primary infection or whether the patients had received treatment were the main obstacles to the organization of this systematic review. Some potential researches were excluded because they did not present concrete values of sensitivity and specificity that would allow correlating the diagnostic accuracy of the protein under test. Thus, most of the evaluated antigens, appear to be beneficial for the quantitative measurement of antibodies to identify and select individuals with multibacillary leprosy and to support the diagnosis of paucibacillary leprosy.

Although all the researchers' efforts to identify *M. leprae* antigens, whether they from the bacterium itself or even recombinants, there is still no antigen with satisfactory sensitivity and specificity to be used as a diagnostic test for both polar leprosy clinical forms. It must be considered that the difference in the modulation of the immune responses of PB and MB forms corroborates this difficulty in the identification of antigens since the antibody production by these clinical forms is inherent and characteristic. As future perspectives, it is possible to optimize these antigens that can be used in diagnostic tests not only to identify leprosy but also to separate clinical forms, providing the correct treatment for people with leprosy.

Conclusion

The present systematic review showed that serine-rich 45-kDa (ML0411) antigen provided the highest values from ELISA assay for leprosy diagnosis, although tested only Indian patients. ND-O-LID conjugate was accurately able to identify new cases of leprosy cases than other recombinant antigens, among people living in endemic or non-endemic areas and household contacts in Brazil, Colombia, and the Philippines. However, low sensitivity values for PB patients remain challenging for tests intended to diagnose the clinical forms that comprise this classification in leprosy.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq - Brazil (RTF: Grant #303345/2018-7) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Brazil (FAPEMIG) (RTF: #APQ-04035-17).

Competing interests

The authors declare that they have no competing interests.

Author's contributions

ALGO conceived the study, participated in the conception, design, data collection, analysis and interpretation of the data and wrote this systematic review. NSG and VGF participated in the design, data collection, data analysis and helped to draft this systematic review. MSC, AGV, CASM, LLB, DCB, and RTF participated in the interpretation of data and helped to draft and critically revise this systematic review and, finally approved the version to be submitted. All authors read and approved the final manuscript.

References

[1] WHO. Weekly epidemiological record [Relevé épidémiologique hebdomadaire], vol. 94. Nova Deli; 2019.
[2] OMS. Estratégia global para hanseníase 2016–2020: aceleração rumo a um mundo sem hanseníase. Nova Deli: Escritório Regional para o Sudeste Asiático; 2017.
[3] Hunter, Brennan. Further specific extracellular phenolic glycolipid antigens and a related diacylphthiocerol from *Mycobacterium leprae*. J Biol Chem 1983;258:7556–62.
[4] Wu QX, Ye GY, Li XY. Serological activity of natural disaccharide octyl bovine serum albumin (ND-O-BSA) in sera from patients with leprosy, tuberculosis, and normal controls. Int J Lepr Other Mycobact Dis 1988;56:50–5.
[5] Duthie MS, Goto W, Ireton GC, Reece ST, Cardoso LPV, Martelli CMT, et al. Use of protein antigens for early serological diagnosis of leprosy. Clin Vacc Immunol 2007;14:1400–8, http://dx.doi.org/10.1128/CVI.00299-07.
[6] Spencer JS, Kim HJ, Wheat WH, Chatterjee D, Balagon MV, Cellona RV, et al. Analysis of antibody responses to *Mycobacterium leprae* phenolic glycolipid I. Lipoarabinomannan, and recombinant proteins to define disease subtype-specific antigenic profiles in leprosy. Clin Vacc Immunol 2011;18:260–7, http://dx.doi.org/10.1128/CVI.00472-10.
[7] Munõz M, Beltran-Alzate JC, Duthie MS, Serrano-Coll H, Cardona-Castro N. Comparison of enzyme-linked immunosorbent assay using either natural octyl disaccharide-leprosy IDRI diagnostic or phenolic glycolipid-I antigens for th tection of leprosy patients in Colombia. Am J Trop Med Hygiene 2018;98:274–7, http://dx.doi.org/10.4269/ajtmh.17-0500.
[8] Moher D. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement David. Syst Rev 2015;207:1–9, http://dx.doi.org/10.1186/2046-4053-4-1.
[9] Meyer GJ. Guidelines for reporting information in studies of diagnostic test accuracy: the STARD initiative. J Personal Assess 2003;81:191–3, http://dx.doi.org/10.1207/S15327752JPA8103_01.
[10] Oliveira MRF, de Gomes AC, Toscano CM. QUADAS and STARD: evaluating the quality of. Rev Saúde Pública 2011;45:6–11.
[11] Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36, http://dx.doi.org/10.7326/0003-4819-155-8-201110180-00009.
[12] Bezerra JM. Desempenho de antígenos nativo, recombinante e sintético, em testes imunoenzimáticos, para diagnóstico e prognóstico de pacientes com diferentes formas clínicas de hanseníase. Tese: Universidade de São Paulo; 2012.
[13] de Souza MM, Netto EM, Nakatani M, Duthie MS. Utility of recombinant proteins LID-1 and PADL in screening for *Mycobacterium leprae* infection and leprosy. Trans R Soc Trop Med Hygiene 2014;108:495–501, http://dx.doi.org/10.1093/trstmh/tru093.
[14] Amorim FM, Nobre ML, Ferreira LC, Nascimento LS, Miranda AM, Monteiro GRG, et al. Identifying leprosy and those at risk of developing leprosy by detection of antibodies against LID-1 and LID-NDO. PLOS Negl Trop Dis 2016;10:e0004934, http://dx.doi.org/10.1371/journal.pntd.0004934.
[15] Marçal PHF, Fraga LA, de O, de Mattos AMM, Menegati L, Coelho A, da CO, Pinheiro RO, et al. Utility of immunoglobulin isotypes against LID-1 and NDO-LID for, particularly igG1, confirming the diagnosis of multibacillary leprosy. Mem Inst Oswaldo Cruz 2018;113:1–8, http://dx.doi.org/10.1590/0074-02760170467.
[16] Leturiondo AL, Noronha AB, do Nascimento MOO, de Ferreira CO, da Rodrigues FC, Moraes MO, et al. Performance of serological tests PGL1 and NDO-LID in the diagnosis of leprosy in a reference Center in Brazil. BMC Infect Dis 2019;19:22, http://dx.doi.org/10.1186/s12879-018-3653-0.
[17] Gama RS, de Souza MLM, Sarno EN, de Moraes MO, Gonçalves A, Stefani MMA, et al. A novel integrated molecular and serological analysis method to predict new cases of leprosy amongst household contacts. PLOS Negl Trop Dis 2019;13:e0007400, http://dx.doi.org/10.1371/journal.pntd.0007400.
[18] Parkash O, Kumar A, Nigam A, Girdhar BK. Detection of antibodies against *Mycobacterium leprae* culture filtrate protein-10 in leprosy patients. J Med Microbiol 2006;55:1337–41, http://dx.doi.org/10.1099/jmm.0.46587-0.
[19] Parkash O, Kumar A, Nigam A, Franken KLMC, Ottenhoff THM. Evaluation of recombinant serine-rich 45-kDa antigen (ML0411) for detection of antibodies in leprosy patients. Scand J Immunol 2006;64:450–5, http://dx.doi.org/10.1111/j.1365-3083.2006.01824.x.
[20] Parkash O, Pandey R, Kumar A, Kumar A. Performance of recombinant ESAT-6 antigen (ML0049) for detection of leprosy patients. Lett Appl Microbiol 2007;44:524–30, http://dx.doi.org/10.1111/j.1472-765X.2006.02099.x.
[21] Roche PW, Prestidge RL, Watson JD, Britton WJ. Antibody responses to the 18-kDa protein of *Mycobacterium leprae* in leprosy and tuberculosis patients. Int J Leprosy Other Mycobacterial Dis 1992;60:201–7.
[22] Triccas JA, Roche PW, Britton WJ. Specific serological diagnosis of leprosy with a recombinant *Mycobacterium leprae* protein purified from a rapidly growing mycobacterial host. J Clin Microbiol 1998;36:2363–5.
[23] Duthie MS, Balagon MF, Maghanoy A, Orcullo FM, Cang M, Dias RF, et al. Rapid quantitative serological test for detection of infection with *Mycobacterium leprae*, the causative agent of leprosy. J Clin Microbiol 2014;52:613–9, http://dx.doi.org/10.1128/JCM.02085-13.
[24] Hunter SW, Gaylor H, Brennan PJ. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from leprosy and tubercle bacilli. J Biol Chem 1986;261:12345–51.
[25] Hunter SW, Brennan PJ. Evidence for the presence of a phosphatidylinositol anchor on the lipoarabinomannan and lipomannan of *Mycobacterium tuberculosis*. J Biol Chem 1990;265:9272–9.
[26] Levis WR, Meeker HC, Schuller-Levis GB, Gillis TP, Marino LJ, Zabriskie J. Serodiagnosis of leprosy: relationships between antibodies to *Mycobacterium leprae* phenolic glycolipid I and protein antigens. J Clin Microbiol 1986;24:917–21.
[27] Burgess PJ, Fine PEM, Ponnighaus JM, Draper C. Serological tests in leprosy, the sensitivity, specificity and predictive value of ELISA tests based on phenolic glycolipid antigens, and the implications for their use in epidemiological studies. Epidemiol Infect 1988;101:159–71, http://dx.doi.org/10.1017/s0950268800029320.
[28] Cho SN, Cellona RV, Villahermosa LG, Fajardo TT, Balagon MV, Abalos RM, et al. Detection of phenolic glycolipid I of *Mycobacterium leprae* in sera from leprosy patients before and after start of multidrug therapy. Clin Diagn Lab Immunol 2001;8:138–42, http://dx.doi.org/10.1128/CDLI.8.1.138-142.2001.
[29] Frota CC, Freitas MVC, Foss NT, Lima LNC, Rodrigues LC, Barreto ML, et al. Seropositivity to anti-phenolic glycolipid-I in leprosy cases, contacts and no known contacts of leprosy in an endemic and a non-endemic area in northeast Brazil. Trans R Soc Trop Med Hygiene 2010;104:490–5, http://dx.doi.org/10.1016/j.trstmh.2010.03.006.
[30] Hungria EM, Bührer-Sékula S, Oliveira RM, Aderaldo LC, Pontes MAA, Cruz R, et al. *Mycobacterium leprae*-specific antibodies in Multibacillary Leprosy Patients decrease during and after treatment with either the regular 12 doses multidrug therapy (MDT) or the uniform 6 doses MDT. Front Immunol 2018;9:1–11, http://dx.doi.org/10.3389/fimmu.2018.00915.
[31] Singh S, Narayanan NPS, Jenner PJ, Ramu G, Colston MJ, Prasad HK, et al. Sera of leprosy patients with type 2 reactions recognize selective sequences in *Mycobacterium leprae* recombinant LSR protein. Infect Immunity 1994;62:86–90.
[32] Araújo JMS. Reações Hansênicas: perfil clínico e resposta imunológica [Dissertação]. Universidade Federal de Sergipe; 2013.
[33] Hungria EM, Bührer-Sékula S, de Oliveira RM, Aderaldo LC, de Pontes AA, Cruz R, et al. Leprosy reactions: the predictive value of *Mycobacterium leprae*-specific serology evaluated in a Brazilian cohort of leprosy patients (U-MDT/CT-BR). PLoS Negl Trop Dis 2017;11:1–17, http://dx.doi.org/10.1371/journal.pntd.0005396.
[34] Carvalho RS, Foschiani IM, Costa MRSN, Marta SN, da Cunha Lopes Virmond M. Early detection of *M. leprae* by qPCR in untreated patients and their contacts: results for nasal swab and palate mucosa scraping. Eur J Clin Microbiol Infect Dis 2018;37:1863–7, http://dx.doi.org/10.1007/s10096-018-3320-9.
[35] Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. Clin Microbiol Rev 2006;19:338–81, doi:10.1128/CMR.19.2.338-381.2006. LK – http://findit.library.jhu.edu/resolve?sid=EMBASE&issn=08938512&id=doi:10.1128%2FCMR.19.2.338-381.2006 &atitle=The+ continuing+ challenges+ of+ leprosy &stitle=Clin.+ Microbiol.+ Rev. &title=Clinical+ Microbiology+ Reviews &volume=19 &issue=2 &spage=338 &epage=381 &aulast=Scollard &aufirst=D.M. &auinit=D.M. &aufull=Scollard+ D.M. &coden=CMIRE &isbn= &pages=338-381 &date=2006 &auinit1=D &auinitm=M.
[36] Geluk A, van der Ploeg-van Schip JJ, van Meijgaarden KE, Commandeur S, Drijfhout JW, Benckhuijsen WE, et al. Enhancing sensitivity of detection of immune responses to *Mycobacterium leprae* peptides in whole-blood assays. Clin Vacc Immunol 2010;17:993–1004, http://dx.doi.org/10.1128/CVI.00046-10.
[37] Britton WJ, Lockwood DN. Leprosy. Lancet 2004;363:1209–19, http://dx.doi.org/10.1016/S0140-6736(04)15952-7.
[38] Reece ST, Ireton G, Mohamath R, Guderian J, Goto W, Gelber R, et al. ML0405 and ML2331 are antigens of *Mycobacterium leprae* with potential for diagnosis of leprosy. Clin Vacc Immunol 2006;13:333–40, http://dx.doi.org/10.1128/CVI.13.3.333-340.2006.
[39] Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol 2019;4:46–54, http://dx.doi.org/10.1038/s41564-018-0295-3.
[40] Lobato J, Costa MP, Reis EDM, Gonçalves MA, Spencer JS, Brennan PJ, et al. Comparison of three immunological tests for leprosy diagnosis and detection of subclinical infection. Leprosy Rev 2011;82:389–401.
[41] da Fabri ACOC, Carvalho APM, Araujo S, Goulart LR, de Mattos AMM, Teixeira HC, et al. Antigen-specific assessment of the immunological status of various groups in a leprosy endemic region. BMC Infect Dis 2015;15:1–9, http://dx.doi.org/10.1186/s12879-015-0962-4.

[42] Penna MLF, Penna GO, Iglesias PC, Natal S, Rodrigues LC. Anti-PGL-1 positivity as a risk marker for the development of leprosy among contacts of leprosy cases: systematic review and meta-analysis. PLoS Negl Trop Dis 2016;10:1–11, http://dx.doi.org/10.1371/journal.pntd.0004703.

[43] Espinosa OA, Benevides Ferreira SM, Longhi Palacio FG, Cortela D, da CB, Ignotti E. Accuracy of enzyme-linked immunosorbent assays (ELISAs) in detecting antibodies against *Mycobacterium leprae* in leprosy patients: a systematic review and meta-analysis. Can J Infect Dis 2018:9828023, http://dx.doi.org/10.1155/2018/9828023.