Determinants for the development and course of leprosy: findings from a prospective cohort study
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Chapter 7

General discussion
All studies presented in this thesis are part of the COLEP study, which is a large double-blind, placebo-controlled chemoprophylaxis trial, performed in Northwest Bangladesh. The study area, Nilphamari and Rangpur districts, had recorded prevalence rates of 3.0 and 1.3 per 10,000 population, respectively, at the beginning of the COLEP study (2002). [1] Surveys done for the COLEP study revealed that the actively found prevalence in the general population was six times higher than the registered prevalence. [2] The field work was conducted by the Rural Health Program (formerly DBLM), which has long-time experience in the study area, both in clinical leprosy services and research. The robust design of the study, the high prevalence in the study area and the experienced field staff, made high quality research possible.

This thesis presents five studies addressing topics related to the current research themes as postulated by the research community and WHO. [3,4]

**Theme 1: Prevention and management of nerve function impairment (NFI) and reaction**

"Effective management of leprosy complications, including reactions and neuritis, can prevent or minimize the development of further disability. The disease and its associated deformities are responsible for social stigma and discrimination against patients and their families in many societies" [3]

As stated by Scollard et al. (2006 [5]), leprosy reactions and the accompanying NFI can be seen as medical emergencies. The acute events of inflammatory response are often a reason to seek medical help. Even when diagnosed with leprosy and during and after MDT, reactions can still occur and potentially damage the nerves in an irreversible manner. Most reactions are recorded within the first year of treatment and decrease in number every sequential year.
NFI may lead to the hallmark deformities of leprosy. Daily activities will be a challenge, and stigma and shame may cause the leprosy patient (and family) to become isolated from his or her social environment. The fear of such a prospect has been related to delay in self reporting, not only increasing the risk of NFI but also the transmission of the bacterium.

From the above follows that prevention and management of NFI and reactions is an important research theme. With leprosy control becoming more and more integrated into general health care services and becoming less specialized, there is a need for simplified procedures at the field level for timely identification and treatment of NFI in leprosy patients.

Much progress has been made by studies like TRIPOD, BANDS, and INFIR mentioned in the introduction. NFI treatment consists of treatment with corticosteroids, which may prevent NFI during reactions and even may result in some recovery of nerve function loss if given early—within 6 months after the event. [6-8] The chances of preventing disabilities increase when health care workers pay special attention to patients who have a high risk of developing NFI. To date, several risk factors for NFI have been identified, [9-11] and an NFI prediction rule was formulated based on data from the BANDS study. [9]

**Prediction of NFI**

Chapter 4 describes an adjustment on the BANDS NFI prediction rule: the variable “longstanding NFI at diagnosis” is replaced with “anti-PGL-I antibodies”. The adjusted prediction rule was better able to identify patients at risk of developing NFI after diagnosis, using “WHO classification” and “anti-PGL-I antibodies” as risk indicators. The adjusted prediction rule can identify a substantially higher number of new NFI cases than either routine or BANDS rule-based surveillance.
and offers increased opportunity to prevent nerve damage in leprosy. However, the number of visits needed to detect one case is higher than with alternative strategies. We consider this operationally feasible and medically justifiable in view of the serious consequences of NFI, including life-long disability.

In contrast to the BANDS rule, the adjusted rule uses two variables that do not include NFI. This offers the possibility of predicting NFI before it actually occurs. We believe that the adjusted prediction rule can be applied in current health services, since it fulfils the need for simplified guidelines and diagnostic protocols. With the adjusted prediction rule, the necessity to continue surveillance beyond the treatment period can be determined. New leprosy patients can be assigned to an NFI risk group, and appropriate surveillance can be planned. Nerve damage can thus be successfully prevented despite the fact that leprosy control has been integrated into general health services.

**Involvement of host genetics**

It is probably impossible to select a single genetic prognostic marker for leprosy because of the diversity and interdependence of the immunological mechanisms involved. [12] The availability of the whole human genome sequence, allowing gene comparison and genome wide-scans, may further increase our understanding of host immunology, potentially leading towards a multiple marker test, which may include one or more genetic markers.

Host genetics are associated with the occurrence of reactions: chapter 2 clearly shows that the function-diminishing SNP TLR1 N248 is strongly associated with ENL reactions. In addition, we found for the PARK_e01(-2599) SNP that all patients with ENL reaction had the TT genotype (unpublished results).

Although, it may not be easy to interpret the relationship of a few SNPs and an infectious disease, our results do illustrate the impact of host
genetics for the development of reactions and even leprosy susceptibility.

In conclusion, there is a definite need for improving the quality of NFI assessment and management. We believe that the adjusted prediction rule including anti-PGL-I antibodies instead of longstanding NFI at diagnosis can improve patient management in general health services. Also, studies on host genetics may one day provide markers to identify NFI risk and potentially provide insight into the mechanisms leading to nerve damage.

**Theme 2: Improved chemotherapy**

"The current treatment of leprosy based on WHO’s recommended multidrug therapy (MDT) for MB and PB leprosy is unlikely to see major changes during the next 10 years or so. However, the longer term role of MDT will be dependent on *M. leprae* remaining sensitive to the component drugs particularly rifampicin" [13]. Judicious use of MDT is thus extremely important.

The WHO technical advisory group [4] noted that the current MDT regimen is still complicated, with the risk that patients fail to take their daily and monthly doses for the (relatively long) treatment period. Moreover, available resources should be used in an optimal manner. Accurate diagnosis and classification of leprosy patients is important for treatment purposes as correct treatment may prevent disabilities, relapse and continued transmission.
Classification for treatment purposes

After its publication in 1966 [14], the standard way to classify leprosy was according to the Ridley and Jopling scale. Starting from 1982 onwards, the WHO has step-wise designed a classification system for treatment purposes, dividing patients into two groups (PB and MB) with matching drug regiments. Initially the WHO classification was based on the Ridley and Jopling scale and microscopy, but nowadays a classification based on skin lesion counting only is promoted. In the WHO classification, “satellite lesions”, small secondary lesions in the vicinity of a larger primary lesion, may be counted as separate lesions. The WHO classification system does not take into account the large variation in the size of lesions.

There are currently two tools available for routine control programmes to help the correct classification of leprosy patients, 1) microscopy on acid-fast stained skin smears or on biopsies can be used to determine the bacterial index (BI). 2) Anti-phenolic glycolipid-I (PGL-I) antibody detection by serology may be used instead of microscopy, since the presence of antibodies to the *M.leprae*-specific PGL-I correlates with the bacterial load. Although microscopy may deliver a definite “proof” of *M.lepraee* infection when positive, the need for laboratory facilities makes it demanding. For individual patient management serological testing may give clinicians a better idea about the systemic bacterial load of a patient. The availability of simple serological tests makes it more field applicable. Unfortunately, the implementation of anti-PGL-I serology field test for routine leprosy control is not very likely, since the focus of classification procedures is very much on reducing complexity and costs.
Skin lesions in classification

In view of the integration of specialized leprosy services into general health care systems, lesion counting will become more and more important. Clear and standardized classification rules while help health workers to prescribe chemotherapy. Chapter 3 critically evaluates classification using a large number of patient characteristics using seropositivity as a proxy parameter for bacterial load and pays special attention to the group of patients with single lesion leprosy. The apparent association of skin lesion size with anti-PGL-I antibodies implies that size does matter: patients with larger lesions are more seropositive. Lesion size may thus be a valuable addition for classification. In contrast, the presence of satellite lesions did not influence anti-PGL-I seropositivity, and we therefore suggest that they should not be counted as separate lesions.

Importance of correct classification

Currently, trials are ongoing to evaluate uniform MDT for all leprosy patients—with both PB and MB patients receiving MB MDT for six months meaning clofazimine as an extra drug for PB patient and only six months instead of twelve months treatment for MB patients [4]. Although it is uncertain if this uniform regiment is going to be implemented, its implementation will not make the need for correct classification obsolete: correct classification remains important for instance to determine risk factors for transmission and reactions.

The WHO classification determines the chemotherapy regimen for a patient, so correct classification is important for the judicious use of MDT. Additional, the uniform interpretation of classification guidelines is needed in order to interpret and compare the epidemiological records:
the WHO strategy guide of 2006-2010 [3] states that the MB rate may be an additional indicator for case detection. Besides, the association between WHO classification and reactions/NFI is clearly demonstrated in chapter 4 and can be used to define risk groups, underscoring the need for correct classification. Therefore, the skin lesion counting system remains an important aspect of the classification of leprosy and subsequent treatment regimens.

**Theme 3: Operational research to improve sustainability and integration of leprosy services**

“A prime component of the WHO strategy is to ensure that leprosy control activities are available and accessible to all affected individuals at their nearest health facility” [3]

This theme is not discussed, since it is outside the scope of this thesis.

**Theme 4: Diagnostics to identify individuals at high risk of developing leprosy**

“Surveillance of the disease will be one of the most important activities to be conducted under low endemic situations. In addition, innovative approaches need to be developed based on a ‘population at-risk’ approach which will help to reduce the disease burden further in the community” [13]

Accurate identification of risk factors in combination with a test for infection could help control activities for monitoring purposes and may justify extended monitoring, intervention and/or treatment of smaller, well-defined groups that are at high risk to develop leprosy.
The latest review by Bakker et al. [15] gives a good overview of risk factors found in cohort studies. In addition, a number of research projects are investigating biomarkers and genetic markers that influence the risk for disease. Before going into detail, one should consider that leprosy has a low incidence: the number of subclinically infected individuals is estimated to be larger, [16,17] although it remains uncertain to what degree. The diversity of clinical symptoms and extent of disease as well as the variable course of infection and disease—from spontaneous clearance via self-healing to full-blown lepromatous leprosy—are a result of the diverse immunological mechanisms active at different stages of infection and disease. The challenge is thus to identify appropriate (sets of) biomarkers that will allow identification of those persons that are prone to develop clinical disease.

The low incidence of clinical disease makes a “population at-risk approach” necessary, since prevalence directly influences the cost per case ratio. So, when applying a biomarker test to define risk groups, the frequency of detecting a real case within that risk group will determine the cost per case for the biomarker test. Thus, it will be extremely important that any diagnostic or predictive test offers a clear advantage over easily obtainable information, such as demographic or contact information. It may be worthwhile to consider a combination of risk factors into a decision model and even to develop a non-tech model based on risk factors that can be determined by interviewing as an alternative for those control programs lacking resources to perform laboratory-based tests. Another aspect in leprosy detection is the role of stigma, which contributes to delay of diagnosis. The fear of social stigma and discrimination against patients and their families will lead people to refrain from participating in surveys to determine their risk status. Leprosy is a disease with a very low incidence, even in so-called “high risk” groups. Labeling someone as “high risk” may have a negative
impact on that person’s quality of life, despite the very real possibility that the person will never develop leprosy. It will therefore be of prime importance to develop a tool with high positive and negative predictive value that can accurately identify high risk groups and so enable health systems to focus their resources.

**Host genetic and immunological risk factors**

In chapter 2 it is shown that host genetics can increase the risk of leprosy and reactions. In the COLEP population, we found that a SNP in the *TLR* gene had a minor association with leprosy and a strong association with reactions.

Chapter 6, describes the potential of an anti-PGL-I antibody serology test. It shows the association between seropositivity and future development of leprosy, especially MB disease, but the predictive value remains low. Furthermore, a significant proportion of persons who did develop leprosy were seronegative at intake or follow-up. It is well known that a substantial number of (PB) patients are seronegative for anti-PGL-I antibodies. This indicates that an anti-PGL-I antibody test will never detect all patients and it remains to be seen whether other tests detecting antibodies will perform any better.

A combination of a serological assay with an assay based on cell-mediated immunity against *M. leprae* might allow detection of both PB and MB and maybe even preclinical leprosy. Geluk et al. (2009 [18]) reported cell-mediated immune (CMI) responses against five *M. leprae* antigens in 59% of the PGL-I seronegative household contacts of BL/LL patients, indicating a serologically undetected but potentially *M. leprae* infected group. Besides the detection of anti-PGL-I antibodies and host genetic factors a CMI test may be used for a multiple marker test for leprosy.
In low-endemic areas, the relative importance of transmission under high-risk groups increases and may justify an intervention in these groups. Serology and host genetic factors may be used to identify risk groups, ideally combined with CMI markers in a multiple marker test, however future research will be needed for such a test.

**Theme 5: A test for infection**

“There is a need for [...] development of epidemiological tools to monitor completeness of case detection and for novel tests for exposure to infection” [12]

The presence of *M.leprae*-specific antibodies is an indication of the presence of the bacterium. Although not all current or future patients are seropositive, seropositivity is associated with the systemic bacterial load and MB disease (Chapters 3 and 6). Ideally, as mentioned above, an assay should include markers for cellular and humoral immune responses in order to identify both responses against *M.leprae*.

Once a reliable test for infection is available, one needs to determine the feasibility of implementation. Of prime importance are the test characteristics, both in terms of sensitivity, specificity and predictive value as well as technical applicability: the test should be robust and easy to perform. With a test that fulfils these criteria one can then detect infected persons. The next step is to decide what intervention should be applied for subclinical infection. Study results from COLEP as well as from a chemoprophylaxis trial in Indonesia [19,20] indicate that one/two dose(s) of rifampicin chemoprophylaxis may not be sufficient to cure subclinical infection. Yet another question is the impact that such early interventions would have on prevalence and incidence of disease; with mathematical modelling one should be able to predict the potential impact.
It is very likely that the number of subclinically infected individuals is much larger than those who develop clinical disease, and that most will never develop clinical disease—the seroprevalence among healthy contacts included in the COLEP study was 6%. Even with a perfect test, it will be demanding to commit sufficient resources to the monitoring of “positive” persons and/or have a justified intervention that will protect against future development of leprosy without many side-effects and increasing the risk of drug resistance in the population. The intervention with a single dose of rifampicin done in the COLEP study did not prevent new infections after it was provided and it could not influence the course of infection in those already infected but seronegative at intake. Further research will be needed to determine the optimal treatment needed for subclinical infected persons.

**Theme 6: Understanding transmission**

*The mode of transmission of the leprosy bacillus remains uncertain, but most investigators believe that* M.leprae *is spread from person to person, primarily as a nasal droplet infection* [3]

Understanding transmission is an ongoing research theme, and is crucial for control and reduction of leprosy. At the introduction of MDT, WHO postulated that early diagnosis and treatment of all individuals with clinical signs would lead to reduced transmission and ultimately elimination of the disease. However, until recently the incidence of leprosy was stable and there is no prove that MDT treatment lowered or even interrupted transmission. This suggests that subclinically infected persons may also play a role in reducing transmission. The disappearance of leprosy from the European continent—even before treatment was available—illustrates that breaking the chain of transmission is possible.
It is generally assumed that multibacillary patients are the most infectious. But, at an individual level, seropositive PB patients may have disease that is behaving more like MB disease. Chapter 3, describes that seropositivity can be used as a marker for more extensive disease, since seropositivity is highly correlated with the extend of clinical signs, like numbers of skin lesions, nerves involved and body areas affected. These clinical signs signify the dissemination of the bacterium in the body of the patient, indicating that seropositivity can be used as a marker for a higher systemic bacterial load, and therefore can be used to identify more infectious patients.

Chapter 6 shows that seropositivity and especially seroconversion has a high association with the future development of MB leprosy. From all 19 MB patients, 8 (42%) were seropositive prior to diagnosis. However, a substantial number of new patients—including MB cases—come from the seronegative contacts and other low risk groups, like non-households and non-relatives. [19] Despite this limitation, serology may contribute to a preventive strategy in which MB disease is targeted specifically. A potential benefit of such a strategy could be the interruption of transmission: MB leprosy is considered as the most infectious form of the disease and MB patients can be expected to transmit bacteria before their clinical diagnosis and treatment.

In order to understanding transmission one could include serological monitoring to identify those at higher risk to transmit the disease. However, for identifying infection serology alone is insufficient, since not all (sub)clinical infections are detected with serology. Future research to understand transmission would benefit form a test for infection, but such a test has still to be developed.
Theme 7: Understanding the development of a protective immune response

"Leprosy provides an excellent opportunity to investigate mechanisms of innate and adaptive immunity in humans" [21]

It is assumed that after infection clearance of the bacteria or self healing are far more likely than the development of disease. However, many aspects of the development of a protective immune response are not known. The overall immune response is a complex interaction between innate, cell-mediated and humoral immune responses.

The humoral immune response, as indicated by (anti-PGL-I IgM) antibodies, does not protect against leprosy. Monitoring a combination of serological and cell-mediated immunological markers could give insight in the balance between the two immune responses. In chapter 6, seroconversion had a strong association with the development of MB disease and not with PB disease, indicating that, along with other markers, monitoring high risk groups or patients may reveal a pattern of immunological changes leading towards disease or disease alterations like reactions.

Host genetic involvement has been proposed to attribute to the different course of infection and of disease. SNP association studies provide small pieces of information that may help to understand the complex involvement and interaction of various aspects of the immune system in this process. Chapter 2 describes the association between an SNP in TLR1, which is part of the innate immune system, and leprosy. A clear relationship was expected between TLR1 functionality, as determined by SNP N248S) and the infection risk. [22] However, results were not that straightforward: homozygous S248 increases and SN decreases the risk of leprosy, but NN showed no influence. So, how
*M. leprae* influences the local functioning and/or expression of TLRs remains unclear.

There is still limited understanding of the development of a protective immune response in leprosy and further research is needed. The availability of the whole human genome sequence, allowing gene comparison and genome wide-scans, may further increase our understanding of host immunology.

**Theme 8: Development of effective, safe, acceptable and inexpensive interventions**

"There is a lack of effective tools to reduce the incidence of leprosy” [4]

As MDT in combination with early detection of clinical disease has been shown to be insufficient to lower the incidence of leprosy, the research community has been involved in developing novel early diagnostic tools as well as prophylactic regimens. The primary research question of the COLEP study is the impact that single dose chemoprophylaxis with rifampicin on leprosy incidence in close contacts of leprosy patients. The study also found that 40% of the participants were vaccinated with BCG. Bacille Calmette-Guérin (BCG) vaccination, originally developed for tuberculosis (TB), has a high global coverage since the start of the WHO Expanded Program of Immunization. It protects against the most severe forms of childhood TB and is currently the only candidate for immunoprophylaxis in leprosy. The well-known protection of BCG against development of leprosy and its potentially ameliorating role in the course of disease may have contributed to the reduction of incidence. [23]

The potential for chemoprophylaxis to reduce transmission of leprosy has been studied first for dapsone and more recently for
rifampicin. [24] Two recent studies with rifampicin chemoprophylaxis showed that a single/double dose of rifampicin is protective against the development of clinical leprosy. [19,20]

Chapter 5 describes the effect of the combination of both immuno- (BCG) and chemoprohylaxis (rifampicin) among contacts of patients. In this high-risk study population, BCG vaccination halves the risk of developing leprosy. Moreover, a similar, additive effect of chemoprophylactic intervention with rifampicin was shown. The combination of these strategies showed a protective effect of 80% and may be successful in reducing the incidence of leprosy.

However, another observation was that both BCG and rifampicin prophylaxis were most effective in the contact groups with the initially lowest risk, such as those groups with more physical and genetic distance to the index patient. This indicates that more extensive regimens are needed to prevent leprosy among close contacts, particularly at household level and blood relatives.

The merit of BCG vaccination for TB prevention has been debated continually and second generation vaccines are being developed with higher protection rates and fewer side effects. [25] The protectiveness of these more TB specific vaccines against leprosy is questionable and the replacement of BCG by newer, more TB-specific vaccines may thus be disadvantageous for leprosy control.

The development of effective, safe, acceptable and inexpensive interventions are needed in order to reduce the incidence of leprosy. Immunoprophylaxis with BCG and chemoprophylaxis with rifampicin are both effective strategies, with BCG now having a high global coverage—at least for the time-being. While rifampicin and BCG are effective as a combination strategy, monitoring of close contacts remains necessary even when both immuno- and chemoprophylaxis are supplied. Future
research will be needed to determine the preventive effect of chemoprophylaxis in the long term and to find an intervention appropriate for all sub-clinical infected persons.

References


