Global Partnership for Zero Leprosy

Global Partnership for Zero Leprosy
Research Agenda Working Group
Subgroup on Diagnostics

Prepared by
Charlotte Avanzi, École polytechnique fédérale de Lausanne (EPFL), Switzerland
Sundeep Chaitanya Vedithi, American Leprosy Missions and University of Cambridge, United Kingdom
Malcolm Duthie, Infectious Disease Research Institute (IDRI), United States
Annemieke Geluk, Leiden University Medical Center, The Netherlands
Deanna Hagge, The Leprosy Mission, Nepal
Ana carla Latini, Instituto Lauro de Souza Lima, Brazil
Milton Moraes, Oswaldo Cruz Institute, Fiocruz, Brazil
Fareed Mizra, Novartis Foundation, Switzerland
Utpal Sengupta, The Leprosy Mission Trust India
Moises Batista da Silva, Pará Federal University, Brazil
Itu Singh, The Leprosy Mission Trust India
Pushpendra Singh, The Maharaja Sayajirao University of Baroda, India
Ravindra Turankar, The Leprosy Mission Trust India, Stanley Browne Research Lab, India

In consultation with
Flavio Alves Lara, Oswaldo Cruz Institute, Fiocruz, Brazil
Kidist Bobosha, Armauer Hansen Research Institute, Ethiopia
Shintouo Cabirou Mounchili, University of Buea, Cameroon
Emmanuelle Cambau, National Reference Center for Mycobacteria of France, Universite Paris Diderot, Paris Hospitals, France
Thomas Cummings, Duke University Medical Center, United States
Andrea de Faria Fernandes Belone, Instituto Lauro de Souza Lima, Brazil
Ida Maria Foschiani Dias-Baptista, Instituto Lauro de Souza Lima, Brazil
André Luiz Leturiondo, Alfredo da Matta Foundation, Brazil
Juliana de Moura, Universidade Federal do Paraná, Brazil
Cynthia de Oliveira Ferreira, Alfredo da Matta Foundation, Brazil
Patricia Rosa, Instituto Lauro de Souza Lima, Brazil
Jenny Laura Ruiz Fuentes, Tropical Medicine Institute Pedro Kourì, Cuba
Fabiana Santana, Instituto Lauro de Souza Lima, Brazil
Eliane Silva, Instituto Lauro de Souza Lima, Brazil
Vânia Souza, Instituto Lauro de Souza Lima, Brazil
Aparna Srikantam, LEPRA -Blue Peter Public Health and Research Center, India
Sujai Suneetha, Institute for Specialized Services in Leprosy (INSSIL), Nireekshana ACET, India
Dhelya Widasmara, Saiful Anwar Hospital and Brawijaya University, Indonesia

Introduction

Although leprosy is caused by an infectious agent (*Mycobacterium leprae* or *M. lepromatosis*), most of the heavily exposed population—the household and family members of patients—will not develop leprosy during their lifetimes. This group is considered at highest risk for developing leprosy, but only 3%–5% will progress to the disease. Inherent to the current method of diagnosis of leprosy (i.e., detection of clinical symptoms such as skin patch with loss of sensation, enlarged peripheral nerves), the disease is often diagnosed late. Furthermore, although multidrug therapy (MDT) is effective, the number of new cases has been stationary for the past 15 years—indicating that treatment does not block transmission. In the past 25 years, immunoprophylaxis (with BCG vaccination) and, more recently, chemoprophylaxis (e.g., single-dose rifampin [SDR]) have proved effective in preventing leprosy in household contacts. Indeed, 2018 WHO guidelines support this chemoprophylaxis approach, which is likely to be a successful, short-term strategy aimed at identifying new cases and treating healthy social and household contacts to impact incidence. Nevertheless, efforts are clearly needed to improve early identification of leprosy patients and to identify and treat infected persons—especially in low-to-middle endemic areas where the use of large-scale chemoprophylaxis would not be cost-effective in controlling transmission and reducing incidence. To reach these goals and contribute to zero leprosy, progress is needed in clinical, laboratory-based diagnosis as well as translation of the latter to rapid, user-friendly field tests.

Overview and Current Activities of Leprosy Diagnostics

Over the past 40 years, biochemical studies identifying PGL-I, the sequencing of the *M. leprae* genome, and consortia such as the Initiative for Diagnostic and Epidemiological Assays for Leprosy (IDEAL) have been landmarks in the development of leprosy diagnostic tests. Initially, detection of humoral and cellular immune host-derived biomarkers with ELISAs were used for detection of antibodies and cyto/ chemokines, respectively (1), whereas molecular diagnostic assays were applied to detect pathogen-derived molecular (DNA/RNA). More recently, for both host immune response-based assays as well as PCR-based assays, technological advances are enabling better performance as well as improved, minimally invasive point-of-care (POC) format through novel versions of the above-mentioned techniques (2-5). Comparison of test platforms as well as large-scale evaluation in multiple endemic areas has been widely investigated for antibody-based tests (6), and the performance of anti-PGL-I Ab based assays has been extensively described in the literature for decades (7). However, for pathogen-based qPCR assays as well as cellular immunity-based rapid field-tests, although both field-tested in multiple areas with different levels of endemicity (2,3,5), there have been few independent and consistently replicated results of large sample size in coherent experimental designs in multicentric studies.

In several studies, data have been presented on pathogen detection using qPCR with different targets (8), host immunity-based serological assays based on using either PGL-I or NDO-LID (9) or cellular assays based on cytokine/ chemokine release assays (2,3). There is vast evidence indicating that anti-PGL-I IgM or NDO-LID can be used in multibacillary (MB) leprosy diagnosis, although seropositivity in endemic areas can be found in numerous individuals who will never develop leprosy. However, PCR improves identification of paucibacillary (PB) leprosy as complementary to histological analysis (5). Moreover, combined detection of humoral (antibodies) and cellular (cytokines) biomarkers significantly improves their diagnostic potential, for both MB and PB leprosy (3). Although most of the current products/tests have been developed "in house," large-scale (population-based) studies using rapid test to detect anti-*M. leprae* antibodies are currently ongoing (EDCTP-funded PEOPLE study). Although rapid tests detecting cytokines/chemokines were field-tested in areas on three continents where leprosy is still endemic (3), larger scale studies are needed to provide proper sensitivity and specificity data.

Among the challenges to leprosy diagnostics that should be the focus of research, the Subgroup on Diagnostics of the Global Partnership for Zero Leprosy (GPZL) Research Agenda Working Group outlined the following:

- The bacteria do not grow in vitro in regular culture media
- There is no definitive gold standard method for diagnosis
- Bacteria silently infect nerves and skin cells, subverting immunological responses; hence, there are few early clear signs of the disease that could distinguish active disease from infection
- There is no reliable marker to estimate infection and risk to disease progression
- Among clinical forms of PB leprosy, the bacteria are virtually undetectable using any testing techniques, although qPCR has demonstrated advances in sensitivity. In addition, indirect methods based on simultaneous detection of host humoral as well as cellular immune response directed against the bacteria provide promise as new tools.
- Clinical presentations among persons with leprosy differ widely, and several other diseases present the same phenotypes—especially for the PB forms

The Subgroup outlined two main diagnostic-related needs to achieve zero leprosy:

1. The ability to conduct early and specific diagnosis of leprosy and *M. leprae* infection to block transmission using affordable, rapid POC tests in low-resourced settings.
2. The ability to screen exposed individuals to detect those who are infected. The use of chemo- and immuno-prophylaxis (see report from Subgroup on Vaccines) in low-to-middle endemic areas could help identify this group more precisely in the future.

Research Priorities and Key Questions

Potential Use of Digital Technologies to Help Improve Clinical Diagnosis

Five clinically recognized forms of leprosy are classified by Ridley and Jopling (10). For operational and treatment purposes, however, the disease can also be presented in PB and MB forms using the number of lesions as a proxy for the number of bacilli found in lesions or specific sites (e.g., ear lobes, elbows, knees). Clinical features of PB and MB leprosy are very different. The chance of a successful diagnosis based entirely on dermatological (sometimes associated with neurological) symptoms is reduced in the PB forms, especially during early presentations since other dermatological diseases such as ptiriasis alba, granuloma annullare, and sarcoidosis can present with similar lesions. In a group of more than 1000 patients referred to a center with expert dermatologists, 90% were diagnosed based on clinical features without skin biopsies for histological or molecular analysis, indicating that intensive education is necessary to train experts for field identification (11). In clinical classification, there continues to be a need for an atlas with high-resolution images.

Among PB forms, laboratory tests are not currently available to confirm either tuberculoid (TT) or borderline-tuberculoid (BT) leprosy. The bacteria are not detected in slit skin smears from lesions or other sites (ear lobes, knee, or elbow). Clinically, PB leprosy is presented as plaque (or up to five plaques and sometimes macula) of varying sizes, generally erythomatous or hypopigmentated with sharply raised and well-defined borders in TT and less defined in BT. These are broad at the periphery and flattened in the center, where the involvement of intradermal neural branches rapidly accentuates, resulting in sensorial autonomic alterations that evolve to hypoesthesia, anesthesia, and dry and hypohydrotic skin, with rarefaction of hairs. The presence of satellite lesions is also observed.

Among the MB forms of leprosy, the detection of the bacilli is possible in borderline-borderline (BB), borderline-lepromatous (BL), and lepromatous leprosy (LL) patients. Thus, when MB leprosy is suspected and microscopy analysis is available, diagnosis is easier. Also, for BB forms, cutaneous lesions are characterized by the presence of erythematous plaques with poorly delineated outer borders and a hypopigmented oval center with well-defined internal borders. Small satellite lesions can also be seen.

Typical of this form is the foveolar lesion, which has a tendency towards symmetrical distribution. BL leprosy shows lesions such as macules, papules, plaques, or nodules. The lesions are numerous, asymmetrical, partially anesthetic, and not as shiny and edematous as the BB form. Some plaques are very large, while others have foveolar appearance and may have nodules. The finding of thickened peripheral nerves is common.

LL is characterized by macules, infiltration, papules, nodules, and tubers. The lesions in general tend to symmetry, and characteristics of systemic disease are present. Another form called indeterminate is considered early and progresses towards either tuberculoid or lepromatous poles. Indeterminate leprosy is characterized by a hypochromic or discretely erythematous macule with altered sensitivity and alopecia or occurs in a hypoesthetic area, with no visible lesions.

- *Key questions*
  - Could the use of high-resolution images and artificial intelligence improve confirmation of suspected leprosy?
  - Could artificial intelligence be used to screen skin biopsies hematoxylin and eosin (H&E) stains or slit skin smears slides for unrecognized patterns to help detect tissue patterns or bacilli to improve diagnosis?
  - Could cutaneous thermography be used as a complementary diagnostic method, with or without ultraviolet photography? Thermography is capable of rapidly and dynamically measuring the thermal energy of large areas of the skin through the generation of images up to 1 million tones, representing differences of up to 0.01°C. It is a non-invasive, safe, and inexpensive technique. It would also make it possible to remotely perform leprosy diagnosis in the most prevalent and poorest areas of the world by sending images to reference centers.

Nucleic Acid-based Tests

qPCR is probably the best method to confirm disease among PB patients. However, two main issues need to be addressed to validate their use for disease confirmation: 1) several different targets are available; and 2) most of the published qPCR data use research reagents and not GMP products, which are designed for diagnostic purposes.

- *Key questions*
  - Is there a method that could improve sensitivity and specificity in qPCR, including reproduction of results? There is an urgent need for independent confirmation, larger sample sizes, and combination of the best methods or mechanisms to harmonize testing of different assays in different laboratories using external quality assessment (EQA). In this regard, minimal requirements for best practices in qPCR in leprosy diagnosis are needed. While specificities from different countries should be considered, tests should be globally validated.
  - Are better sampling methods available for direct/indirect detection of *M. leprae* or DNA/RNA for use in diagnostic confirmation? Current methods rely on slit skin smears and biopsies that are invasive and painful. Novel, less invasive, and affordable methods are needed.
  - Could other diagnostic methods be developed? The use of loop-mediated isothermal amplification (LAMP) in leprosy molecular diagnosis is a relatively new DNA amplification technique. Because of its simplicity, ruggedness, and low cost, LAMP could be soon the method of choice for molecular diagnosis of leprosy but needs extensive validation.

Drug-Resistance Surveillance

The number of new cases of primary resistance, especially for rifampicin, depends on treatment adherence and completion rates for MB cases. It is estimated that resistance is increasing in different countries, although no systematic surveys/queries have been performed. PEP protocols are spreading, and their impact on drug resistance needs to be evaluated in the long term.

- *Key question*
  - Is the number of *M. leprae* resistant strains increasing, especially in endemic countries?
  - Are there other mechanisms for drug resistance, especially for clofazimine?

Reactions and Relapses (including in the context of resistance)

Leprosy is a phenotypic diverse disease, and patients can undergo reactional episodes. One of the most difficult issues is to discriminate relapses from reactions. Since reemergence of the disease could be associated with resistance, direct screening is necessary to ensure adequate treatment.

- *Key questions*
  - Since qPCR or other molecular available techniques could be developed to directly detect MB leprosy, as well as primary resistance to avoid ineffective treatment, is it possible to develop a duplex or triplex qPCR also targeting the most frequent resistant SNPs in rpoB?
  - Could a new test be developed to detect bacterial viability? The direct detection and estimation of molecular bacilli viability in fresh or fixed clinical samples would help improve management of relapse cases (live mycobacteria) by distinguishing from reactional states (dead mycobacteria)
  - Concerning reactions, is it possible to define markers or a score to estimate the a patient's risk of developing reactions?

Diagnostic test based on detection of host immunity

Serological methods detecting antibodies against *M. leprae* antigen such as NDO-LID or PGL-I are not sensitive enough to detect PB leprosy. Besides the presence of anti-M.leprae antobdies is not predictive for disease. Current strategies such as detection of blood-based cytokines by POC lateral flow assays offer diagnostic advantages and have been tested in different countries. These strategies should be further evaluated in larger study designs. For serological assays, some strategies can be used to achieve specificity and higher sensitivity. These include 1) employing conformational or linear immunodominant epitopes selected from products of the patient's immune response and 2) using these epitopes as bait for specific antibodies on label-free biotechnological platforms (12).

- *Key questions*
  - Can large scale multicenterc studies be undertaken to validate diagnostic potential for MB and PB leprosy of POC lateral flow assays for (simultaneous) detection of multiple cytokines/ chemokines and provide proper data on specificity and sensitivity of lateral flow assays, using a defined biomarker signature including markers for humoral and cellular immunity?
  - Can large scale multicenter longitudinal studies be conducted for early detection of leprosy in high risk populations using a defined biomarker signature including markers for humoral and cellular immunity?
  - Can large scale multicenter longitudinal studies be conducted for monitoring treatment responses in patients using a defined biomarker signature including markers for humoral and cellular immunity?

Other Diagnostic Issues

The use of genomics has pinpointed novel pathways that are activated or deactivated upon infection either in blood or tissue (skin and nerves). Also, SNPs have been identified as being associated with disease outcome (13).

- *Key questions*
  - Although a panel of blood-based host transcriptomic biomarkers has been described, can more extensive data be obtained (particularly data on infected individuals developing disease) in order to determine markers associated with (early) disease?
  - Can a panel of SNPs be used to estimate the risk of developing disease?

There are no tools, culture media, or techniques available to aid *M. leprae* growth, making the identification and characterization of *M. leprae* difficult. Recently, tick-cell lines have been described as tools to grow *M. leprae* (14).

- *Key question*
  - Would the use of tick-cell lines be feasible for confirmation of *M. leprae* diagnosis?
  - Would their use be feasible for antibiotic resistance and drug discovery screening?

Testing for Infection

Achieving zero leprosy will require better tools for disease control. It will be necessary to predict among the risk population which individuals have the highest chance of progressing to disease. Defined markers are needed to test whether a specific panel, signature, or response could anticipate leprosy progression among contacts or the general population. It is important that these tools be used in the near future in low- and middle-endemicity countries/areas where screening of risk populations prior to chemo- and immunoprophylaxis would be cost-effective.

- *Key questions*
  - Could a panel of genetic polymorphisms or transcripts or metagenomic markers be defined to scrutinize high risk contacts?
  - Is it possible to have a next-generation skin test (for example, based on recombinant proteins) to screen infected people?
  - Can novel, low-complexity lateral flow assays based on fingerstick blood provide a means for POC triage testing for infection by measuring both antibodies and cyto/chemokines in capillary blood?
  - Can a combined field-friendly test with a smartphone app be developed for follow up of at-risk individuals and patients to increase testing and population coverage in leprosy endemic areas.

Non-human Reservoirs

Issues surrounding non-human reservoirs for leprosy deserve attention and may impact diagnosis. Recently, armadillos and red squirrels were reported as natural hosts that also develop the disease after infection with *M. leprae* or *M. lepromatosis*. These results have provided novel hypotheses concerning *M. leprae* transmission that could influence leprosy epidemiology and control.

- *Key questions*
  - Are there reservoirs and transmission routes other than human-human in leprosy? An improved and integrated view of the natural course of the disease could help establish life cycles.
  - Does leprosy in non-humans exhibit an infection stage and later an active disease stage (a two-step leprosy progression) that could be used as model of leprosy development?
  - Could non-human models be tested for leprosy progression?

Genomics could be used to better understand phylogeography and perhaps depict novel virulence factors. Whole, large-scale genomics could be used to help determine strains/SNP type/haplotype associations isolated from different clinical forms of the disease.

Conclusions/Recommendations

Early diagnosis can help stop transmission and improve leprosy control. Although novel tools with the potential for use in leprosy control exist, they must be scalable, GMP produced, field friendly (i.e. low complexity), low cost, and adaptable to different endemicity

As research priorities to ensure the capability for early diagnosis needed to achieve zero leprosy, the Subgroup on Diagnostics recommends the following

- Diagnostic assays (qPCR for pathogen, host immune response assays, host transcriptomic assays) should be harmonized and validated globally through multicentric studies. As part of this effort, standardization and quality assurance programs should be implemented to compare these tools providing grants are available for these efforts.
- Less invasive sampling methods should be developed
- Although reasonable sensitivity and specificity have been achieved with currently available methods, new methods using biomarker discovery, mycobacteria viability, cell culture, and risk factors modeling should be developed for improved (next-generation) diagnostic tools
- Transmission research (intermediary host, vectors) may impact diagnostics, epidemiology, surveillance, and control and should be prioritized
- Tools should be used either to confirm leprosy when patients present suspicious lesions or to screen and follow-up high risk individuals.

Longitudinal studies will allow identification of better markers associated with disease progression. Future studies should involve evaluation of several assays at different laboratories/field sites globally using identical protocols and allowing overall accessibility in open (multi-disease) platforms for independent confirmation and validation.

References

1. Geluk A, Bobosha K, van der Ploeg-van Schip JJ, et al. New biomarkers with relevance to leprosy diagnosis applicable in areas hyperendemic for leprosy. J Immunol 2012;188(10):4782-4791.
2. van Hooij A, Tjon Kon Fat EM, Richardus R, et al. Quantitative lateral flow strip assays as user-friendly tools to detect biomarker profiles for leprosy. Sci Rep 2016;6:34260.
3. van Hooij A, Tjon Kon Fat EM, Batista da Silva M, et al. Evaluation of immunodiagnostic tests for leprosy in Brazil, China and Ethiopia. Sci Rep 2018;8(1):17920.
4. Corstjens PLAM, van Hooij A, Tjon Kon Fat EM, et al. Fingerstick test quantifying humoral and cellular biomarkers indicative for *M. leprae* infection. Clin Biochem 2019;66:76-82.
5. Barbieri RR, Manta FSN, Moreira SJM, et al. Quantitative polymerase chain reaction in paucibacillary leprosy diagnosis: a follow-up study. PLOS Negl Tropl Dis 13(3): e0007147. https://doi.org/10.1371/journal.pntd.0007147.
6. van Hooij A, Tjon Kon Fat EM, van den Eeden SJF, Wilson L, Batista da Silva M, Salgado CG, et al. Field-friendly serological tests for determination of M. leprae-specific antibodies. Sci Rep. 2017;7(1):8868.
7. Spencer JS, Kim HJ, Wheat WH, 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 Vaccine Immunol 2011;18(2):260-267.
8. Martinez AN, Talhari C, Moraes MO, Talhari S. PCR-based techniques for leprosy diagnosis: from the laboratory to the clinic. PLoS Negl Trop Dis 2014;8(4):e2655.
9. Penna ML, 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(5):e0004703. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0004703

10. Ridley DS, Jopling WH. Classfication of leprosy according to immunity: a five-group system. Int J Lepr Other Mycobact Dis 1966;34:255-273.
11. Barbieri RR, Sales AM, Hacker MA, et al. Impact of a reference center on leprosy control under a decentralized public health care policy in Brazil. PLOS Negl Trop Dis October 12, 2016. https://doi.org/10.1371/journal.pntd.0005059
12. de Santana JF, da Silva MRB, Picheth GF, et al. Engineered biomarkers for leprosy diagnosis using labeled and label-free analysis. Talanta 2018;187:165-171.
13. Fava VM, Schurr E. The complexity of the host genetic contribution to the human response to *Mycobacterium leprae*. In: Scollard DM, Gillis TP, eds. The International Textbook of Leprosy. Available at https://www.internationaltextbookofleprosy.com/sites/default/files/ITL_8_1%20FINAL.pdf.
14. Ferreira JDS, Souza Oliveira DA, Santos JP, et al. Ticks as potential vectors of *Mycobacterium leprae*: use of tick cell lines to culture the bacilli and generate transgenic strains. PLoS Negl Trop Dis 2018;12(12):e0007001.