Lepr Rev (2019) **90,** 183–200

WORKSHOP REPORT

Meeting report: the Leprosy Research Initiative Spring Meeting

LRI Scientific Review Committee, LRI Steering Committee

Accepted for publication 4 June 2019

On 4th and 5th April, 2019 the fourth annual Leprosy Research Initiative (LRI) Spring Meeting was held in Veenendaal, the Netherlands with a total attendance of 75 participants from 21 countries among whom were representatives from the Turing Foundation (LRI's co-funder), the Global Partnership for Zero Leprosy (GPZL), the European and Developing Countries Clinical Trial Partnership (EDCTP), Novartis Foundation and R2STOP. As in previous years, the meeting was devoted to presentations on the progress and results of LRI funded projects, and representatives of all funded projects were invited to participate. In addition, for the second time representatives of projects funded by R2STOP participated in the meeting. Moreover, it serves as a meeting point where researchers can meet each other and can make new connections.

In total there were 33 presentations: 22 long and 11 short presentations. Long presentations were from participants who presented data from their research, whereas short presentations were from recently started projects. In addition to the progress presentations, the representative from the EDCTP presented the upcoming EDCTP funding opportunities. The afternoon of the second day was devoted to the GPZL, starting with a plenary session with speakers addressing different aspects of the GPZL and LRI research agenda, followed by discussion groups.

Many positive features about this fourth LRI Spring Meeting were noted: first, since several projects were nearing completion or were already completed more and definite data could be presented; second, a very enthusiastic and engaged group of researchers participated in very valuable discussions following the presentations; third, the meeting provided ample opportunity to interact; fourth, seeing quite a number of young investigators from many different countries was a hopeful sign for the future of leprosy research.

Basic Science

The meeting featured 12 reports on LRI-funded basic science projects, and three presentations on R2STOP-funded basic science projects, all with direct relevance to clinical and field work in leprosy.

Correspondence to: Nienke Veldhuijzen, Leprosy Research Initiative, Amsterdam, Netherlands (e-mail: n.veldhuijzen@leprosyresearch.org)

0305-7518/19/064053+18 $1.00

Probably the greatest 'buzz' surrounded the report of an attempt to develop a mouse footpad model to assess potential post-exposure chemoprophylaxis (PEP) regimens (Annex 1). Dr. Shannon Lenz (National Hansen's Disease Research Program, USA) presented data from experiments in which immune-deficient nude mice had received a low dose of *M. leprae,* and molecular methods were used to count the bacilli, making the model much more sensitive. The unexpected finding, that a single dose of rifampicin (SDR) did not prevent the multiplication of *M. leprae*, could be explained by the model reflecting the outcome in highly sensitive and/or highly exposed individuals who have already developed early infection. This finding thus appears to provide laboratory evidence consistent with the observation that SDR PEP provides the least benefit to the closest contacts. Additionally, in this model success was achieved in preventing the development of infection when 2-drug regimens were given once a month for 3 months. This is welcome news regarding the multi-drug, multi-dose regimens being considered for 'PEP++' trials. Together, the results indicate that further studies using this model might be valuable in planning future PEP approaches.

Understanding early events in the development of leprosy, and potential methods for early detection, were the objectives of several studies underway. One presentation in this area that generated great interest was given by Ms Anouk van Hooij (Erasmus University/LUMC, Netherlands), concerning an extensive study of diagnostic tests that combine the detection of markers of cellular immunity (CMI) with tests for antibodies to *M. leprae* (Annex 2). Their findings are very promising for the development of better diagnostic field tests using a combined assessment of humoral and cellular immunity, as well as for improved understanding of the immunological changes that differentiate contacts who develop infection with *M. leprae* from those who resist infection.

Several reports detailed progress in the application of molecular methods for diagnosis and in understanding the development of infection. Dr. Milton Moraes (FIOCRUZ, Brazil) reported that quantitative PCR does not appear to have good predictive value as an early diagnostic test in household contacts, but is very useful in confirming the diagnosis of suspected leprosy, especially when combined with histopathology. Further work is underway to standardise this method and better define the cut-off values that can reliably be used to verify the presence of low numbers of *M. leprae* in biopsies. Discussion emphasised the need for markers to distinguish subclinical infection with *M. leprae* from simple exposure to the organism.

Another collaborative study (India and the USA), presented by Ms Purna Dwivedi (Department of Microbiology & Biotechnology Centre, Maharaja Sayajirao University of Baroda, India) and funded by R2STOP, reported early results in efforts to determine if there are pathologic variants of *M. leprae* associated with different pathological outcomes. They have found that samples from India, all of which had high bacterial loads, were all of one genotypic group. Future plans of this study are to assess whether there are genomic differences in the *M. leprae* associated with PB vs MB disease.

Two presentations concerned studies of genes associated with susceptibility to leprosy. Prof. Marcelo Mira and colleagues (Pontifical Catholic University of Paraná, Brazil), looking for mutations within human genes that may be associated with leprosy, reported on an analysis of 73 human genes. Analysis of variants in the genes of the individuals included in the study led to the identification of 37 DNA changes distributed between 24 genes that may increase the chance of an individual developing leprosy after contact with *M. leprae*. Work on this continues, with hope that it will ultimately lead to identification of markers that will be useful in the prevention and management of leprosy. In a second (short) presentation by Prof.

Marcelo Mira and colleagues, the group presented their plans to look at variants in a family with many affected persons, the Piaui family.

A study funded by R2STOP has attempted to identify pathogen-based transmission patterns of *M. leprae*. Ms Maria Tio-Coma (representing a collaboration between The Leprosy Mission International, Bangladesh; LUMC, The Netherlands and Erasmus University, The Netherlands) presented preliminary results showing that a high percentage of skin smear and nasal swab samples from MB and PB patients and household contacts are PCR (RLEP)-positive, and that whole genome sequencing, genotyping and testing for mutations can be performed on a large percentage of them. This group has also published their findings of positive PCR, sequencing, and genotyping of *M. leprae* from soil samples. These technological advances hold great promise for further studies of potential environmental reservoirs of *M. leprae*, although there is no evidence yet regarding viability or infectivity of the bacilli found in soil.

The earliest events after *M. leprae* infection cannot be observed in human studies, but can be studied in animal models in which the time, dose, and route of infection are known. Dr. Pushpendra Singh (Department of Microbiology & Biotechnology Centre, Maharaja Sayajirao University of Baroda, India) reported on preliminary results using a novel approach to detect molecular immunological events occurring just four months after infection with *M. leprae* in susceptible vs resistant armadillos. This is far earlier than that can be studied in human infection, due to the long incubation time in humans. When results in these two armadillo groups were compared, up- or down-regulation of 72 genes was observed at this early time-point. The findings called particular attention to genes controlling 'notch' and other molecular signalling mechanisms. This very innovative approach requires highly sophisticated techniques for molecular analysis of gene activation, and doing this in armadillos entails unique difficulties. However, the armadillo offers a model of both naturally susceptible and resistant individuals, and thus offers great promise in unravelling early changes that might become the basis for early diagnostic tests for human leprosy.

Leprosy reactions remain a major problem in the clinical management of leprosy, and immunological and patho-genetic characteristics were the subject of two presentations. A study of helminth infections and their relationship to reactions offered a look at one possible trigger for reactions. Dr. Deanna Hagge and colleagues (Mycobacterial Research Labs, The Leprosy Mission Nepal) assessed the correlation between leprosy reactions and helminth infections and the impact of de-worming on the development of reactions. Their findings suggest that helminth infections may affect the immune system in such a way as to suppress reactions. Both helminths and leprosy bring unique challenges for research, but these findings are of value both clinically and in suggesting further avenues for the study of the immunologic mechanisms underlying leprosy reactions.

The other report considered possible pathogen variations related to reactions, by examining RNA transcripts from *M. leprae* to determine if any are associated with leprosy reactions. Ms Madhusmita Das and colleagues (Schieffelin Institute of Health- Research & Leprosy Centre, India) amplified RNA from *M. leprae*, and the over-or under-expression of many genes was identified in patients with Type-1 or -2 reactions when compared to those with no reaction. From these studies, a small number of protein antigens appear to be of particular interest with respect to Type 2 reactions. These will be evaluated for their possible utility in the diagnosis and study of Type 2 reactions.

Dr. Jessica Fairley (Emory University, USA) gave a short presentation on different host-pathogen metabolite signatures for patients with active leprosy, leprosy reactions and

helminth co-infections. Future work will involve comparison of metabolic pathways among the groups.

In a delightful and provocative presentation, Prof. Bill Jacobs (Georgia State University, USA) reported on his laboratory's attempts, funded by R2STOP, to find a way to insert genes into *M. leprae* that would enable it to grow in culture, so that rapid progress could be made in the development of diagnostic tests, drug development, and vaccines. As a first step, his laboratory has studied *M. haemophilum*, which needs a special medium to provide substances needed for iron metabolism in order to grow in culture. They have succeeded in inserting iron-metabolism genes from other mycobacteria into *M. haemophilum*, and the resulting organism is able to metabolise iron normally and can grow in ordinary media. They have not yet succeeded in inserting 'missing' genes into *M. leprae*, but are very committed to continuing this line of research.

Clinical and public health research

The clinical presentations focused in particular on the understanding and management of reactions and the prevention of nerve damage and disability, which remain the most significant challenges in clinical leprosy. Better identification of new cases is another challenge requiring further research – this has been given a greater emphasis recently with the drive to give chemoprophylaxis to contacts of new cases, who must be identified before their contacts can be traced and treated. The public health presentations developed the theme of the prevention of leprosy through post-exposure prophylaxis (PEP), the treatment given to contacts to prevent new cases and eventually reduce transmission. In total, nine presentations were given, four long and five short presentations.

Dr. Marivic Balagon (Leonard Wood Memorial Center for TB & Leprosy Research, Philippines) presented a study being carried out in Cebu, Philippines, which looks at the possibility of patients themselves recognising a leprosy reaction and possible nerve damage, and the need for prompt treatment. This is especially important for patients living a long way from the nearest health unit, and in low endemic areas where health staff may be very unfamiliar with leprosy and its complications. Patients were taught how to score their skin lesions using the Lesion Assessment Severity Index (LASI), giving a score of 0–15. This score correlated well with a score done by one of the clinic staff. The scores also correlated closely with the development of a reaction and nerve involvement was strongly associated with reaction in an overlying lesion (Annex 3). Final follow-ups and analysis will follow.

A collaboration between the Infectious Disease Research Institute (IDRI, USA) and The Leonard Wood Memorial (Philippines), presented by Dr. Malcolm Duthie (IDRI, USA), found that the idea of serological screening of family/community members exposed to leprosy was highly acceptable among the general population, and that the great majority of patients also accepted serological surveillance during and after treatment as a means to assess progress. The results of serological screening during multi-drug therapy (MDT), using the LID-1 antigen, showed a clear decline in antibody levels among MB patients during the first 24 months and longer, although the number of patients followed beyond 24 months was quite small. Notably, patients who developed reactions during treatment and follow up had higher initial antibody levels that in those who did not develop reactions. Altogether, these findings suggest that such screening and follow up may be useful in medical management of leprosy.

Prof. Paschal Kum Awah (representing a collaboration between FAIRMED, Cameroon and the University of Yaoundé, Cameroon) presented a study from Cameroon, still in its early stages, which looked at the diagnosis of so-called 'Skin-NTDs' – namely, neglected tropical diseases presenting with skin lesions, including leprosy, Buruli ulcer, yaws and cutaneous leishmaniasis. An important innovation was to use the recently developed SkinApp from NLR (Netherlands) to aid diagnosis, and then another smartphone app to collect and upload the data. The goal is to improve the diagnosis and reporting of leprosy and related skin diseases. Obtaining ethical clearance took more time than expected and led to some delays, but the study is now underway.

Improving early detection through community-awareness methods was the focus of a study in India. In this study, Dr. Karthikeyan Govindasamy (The Leprosy Mission Trust, India) and colleagues evaluated the effectiveness of three types of community-awareness methods for the early detection of leprosy. The different awareness activities targeted non-formal health providers, the index patient or the community at large, resulted in more new cases, fewer impairments and a higher proportion of PB cases – all suggesting earlier detection. It may be noted that activities targeting the whole community had the largest effect.

Ms Barbara de Barros (London School of Hygiene and Tropical Medicine, UK) presented on behalf of the ENLIST consortium, which is about to start two randomised controlled trials of methotrexate in erythema nodosum leprosum (ENL). In these studies, methotrexate plus prednisolone will be compared with standard treatment (prednisolone alone). The studies will be conducted across 7 countries. ENL is difficult to treat and often becomes a chronic condition with severe damage to quality of life. One study will look at the treatment of an acute attack, while the second study will treat recurrent and chronic cases. The goals include identifying a better treatment regimen for ENL, and at the same time reducing the use of steroids, which are responsible for most of the adverse effects of current treatment, including a significant mortality.

Mr David Prakash Kumar (Schieffelin Institute of Health- Research & Leprosy Centre, India) presented work on a new offloading device to promote healing of plantar ulcers. The new device uses computer-aided-design and 3D printing to produce aesthetically pleasing products, in order to be more acceptable to patients.

Mr Jiptha Boiragee (The Leprosy Mission International, Bangladesh) presented a project on patient migration in Bangladesh, which is nearing completion. The project used interviews to find out why so many new cases are registered in the capital, Dhaka, when they actually live elsewhere. Almost two-thirds (62%) of names on the MDT registers are from out of the area. The reasons included the lack of adequate treatment in the more remote areas, the perception that better treatment and better employment opportunities would be available in Dhaka, and the fear of stigma and discrimination in the home area. Better mapping of where new cases come from may help to improve services outside the capital.

Two presentations focused on the implementation of PEP. Before the presentation of the first PEP implementation study, results were presented of an R2STOP-funded study which lead to the development of the PEP study protocol. This study on leprosy transmission in the Comoros Islands looked at the use of PGL-1 antibodies and PCR testing for the diagnosis of new cases. Ms Sophie Braet (Institute for Tropical Medicine, Belgium) presented the results showing a surprisingly high test positivity in both PB and MB cases, but interestingly nasal swabs were much less positive on PCR. Newer methods of DNA analysis (for both SNPs and VNTR) allowed better identification of clusters with similar strains of *M. leprae*, than has been achieved previously. The PEP implementation study, the so-called PEOPLE project, is

now starting to build on these results and was presented by Prof. Bouke de Jong (Institute for Tropical Medicine, Belgium). The PEOPLE project is jointly funded by LRI and EDCTP and involves a cluster randomised controlled trial of PEP given according to four different strategies: (1) control group with no PEP; (2) PEP given to all household contacts of incident leprosy cases; (3) PEP given to everyone living within 100 meters of an incident leprosy case; (4) everyone living within 100 meters of an incident case is tested for anti-PGL-1 antibodies and all positives are given PEP. In this project PEP is given at a higher dose (1200 mg of rifampicin for adults, instead of 600 mg) but it is still a one-time dose. During the first seven weeks of enrolment, they had already recruited 10,000 contacts of a total sample size of about 144,000 contacts. The study will take place in the Comoros and Madagascar.

The second PEP project was presented by Dr. Anne Schoenmakers (NLR, Netherlands). The so-called PEP4LEP project is also funded jointly by LRI and EDCTP. This is an implementation trial looking at doing contact examination and providing PEP through a skin camp approach, as compared to a health-centre-based approach. The skin camp approach will involve inviting about 100 neighbours living near an incident case to come for screening and PEP, if eligible (people are eligible once all contra-indications to SDR have been excluded). In the health centre approach, incident cases will be invited to bring their household contacts for screening at the health centre; those eligible will be given PEP. This study will take place in Ethiopia, Tanzania and Mozambique, and will evaluate the effectiveness, acceptability and cost-effectiveness of the two approaches.

Social Science

The social science session consisted of eleven presentations (seven long, six short) from ten countries. The topics ranged from diagnostic delay to inclusion and resilience.

Diagnostic and treatment delays are an important issue to address in order to improve early detection. One study from Nepal looked in detail at this topic. Dr. Ulla-Brit Engelbrektsson and colleagues (International Nepal Fellowship, Nepal) reported an average delay of 28 months in Nepal. They found that the first contact is often within traditional health services, and that the longest mean delay was after the start of health-seeking within the biomedical sector. Most consultations, by far, took place within the private health services. However, most of the leprosy training is provided in government health facilities and they concluded that awareness raising in society at large, and within the public and private health sectors is needed (Annex 4).

Among the neglected tropical diseases, leprosy is well-known for requiring long-lasting management of impairments and other complications. Traditional impairment management is dependent on health professionals, but this is not feasible or sustainable in many contexts. The Spring Meeting highlighted a number of studies looking at innovative approaches to managing leprosy-related impairments.

Dr. Sathish Kumar Paul presented initial results of a study investigating the effectiveness of a telephone help-line to support persons affected in managing the complications of leprosy. With the increasing availability of mobile phones, mHealth approaches are increasingly becoming feasible and acceptable. Using a toll free number and dedicated staff the project received calls (435 to date) and also called individual patients to check how they are doing - 452 calls with 381 (84%) being picked up. The system uses existing mobile phone networks and is built on the Android platform so will be easy to scale up to other areas. The phone service

complements the care given at hospitals and clinics. So far, the group using the phone service has fewer defaulters compared to the control group. Dr. Moges Wubie and colleagues (ENAPAL and Debre Markos University, Ethiopia) explored family-based approaches for the prevention and self-management of disabilities related to three different NTDs, including leprosy. In many countries, the traditional self-care group approach of people gathering together is not feasible dues to financial or geographical barriers, or due to private barriers, including not wanting to disclose problems. Family-based support may be a sustainable, feasible and acceptable strategy. Dr. Bob Bowers (The Leprosy Mission International, Bangladesh) presented preliminary findings on a study looking at the long-term attributable impact of community based rehabilitation for leprosy-affected persons.

Early results from a study in India and Indonesia, presented by Mr Kevin De Sabbata (Athena Institute, Netherlands) showed that at least half of people with erythema nodosum leprosum (ENL) are initially misdiagnosed; most conceal the reaction and one-third are unemployed due to pain. Persons affected say that lifestyle, stress and overwork all play a direct role in reactions. They also report that effective management of ENL is a challenge due to distance to health services and non-availability of anti-reaction drugs. Further work will focus on improving the management of reactions in these settings.

Inclusion of persons affected in society is another important topic of research. Despite the endorsement of the United Nations Principles and Guidelines for the Elimination of Discrimination against Persons Affected by leprosy and Their Family Members, in 2015, implementation is lagging behind. Moreover, stigma denies people with disabilities their dignity and potential, and this is a barrier to social inclusion in the community. Mr Paulo Hansine (representing IDEA Mozambique, Niger and Nigeria) presented results of a project to develop and implement effective tools (including video testimonies) to educate persons about their rights and increase awareness about leprosy. The findings indicated that stigma is reducing and persons affected by leprosy report less exclusion. Mr Sunarman Sukamto (NLR, Indonesia) reported results of a study in Indonesia looking at the enabling and disabling factors faced by persons affected by leprosy, women with disability and persons with mental health conditions. The most frequently reported disabling area was access to public facilities. Implementation of inclusion policies is weak. Women raised more issues than men. Again, self-stigma among persons affected by leprosy was higher than other groups. A community development study in Uganda, presented by Ms Carolyne Maholo (GLRA, Uganda) shows that data about leprosy and lymphatic filariasis (LF) beyond impairment-related data is scarce, making it difficult to assess participation and inclusion (Annex 5). Both groups tend to be excluded from disability mainstreaming programmes and persons affected by leprosy have less social participation (higher score on the Participation Scale) than those affected by LF. The results indicate that they face the same challenges as persons with disabilities, so community-based rehabilitation can be adopted to empower people affected by leprosy and LF. Mr Sarju Rai (Atma Yalma, Indonesia) presented results of a study identifying successful positive deviant[1] strategies employed by people affected by leprosy, schizophrenia, HIV and diabetes) to manage and overcome stigma in their lives and foster social inclusion. Positive deviants demonstrate three steps: first, the individuals empower themselves through self-acceptance, positive spiritual belief, active use of technology, and support from healthcare

[1]**Positive deviance** is an approach to behavioural and social change based on the observation that in any community there are people whose uncommon but successful behaviours or strategies enable them to find better solutions to a problem than their peers, despite facing similar challenges and having no extra resources or knowledge than their peers. These individuals are referred to as positive deviants.

providers; second, the persons reclaim control of their lives and life decisions by selective disclosure, resilience, and indifference to others' stigmatising responses; and third, the persons developed passion and desire to help other stigmatised people through advocacy and peer support. Based on the discussions with the stakeholders, life-skills training and community sensitisation were the best possible interventions to reduce stigma and create positive experience among those stigmatised people. Religion is another factor that may influence the presence of stigma toward persons affected by leprosy. As for the general population, religion plays a tremendous role in the lives of people in many leprosy-endemic countries. Mr Ibrahim Hassan (TLM, Nigeria) presented a study in Nigeria that is investigating how religion influences norms, values and perceptions on disability, leprosy and disease. The first phase of the study looked at Christian faith communities, while the current phase is looking at Muslim faith communities. From this study it appears that Muslims and Christians in Nigeria have a lack of adequate information about leprosy and poor interaction with people affected by leprosy, resulting in stigma. In addition, Muslims and Christian have similar infrastructures and a similar willingness to learn about leprosy and participate in strategies for reducing stigma.

During the final presentation of the Spring Meeting, Dr. Zoica Bakirtzief da Silva Pereira (Federal University of Santa Maria, Brazil) discussed the findings of a study in Brazil and India focusing on resilience or psychosocial strength as a way to prevent the deterioration of mental wellbeing. Three levels of resilience building activities were identified during the first two phases of the study: the intrapersonal focus, the interpersonal focus and the structural (system level) focus. A pilot intervention based on these findings is being implemented in India.

Conclusion

These presentations illustrate the range of research projects currently supported by LRI. Of particular note is the significant degree of collaboration between scientists in the north with counterparts in endemic areas. It is also encouraging to see an increasing number of projects in African countries, which were very poorly represented in the first rounds of LRI funding. LRI acknowledges the continuing support of the Turing Foundation and this reports also indicates the fruitful partnership that has been developed with the European and Developing Countries Clinical Trial Partnership (EDCTP), currently helping to fund two large clinical trials of post-exposure prophylaxis in eastern Africa.

Please see the LRI website https://www.leprosyresearch.org/ for further details.

Acknowledgements

The following are members of the independent Scientific Review Committee: Prof. W.R. Faber (Chair), Dr. J.H.M. Dekker, Dr. G.J. Ebenezer, Dr. B.E. Ebenso, Prof. V.P.M.G. Rutten, Prof. Dr M.L.W.R. de Oliveira and Prof. T.S. van der Werf. The following represent the partner organisations on the LRI Steering Committee: Dr. D. Scollard (Chair), Dr. W.H. van Brakel (NLR), Dr. P. Steinmann (effect:hope), Dr. C. Kasang (GLRA), Mrs J. Ebenso (TLMI) and Dr. P.R. Saunderson (ALM).

The five Annexes display five project Abstracts, as examples of the LRI-supported projects.

Annex 1

The efficacy of different chemoprophylaxis regimens in a highly susceptible subclinical model of leprosy

Shannon M. Lenz*,**, J. Hunter Collins*, Nashone A. Ray*,
Angelina T. Deming*, Ramanuj Lahiri*, and Linda B. Adams*
**National Hansen's Disease Programs – Laboratory Research Branch, Baton Rouge, LA, USA*
***IHRC, Inc. Atlanta, GA, USA*

Background and Aims: Despite successful use of MDT, leprosy incidence has remained constant at around 200,000 cases annually, indicating continuing transmission. Subclinical infections in household contacts is one potential source of ongoing transmission, and numerous post-exposure prophylaxis (PEP) regimens have been proposed to control this source. Here we looked at the effectiveness of different PEP protocols using low-dose infections in athymic *nu/nu* mice as a model of subclinical infection in susceptible contacts.

Methods: Drug Study 1: Mice were inoculated in hind footpads (FP) with 6×103 *Mycobacterium leprae* and treated with vehicle, single dose rifampicin (10 mg/kg; SDR), single dose ROM (10 mg/kg rifampicin, 150 mg/kg ofloxacin, 25 mg/kg minocycline), or single dose PMM (10 mg/kg rifapentine, 25 mg/kg minocycline, 150 mg/kg moxifloxacin) by gastric gavage at either one day or two months post-inoculation. FPs were harvested at 2, 4, 6, 8 and/ or 10 months post-treatment. Following DNA/RNA extraction from FPs, bacterial enumeration and viability were determined by RLEP PCR and *esxA* gene expression, respectively.
Drug Study 2: Mice were inoculated as above and treated with vehicle, rifampicin (10 mg/kg) + moxifloxacin (150 mg/kg), rifampicin (10 mg/kg) + clarithromycin (100 mg/kg), rifapentine (10 mg/kg) + moxifloxacin (150 mg/kg), or rifapentine (10 mg/kg) + clarithromycin (100 mg/kg) by gastric gavage. Mice received 3 monthly doses of antibiotics. FPs were harvested at 1, 3, 6 and 9 months post-treatment. RLEP PCR was used for molecular enumeration of the bacilli on extracted DNA.

Results: The first study found that none of the single dose regimens were able to effectively control bacillary growth long-term. SD-PMM was the only treatment that was able to initially control growth. However, bacteria were still viable in all treatment groups, further confirming the inability of the single dose regimens to impact bacterial multiplication. The final study showed that all of the three-dose regimens were equally very effective ($\sim 10^2$ bacteria compared to $\sim 10^8$ bacteria for vehicle) at controlling bacterial growth. Even the regimens containing a lower paediatric dose (equivalent to a human dose of $\sim$330 mg) of clarithromycin were able to efficiently prevent bacillary growth, indicating that regimens containing the adult dosage (500 mg) would most likely be very effective as well.

Conclusions: While SDR and other single dose PEP regimens may be effective in the more resistant contacts, they may not be sufficient in highly susceptible contacts. Due to the

slow-growing nature of *M. leprae*, multiple doses of antibiotics may be required to ensure that administration occurs while the bacteria are metabolically active. In our studies, none of the single dose regimens prevented *M. leprae* growth in the nude mouse FP; whereas, all of the three-dose combination therapies were effective. These results suggest that in highly susceptible individuals with subclinical disease, multiple doses of PEP would be needed to control multiplication and decrease transmission. Further studies in mice with different levels of immunosuppression could determine if these findings are applicable to other groups of contacts.

Annex 2

Identification of Biomarkers for application of immunodiagnostic tools for early detection of leprosy in the field

Anouk van Hooij*, Susan van den Eeden*, Renate Richardus*,¶, Louis Wilson*, Kees L.M.C Franken*, Roel Faber¶, Meruva Khatun§, Korshed Alam§, Jan Hendrik Richardus¶ and Annemieke Geluk*
**Department of Infectious Diseases, Leiden University Medical Center, The Netherlands*
¶Department of Public Health, Erasmus MC, Rotterdam, The Netherlands
§Rural Health Program, Leprosy Mission International Bangladesh, Nilphamari, Bangladesh

Background and aim: Leprosy remains persistently endemic in several low and middle income countries and transmission of its causative agent *Mycobacterium leprae* is still ongoing, as indicated by the unabated rate of leprosy new case detection.

The clinical outcome after *M. leprae* infection is determined by host factors resulting in a spectrum of disease ranging from lack of T cell immunity concomitant with large numbers of bacteria and antibodies against *M. leprae* antigens in multibacillary (MB) leprosy, to paucibacillary (PB) leprosy characterised by strong pro-inflammatory, T- cell immunity. This spectrum of pathology compels leprosy diagnostic tests to be based on multiple, diverse biomarkers. However, sensitive and specific biomarkers for leprosy are limited. Therefore, as part of our research line in immunodiagnostic tests, we aimed to identify new biomarkers associated with leprosy and *M. leprae* infection.

Methods: Blood samples from a cluster randomised BCG vaccination field-trial in Bangladesh were assessed for the concentrations of host proteins in supernatants of *M. leprae*-antigen-stimulated whole blood of LL/BL and BT/TT leprosy patients, contacts of leprosy patients and healthy endemic controls without known contact to patients.

First, we applied user-friendly lateral flow assays (LFAs) that quantitatively detect anti-PGL-I IgM antibody (humoral immunity), IP-10, CCL4, IL-10 and CRP (cellular immunity), to supernatants of whole blood samples. Simultaneously, we conducted studies in other areas with variable endemicity (Brazil, China and Ethiopia), using similar UCP-LFA.

Results: In Bangladesh, combined detection of these host blood-based biomarkers significantly improved the diagnostic potential, particularly for paucibacillary leprosy, the majority grouping of leprosy patients in Bangladesh. Similarly, multi-biomarker based assessment increased the diagnostic potential for leprosy along the spectrum also in other areas with varying leprosy endemicity such as Brazil, China and Ethiopia (Figure 1). Thus, these data clearly demonstrate the added value of cellular markers (cytokines and chemokines) for leprosy diagnostics.

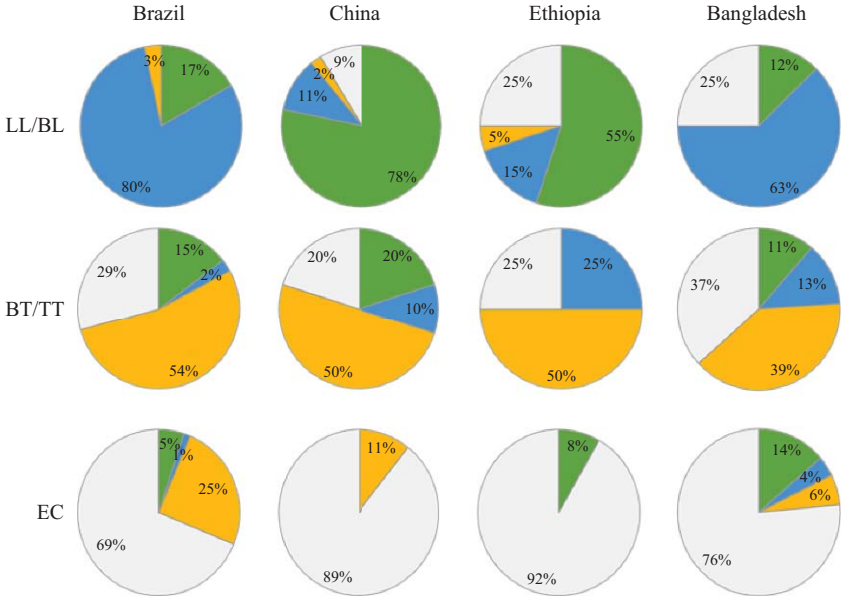

Figure 1. *Percentage of test groups detected with UCP-LFA.* : *positive for PGL-I;* : *positive for PGL-I and Cellular markers;* : *positive for cellular markers;* : *no markers positive.*

Interim conclusions and next steps: These data on combined assessment of host biomarkers for humoral and cellular immunity pave the way for implementation of user-friendly, point-of-care tests that can be applied in low-resource settings for various applications in leprosy diagnostics (early diagnosis of disease, detection of *M. leprae* infection, classification and timely detection of leprosy reactions). However, based on the currently identified markers, it remains a challenge to discriminate PB disease from *M. leprae* infected contacts without disease. Thus, using multiplex cytokine arrays for assessment of the levels of 60 proteins, we are currently extending the number of biomarkers with potential for application in field-friendly multi-biomarker UCP-LFA, particularly those compatible with fingerstick blood. Within the 2019-2021 LRI study we aim to apply our UCP-LFA version 2·0 to quantitatively assess the direct effect of SDR on *M. leprae* infection in contacts of leprosy patients.

Annex 3

Inflamed Skin Lesions as Patient Self-Help Proxy Indicator to Detect Early Signs of Nerve Abnormalities in Leprosy

Marivic Balagon, Florenda Roferos, Junie Abellana, Tonet Taborada, Riza Sanchez and Armi Maghanoy
Leonard Wood Memorial Center for TB & Leprosy Research, Cebu, Philippines

Introduction: Frequent monitoring of leprosy cases under treatment is necessary to detect reactions and nerve damage at an early stage, so that effective treatment can be administered and disability prevented. In many contexts, it is difficult for patients to attend the clinic frequently and even if they do, the health worker's capacity to carry out nerve function assessment may be sub-optimal, leading to poor outcomes.

We hypothesised that if patients could be taught to identify inflamed skin lesions, these may be a pointer to incipient nerve damage, leading to timelier and more efficient case management.

Methods: This prospective, observational cohort study recruited both PB and MB leprosy patients. Skin lesions were scored on a 0–15point scale; nerves were noted to be either close to or distant from any particular skin lesion and nerve function was also scored on a 0–15-point scale. Lesion scores done by the patient and health staff used lesion size, colour, swelling, tenderness and ulceration as parameters; while the nerve scores (done independently by another health worker) used nerve enlargement, tenderness, pain, sensory and motor function as parameters. Patients were taught to score their skin lesions and this was compared with scoring done by the health staff, and with the nerve scores, done independently by other staff.

Lesions on the same side of the nerve in question were considered "near", while lesions on the opposite side of the nerve in question were considered "distant" from the nerve. The goal was to study 300 nerve/lesion pairs involving a reaction.

Results: So far, 660 nerve/lesion pairs have been studied in 200 patients, 91% of whom are smear positive, MB cases. Signs of inflammation were noted in 249 skin lesions and the scoring done by patients and health staff was not significantly different. Of these 249 inflamed skin lesions, 146 were Type 1 or reversal reactions, while 103 were Type 2 or ENL reactions.

In general, the lesion score was less than four in the absence of a reaction, but above four at the start of a reaction and above five at the peak of the reaction. The nerve score remained low in the absence of a reaction, and also when a reaction occurred in a skin lesion "distant" from the nerve. However, the nerve score was significantly higher when the skin lesion "near" the nerve in question became inflamed.

Conclusion: The results show that patients can reliably assess the state of their own skin lesions and that this could help identify cases with incipient nerve damage. Based on these

findings, it seems practical and logical to encourage incoming patients to regularly use the tool in monitoring their lesions at home and transmit the scores by mobile phone for timely intervention and management. Likewise, it would be helpful to translate these findings into routine practice at a larger scale by partnering with program managers and by conducting "training of trainers" involving patients and primary health workers. Setting intervention guidelines based on actual findings is also recommended.

Annex 4

Delays in diagnosis and treatment of leprosy in Nepal

Ulla-Britt Engelbrektsson and Dhakaram Budha Magar
International Nepal Fellowship, Nepal

Background and aim: In 2009, Nepal reached the leprosy elimination goal. Expectations were that the registered point prevalence ratio would gradually decline. This, however, did not happen. In 2015, the International Nepal Fellowship (INF) launched a research project (financed by LRI) to better understand delays in diagnosis and treatment. The project, now completed, was carried out by regular INF leprosy staff with some support from outside.

Delay = *The time span between the person becoming aware of a leprosy-related symptom and diagnosis/start of MDT treatment*.

Methods: After informed consent, patients in the process of starting MDT from two of Nepal's five Development Regions, the Western and the Mid-Western, were interviewed at their place of: regular treatment (field patients); inpatient care (inpatients); diagnosis (outpatients) - three cohorts totalling 400 patients. The main focus was upon the patient's perceptions of the development of his/her leprosy-related symptoms, health-seeking actions and experiences, and thoughts on how the delay could have been shortened. Open-ended questionnaires were used with additional information from clinical files, health workers, and other key informants - a mixed patient-centred qualitative and quantitative approach supplemented by two delay-focused community studies.

Results: An increased understanding that in direct replies to questions on delays the early stages are often underreported and thus a need for in-depth probing. We learnt that the mean delay of the three cohorts was: 25·3 (field patients); 29·8 (inpatients); and 28·1 months (outpatients), respectively.

In Nepal, most patients present voluntarily. They are commonly blamed for the long delays. However, in none of the cohorts did 'the Patient Delay' exceed half of the mean delay. Commonly, initial symptoms were not bothersome and actions were only taken in connection with additional signs and symptoms. Before those there might have been some home treatment, mostly Ayurvedic. After 'the Patient Delay' there was 'the Health Services Delay' (delay after the start of health- seeking outside the home). Health Services include traditional, mostly shamanistic, and biomedical services. Shamanistic consultations, when they took place, were usually the first health-seeking step outside the household sphere. However, they also took place in between biomedical consultations. The delays of those who had consulted traditional healers were considerably longer than those who had not. The longest delays, however, were within the biomedical sphere, public and private. The initial biomedical consultation was mostly within the private sector, most often a local medical hall where the person on the other side of the counter might or might not have been medically trained. A large proportion had only visited private health facilities. Most patients reported several

misdiagnoses and inappropriate treatments with consequent worries, costs, and a worsening of the condition. The diagnosis of leprosy, when it finally came, was a big and frightening surprise to most.

There are three major geographical terrains in Nepal: the Plains in the south (most developed, health services included); the Hills; and the Mountains. The number of new patients is far higher in the Plains than in the other two parts. Yet, the great majority in need of inpatient care for leprosy complications is from the Hills and Mountains. This corresponds to their delays commonly being longer.

Conclusions and next steps: To reach the aim of Zero leprosy, there is a need for increased leprosy awareness in society as a whole combined with an upgrading of diagnostic performances within both the public and private health services. An enormous task! The focus should not be on numbers only, i.e. not solely on high-prevalence districts in the south. The question is how it can be done in a context sensitive and sustainable way. Meanwhile, in Nepal, the research findings have been extensively disseminated through mini-workshops and sessions with administrators and practitioners within the health sector, the leprosy sector in particular. And, a wider audience has been reached through publications and conference-participations. Moreover, tailor-made leprosy awareness and capacity building material has been produced for the general public, for patients and their families, and for various groups of health workers.

Annex 5

Inclusion of people affected by leprosy and lymphatic filariasis in community development

Maholo Carolyne Sserunkuma*, Kawikizi Moses**,
Barbara Batesaki Ssembatya***
**Kyambogo University,*
***German Leprosy and TB Relief Association (GLRA),*
****Uganda National Alliance against Leprosy (UNALEP)*

Leprosy and Lymphatic Filariasis (LF) are among the neglected tropical diseases in Uganda. They cause disfigurement and disability. However, affected persons are hardly included in community development programs. In 2015, 25% of newly detected leprosy patients had grade 2 disabilities (G2D). Persons with disabilities (PWDs) suffer exclusion due to their impairment and barriers experienced in society. The exclusion restricts productivity causing a poverty and deprivation trap. Effective participation of people affected by leprosy and LF requires understanding of their needs, priorities, capacities and barriers for intervention.

Objectives: The study sought to explore the nature and extent of participation of people affected by leprosy and LF in community development programs in Northern Uganda and identify strategies to promote inclusion.

It specifically meant to:

1. Identify the existing community development programs
2. Establish the level and nature of participation in Health, Education and Livelihood community development programs
3. Identify factors that enable and hinder participation
4. Identify challenges experienced
5. Identify appropriate strategies and priorities in enhancing participation and inclusion.

Methods: The study used a descriptive research design with qualitative data to establish the nature of participation, enablers and barriers to participation, challenges experienced and priorities to develop appropriate strategies for inclusive development. Quantitative data were used to establish various demographic aspects of the people affected by leprosy and LF, level of disability and level of participation for an adequate conclusion of the study.

It included people affected by leprosy: released from treatment (n = 294), on treatment (n = 11) and new cases (n = 5); people with LF: elephantiasis (n = 167), hydrocele (n = 179) as well as other PWDs (n = 359). Complementary data were obtained from the district; TB/leprosy supervisors, vector control officers, community development officers, disability councillors as well as the leadership of the disability unions.

Data were collected using the Participation Scale (n = 1016), interviews (n = 38), and focus group discussions (n = 18).

Results:

Participation restriction:

Level of Participation	Leprosy (n = 310)	Lymphatic Filariasis (n = 342)	PWDs (n = 359)
0–12: No significant restriction (n = 269)	17·7%	39·2%	22·3%
13–22: Mild restriction (n = 182)	16·1%	20·8%	17%
23–32: Moderate restriction (n = 135)	15·5%	13·5%	11·4%
33–52: Severe restriction (n = 251)	31·6%	18·7%	24·8%
53–90: Extreme restriction (n = 174)	19%	7·9%	24·5%

The majority (76·7%) of respondents reported benefit from health facilities. People affected by leprosy, LF and PWDs do not benefit from most mainstream community development programs while only 15 benefited from the disability grant.

Improvement in health (62·8%), improved food security (32%) and starting to earn (21·1%) are the changes realised by those who participate in community development.

Knowledge and information of existing services (52·7%), distance to service centres and affordability (16·7%) and friendliness of service providers (15·5%) are enablers of participation in community development.

Limited knowledge and information of the existing services (56·9%), distance (37·6%), long processes involved (16·5%) and negative attitudes from service providers (15·2%) are barriers to participation.

Negative attitudes (52·3%), limited funds to afford attendance (51·8%), long distances to services (38·3%), are challenges to participation in community development.

Conclusions: People affected by LF have low levels of participation restriction, while those with leprosy experience as much participation restriction as other PWDs. People affected by leprosy and LF hardly benefit from community development programs. Distance, attitude and affordability of services enable participation while negative attitudes, limited information and distance are the main barriers to participation.