Risk factors, early detection and treatment of neuropathy in leprosy

Risicofactoren, vroege opsporing en behandeling van neuropathie in lepra

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ISBN: 978-94-6332-074-0

Printing: GVO, Ede

The studies presented in this thesis were funded by The American Leprosy Mission, the German Leprosy and TB Relief Association, the Netherlands Leprosy Relief, the Ordre de Malte, and the Turing Foundation. This thesis was printed with financial support of the Department of Public Health, Erasmus Medical Centre, and of the Erasmus University Rotterdam.

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Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 2 november 2016 om 13.30 uur

door

Inge Margriet Wagenaar
geboren te Wageningen

Erasmus University Rotterdam

Erasmus

Promotiecommissie

Promotor: Prof.dr. J.H. Richardus

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Chapter 1

General introduction

1. General introduction into leprosy

i. Leprosy

Leprosy is an infectious disease that affects the skin, nerves and eyes and is caused by the *Mycobacterium leprae*. Even though the disease has been well curable since the introduction of multidrug treatment (MDT) in the early 1980's, leprosy remains a public health problem in different parts of the world. Skeletal remains from 2000 B.C. discovered in India provide evidence that leprosy already exists for millennia. From India, the disease spread to other parts of the world [1], including to Europe where it reached its peak during the 13th and 14th century [2]. Leprosy disappeared from Europe in the eighteenth century, even before there was any treatment for leprosy available. Leprosy is now only known as a disease of the poor, and is mostly prevalent in low resource countries. The causative agent of leprosy, *M. leprae*, was discovered in 1873 by Armauer Hansen. It was the first time a bacillus was identified as a cause of disease in humans [3].

Even though leprosy is one of the oldest diseases known to man, there is still an incomplete understanding on the mode(s) of transmission, the mechanisms behind the immune response, nerve damage and reactions and unfortunately so far no effective vaccine has been developed to prevent clinical leprosy. Studying *M. leprae* is complex, since the organism has never been successfully cultured in vitro.

ii. Symptoms and diagnosis

Hypopigmented skin lesions are often the first symptoms of leprosy [4], however, due to low awareness or fear of stigma patients often wait to seek medical help until they experience more advanced symptoms caused by nerve function impairment, such as numbness or muscle weakness, [5,6]. The diagnosis for leprosy can be made when at least one of the three following cardinal signs is present: enlarged peripheral nerves, diminished touch perception in skin lesions or bacilli in skin smears. From skin smears or biopsies the bacterial index (BI) is derived which expresses the density of *M. leprae* on a logarithmic scale from 1 to 6 [7]. This can be used for diagnosis and to determine the effectiveness of therapy.

iii. Transmission

The incubation time of leprosy is usually long with reported maxima of 20 years, but is in most cases between 2 and 5 years [8]. The long incubation time makes it difficult to determine when and under what circumstances the disease was contracted, and therefore transmission of leprosy is not well-understood. The main transmission route is presumed to be from person-to-person through nasal droplets via the upper respiratory tract [9], although abraded skin is considered a possible port-of-entry as well [8]. Furthermore, *M. leprae* has been found to survive in soil for 46 days [10], which could also be a factor in transmission.

Only patients with the lepromatous type are considered infectious (see §3.i), and they remain infectious until the start of treatment with multi-drug therapy (MDT). Being a contact of a leprosy patient increases the risk of contracting the disease, depending on the intensity and duration of the contact [8,11]. Household members of lepromatous patients have the highest relative risk: 8-10 times the risk of the general population. In non-endemic areas, transmission within households plays an important role [12].

iv. Treatment

In the 1940's, dapsone was discovered as treatment against *M. leprae*. However, the bacteria developed resistance against the monotherapy and therefore a multi-drug therapy was introduced in 1982. The MDT is provided for free by the World Health Organization (WHO) since 1995. The WHO advises a therapy of 6 months for paucibacillary (PB) leprosy, with a daily dose of dapsone and a monthly dose of rifampicin. Multibacillary (MB) leprosy patients should follow a treatment of 12 months with daily doses of clofazimine and dapsone, together with rifampicin plus a high clofazimine dose once a month [13]. In line with older WHO guidelines, in some countries 24 months of MDT is given to MB patients.

v. Prevention

Even though there is no effective vaccine against leprosy, the BCG vaccine against tuberculosis also protects against leprosy [14,15]. The efficacy differs between populations, as case-controls studies have shown that vaccine protection lays between 20 and 90% [14].

2. Epidemiology

i. Prevalence

Between the introduction of MDT in 1982 and the early 2000's, the leprosy prevalence dropped by 90% [16]. However, in the last decade the decrease in prevalence has stabilized. The prevalence is defined as the number of leprosy cases on treatment, and thus depends on which treatment guidelines are followed [17]. A more stable indicator is the new case detection rate (NCDR), which shows the number of new detected cases in one year per 100 000 population. Elimination of leprosy, defined as less than 1 leprosy case per 10 000 population, was reached in 2000 at global level, but this target has not been reached in many regions of leprosy endemic countries.

In 2014, the total number of reported new leprosy cases was 213 899 [18]. Eighty-nine percent of these new cases were concentrated in eight countries: Bangladesh, Brazil, Democratic Republic of Congo, Ethiopia, India, Indonesia, Nepal and Nigeria. India alone accounted for 59% of new detected leprosy cases in 2014. In total, 13 countries had a new case detection of >1000.

Details of the eight countries with the highest number of new cases are shown in Table 1. The PB/MB ratio differs between countries. Leprosy is more frequently seen in males, the male-female ratio is about 1.5 to 2:1 [19]. Also, leprosy is seen most often in the age group 20-30 years [20], but the disease has been diagnosed in children as young as three weeks old [21]. Table 1 also contains the proportion of new patients with Grade 2 Disabilities – i.e. visible impairments and/or deformities. This is a useful indicator of delayed detection of leprosy.

Table 1- New case detection, prevalence and patient details for the eight countries with the highest new case detection rates in 2014 [18]

Country	New case detection	Prevalence	% MB	% female	% children	% grade 2 disabilities
India	125785	88833	53	37	9	5
Brazil	31064	25738	66	45	8	7
Indonesia	17025	19949	83	37	11	9
Ethiopia	3758	3758	74	NR	13	10
Bangladesh	3622	3310	46	38	5	11
Congo	3272	3231	64	46	12	14
Nepal	3046	2382	52	37	6	4
Nigeria	2983	3147	93	43	9	14

ii. Countries

The TENLEP study described in this thesis took place in four of the countries listed in Table 1: India, Bangladesh, Nepal and Indonesia. The new case detection rate (NCDR) of these countries is shown in Figure 1, together with the location of the centres where the studies were carried out.

In India (Figure 1.A), we worked together with two centres. The first is the Foundation for Medical Research (FMR), a research institute located in Mumbai, which was cooperating with a referral centre in Mumbai to include patients in our studies. The second is JALMA institute, a specialised governmental leprosy hospital located in Agra. Several studies within this thesis were done in Bangladesh (Figure 1.B), all in Nilphamari Leprosy Mission Hospital, a specialized hospital with multiple outpatient clinics in the Rangpur district. In Nepal (Figure 1.C), also two centres were involved. The first is Anandaban Leprosy Hospital, located near the capital Kathmandu, also run by The Leprosy Mission. They are holding weekly clinic days in Kathmandu. The second is Lalgadh Leprosy Services Centre, a more rural hospital, which is one of the busiest leprosy centres in the world. In Figure 1.D the centre in Indonesia is shown. We worked together with the dermatology department of Dr. Soetomo hospital in Surabaya. Most patients came from the island of Madura, and were visited monthly by the staff.

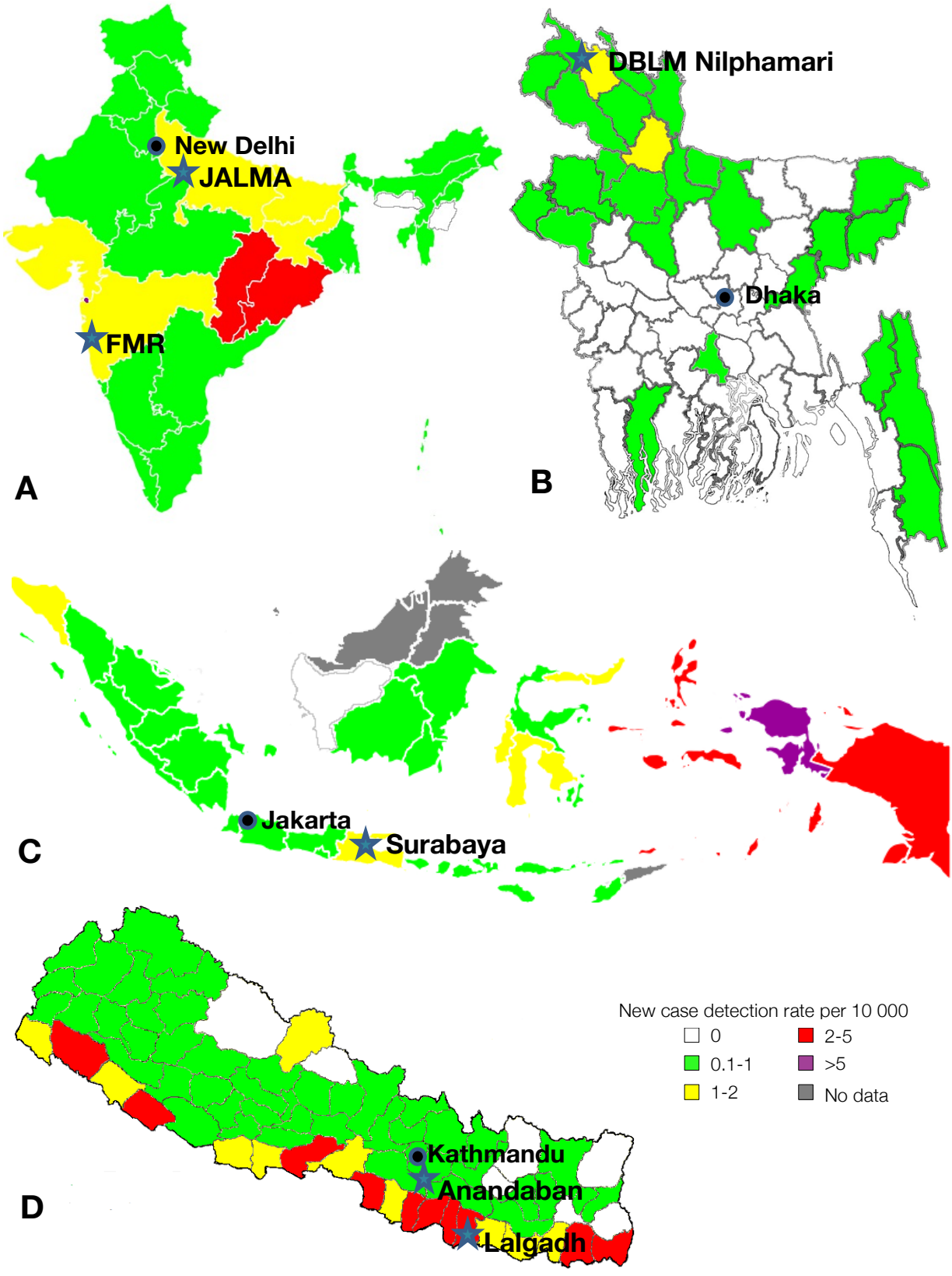

Figure 1- Country maps indicating the new case detection rate in 2014 per district per 10 000 inhabitants and the location of the hospitals for (A) India, (B) Bangladesh, (C) Indonesia, and (D) Nepal

3. Immunology and leprosy reactions

i. Immune response and classification

Leprosy is classified in two ways, the Ridley-Joplin and the WHO classification. The five-group Ridley-Jopling system is based on the characteristics of skin lesions and bacterial load and is used for research and clinical purposes [22]. The patient's immune response to the bacilli determines which type develops. At one end of the spectrum, patients with a mild form of the disease, the tuberculoid (TT) form, can be found. These patients show a strong cell-mediated immune response and have few well-defined skin lesions and no or low bacteria counts. At the other, more severe end, the lepromatous (LL) patient's immune system has a weak or absent response to *M. leprae*, resulting in multiple macules and/or nodules with many bacilli present [23]. In between these two extremes, the majority of the patients are classified in one of the three unstable borderline forms: borderline tuberculoid (BT), borderline borderline (BB) and borderline lepromatous (BL). The cellular immunity becomes weaker the more one gets to the lepromatous side. The intensity of this response can change and therefore patients can switch between the three borderline types, although most patients will move towards the lepromatous side (downgrading).

It is not always possible to carry out and examine a skin smear test under field circumstances, therefore the WHO advises to use a simpler two-group classification system in these cases. When a person has up to five skin lesions the disease is classified as paucibacillary (PB) and patients with more than five lesions fall under the multibacillary (MB) group [13]. The PB group contains TT and BT types and the MB group includes BT, BB, BL and LL types [24,25]. If a skin smear is positive the patient should always be treated as an MB case.

Next to determining which leprosy type develops, immunology is also very important regarding the susceptibility for leprosy. Analyses of *M. leprae* DNA in nasal swabs show that up to 31% of the general population in endemic areas carry the bacilli in their nose [9,26-28]. However, it is assumed that less than 1% of people exposed to *M. leprae* will develop clinical leprosy [20,29]. This means that a vast majority of the population has a strong immune response, leading to containment and spontaneous clearing of the disease. The factors leading to the differences in immune response between persons are not completely understood, but genetics, nutritional status and socioeconomic status are thought to influence the host reaction to the leprosy bacilli [30-33].

ii. Reactions

In part of the leprosy patients, the antigens of the (dying) leprosy bacilli generate an increased activity of the immune system leading to acute inflammation of skin and nerves. These reactions can occur before MDT, but commence mostly during or after treatment. When not treated in time, the nerves can be severely damaged during these reactions – i.e. neuropathy, which can

lead to disabilities. Leprosy is curable, but even when taking MDT the risk on neuropathy due to reactions is still present, until years after treatment has ended [34-36].

There are two types of reactions. Type 1 reactions, also called reversal reactions, arise in around 25-30% of the patients with borderline leprosy [37]. The number of skin lesions may increase and they become inflamed, red, swollen and tender. The peripheral nerves become swollen and the resulting intra-neural pressure may affect their function, leading to anaesthesia or paralysis of hands, feet and eyes [38].

Type 2 reactions, or erythema nodosum leprosum (ENL), only occur in lepromatous and borderline lepromatous types, respectively in approximately 10 and 50% of the patients [19,39]. ENL is seen as a chronic disease, since reactions can relapse over several years. Painful red papules or nodules appear on the skin. Next to involvement of nerves and skin, also other organs as testes, eyes, kidneys, joints and lymph nodes can be affected [40]. The reactions cause fever and general malaise and severe ENL can be life-threatening.

Both type 1 and 2 reactions are treated with corticosteroids. The WHO advises a 12-week course of prednisolone, however, studies show that longer treatments may be more effective [13,41]. When patients with severe type 2 reactions do not respond well to prednisolone, thalidomide or clofazimine are given.

iii. Nutrition and immunology

As mentioned, nutritional status may influence the effectiveness of the immune response towards the leprosy bacilli or its antigens. The relation between nutrition, immunology and infections has been acknowledged for decades [42]. The essential defence against *Mycobacterium leprae*, an intracellular micro-organism, is dependent on the cell-mediated immune response [24]. Protein-energy malnutrition (PEM) as well as inadequate intake of vitamins and minerals are related to a reduced cell-mediated immunity [43-46].

For centuries (mal)nutrition has been linked to susceptibility to leprosy, for example by the Dutch physician Fortestu in the 17th century [47]. Various foods as fish, salt, pork meat and alcoholic drinks have been 'blamed' for causing leprosy [48]. However, until this day only little research has been carried out on the exact role of nutrition on the susceptibility and development of leprosy and thus this relation remains unclear. With the long incubation time of *M. leprae* it is very hard to study the nutritional status at the moment of infection. In addition, the relation between nutrition and infection runs both ways: infections may have major impact on nutritional status as well. This makes it almost impossible to distinguish between cause and consequence.

4. Leprosy neuropathy

i. Neuropathy and nerve function impairment

Leprosy is regarded as a disease of skin and nerves, but it is the neurological aspect that makes leprosy a disease with a high impact. Neuropathy can arise gradually or can be a consequence of reactions. New patients often present with some level of nerve function impairment (NFI), the percentage varies between 2% and 55%, with lower figures for PB cases [34,49-51]. On top of that, 2.4-29% of the patients develops neuropathy during or after treatment [35,50,52], also depending on classification and pre-existing nerve function impairments.

M. leprae bacilli settle and reproduce in the Schwann cells [53,54]. The severity and extensiveness of neuropathy depends on the type of leprosy. Patients on the tuberculoid end of the spectrum often have only one or few nerves affected. Granulomatous inflammation causes enlargement of the nerves, oedema and loss of nerve tissue and structure. The damage occurs fast and severe.

At the lepromatous end, often multiple nerves are bilaterally and symmetrically involved [55]. The lack of cell mediated immunity results in extensive infiltration of bacilli. The continued multiplication and high bacterial load cause damage to the nerves. This process is very slow, the nerve structure and function are often maintained for a long period of time. In borderline leprosy, the worst of the two above mentioned classes is combined: the wide involvement of nerves as in LL with the formation of granulomas and accompanying destruction of nerves as in TT. Patients in the borderline groups have therefore a high chance on severe neuropathy [53].

Generally in leprosy, sensory nerves are affected before motor nerves. Older histopathological studies showed that neuropathy occurs first in the small nerve fibres (unmyelinated C-fibres followed by myelinated Aδ-fibres), which conduct pain, warm and cold sensation and are responsible for the autonomic function (i.e. sweating) [56-58]. Subsequently, the large fibres (Aβ-fibres) are affected, responsible for vibration, touch and pressure perception. The INFIR study found, however, that the order of first affected modality and nerve fibre differs from patient to patient [59]. When autonomic nerves are affected, sweat gland function is lost, which leads to dryness of the skin and an increased risk of ulceration.

The specific nerves affected by leprosy are the facial nerve, the ulnar, median and the radial cutaneous in the arm and hand, and the posterior tibial and the sural in the leg and foot. The most commonly affected nerves are the posterior tibial, sural and ulnar nerve [35,60,61].

To estimate which patients are at risk of developing NFI, Croft and colleagues developed the Clinical Prediction Rule. They established that MB patients who already have some nerve function impairment have the highest risk for new NFI and should have regular follow-ups in the first 2 years after diagnosis [62].

ii. Disabilities

When nerve function impairment is not treated within 6 months of onset, the damage can become irreversible and this will have severe consequences. Insensible feet and hands are extra vulnerable for burns and ulcers, with amputation as a result in the most extreme cases. This neurological aspect of leprosy, and the possibility of developing chronic disabilities, is what make leprosy such a serious disease. The disabilities make patients vulnerable for stigma and exclusion. Early detection of leprosy and neuropathy is therefore very important.

iii. Diagnosis and nerve function assessment

Diminished sweat function or blood flow (autonomic), pain, touch, pressure, warmth, cold and vibration sensation (sensory), and muscle strength (motor) can be assessed to detect leprosy neuropathy. Common methods used in the field to detect sensory nerve function impairments are monofilament testing (MFT) and ball point testing (BPT); both assess touch sensation on the hands and feet. A standard set of Semmes-Weinstein monofilaments consists of 6 filaments, with increasing thickness and pressure (from 70 mg to 300 mg). This is a reliable and standardized method to test for sensory impairments, in contrast to a BPT for which pressure is not standardized and which can only provide a yes/no outcome, although the low costs and high availability give BPT a huge advantage.

For the detection of motor nerve function impairments, voluntary muscle testing (VMT) is performed by examining the ability of the patient to move a body part to a given position and to hold that position against resistance applied by the tester [63]. The modified 0–5 MRC scale is used, ranging from full range of motion and full resistance to complete paralysis.
Both VMT and MFT require training and practice, but then both can be executed reliably [64-66]. When the nerve function is impaired according to one or both of these tests, it is seen as clinically impaired.

The INFIR study showed that neuropathy was more widespread than could be detected with the MFT and VMT [67]. Before the nerve function impairment is clinically detectable, the majority of nerves already showed some subclinical neuropathy as can be detected with more sensitive methods. The warm detection threshold (WDT) and nerve conduction studies (NCS) were able to detect subclinical neuropathy up to 12 weeks before the clinical neuropathy was noticeable with MFT or VMT.

Nerve conduction studies basically assess how fast an electrical pulse is conducted through a nerve. This can both be done for sensory and motor nerves, and the latency, amplitude and velocity of the pulse are recorded. The disadvantage of these two methods is that they are very expensive and require electricity and acclimatized conditions. When WDT and/or NCS are impaired, it is seen as subclinical impairment.

iv. Treatment of nerve function impairments

Nerve function impairment of recent onset (≤ 6 months) can be treated with corticosteroids [35, 38]. The standard treatment recommended by the WHO starts at 40-60 mg per day and is decreased over 12 weeks [68]. Ideally, each patient would receive an individual, tailored therapy, but this is only possible in referral centres. In the non-specialized clinics in the field, standard treatment is used.

Several studies have been conducted to examine treatment of clinical NFI using prednisolone. In one cohort, the WHO recommended 12-week prednisolone regimen was found not to be successful in the prevention and reversal of NFI in MB patients [69]. Also a 16-week prednisolone regimen, usual practice nowadays, was found not to be very effective in two randomized controlled trials (RCT) [70,71]. There is reason to assume that longer steroid treatment might be more beneficial, since one RCT showed that a 5-month corticosteroid regimen was significantly more effective than a 3-month regimen. Since steroids can have severe side effects as diabetes, peptic ulcer, osteoporosis, glaucoma, cataract, psychosis, and hypertension, it is important that there is clear evidence for longer use of steroids. A Cochrane review concluded, however, that the evidence of the RCTs is not enough to draw robust conclusions about the long-term effect of corticosteroids on reactions and NFI in leprosy [53]. More research needs to be done to determine the optimal dose and duration of prednisolone for treating clinical NFI in leprosy.

Interestingly, several studies have reported spontaneous recovery of nerve function impairments, even for old impairments. The Tripod studies showed that after 12 months 75% and 50% of the nerves spontaneously healed in mild and long-standing impairment, respectively. This was also seen in the AMFES trial in Ethiopia, where acute neuropathy recovered spontaneously in 42% and old neuropathy recovered in 23% of the cases [35,72].

5. The TENLEP study

Nerve function impairment is a vicious but frequent consequence of leprosy. Unfortunately, optimal treatments to restore and prevent NFI have not unequivocally been established yet. For this reason, the TENLEP study (Treatment of Early Neuropathy in LEProsy) was set up in four Asian countries: Bangladesh, India, Indonesia and Nepal. The study consists of two randomized controlled trials, one focusing on restoring and one on preventing NFI. The aim of the first trial was to assess whether prednisolone treatment of 32 weeks duration is more effective than treatment of 20 weeks duration in restoring nerve function in patients with clinical sensory and/or motor NFI of recent onset (<6 months). The second trial was designed to determine whether prednisolone treatment of early subclinical neuropathy, as detected with WDT and NCS, would prevent clinical sensory and/or motor function loss in leprosy patients. In both trials, patients were followed for 18 months. The first patient was taken in in June 2012 and the data collection was completed in October 2015.

In addition to the primary aims of the study, the information gathered from this study may be useful to refine the clinical prediction rule as designed by Croft et al [73]. Guidelines for prophylactic prednisolone could be developed for certain high risk groups to make steps forward in the prevention of disabilities and deformities in newly diagnosed leprosy patients. However, this is outside the scope of this thesis.

The study took place in six leprosy centres: Anandaban Leprosy Hospital and Lalgadh Leprosy Services Centre in Nepal, the Foundation for Medical Research and the JALMA institute in India, Dr. Soetomo hospital in Surabaya, Indonesia, and Nilphamari-Leprosy Mission Hospital in Bangladesh (see maps in §2.ii).

6. Research questions

The first objective of this thesis is to study methods that help early detection and treatment of neuropathy in order to prevent irreversible nerve function impairment and the resulting disabilities in leprosy patients. Regarding risk factors for leprosy we studied differences in diet and food security between leprosy patients and a control group without leprosy. The research questions of this thesis are:

1. How can the detection of nerve function impairment in leprosy at field level be improved?
2. How can irreversible nerve function impairment best be prevented in leprosy?
3. Which nutritional factors possibly underlie the development of clinical leprosy?

This thesis is divided in three sections. In the first part, the focus lays on methodological studies. The TENLEP study protocol is presented in **Chapter 2**, describing the details of the assessments and treatment for both the Clinical and Subclinical trial in depth. Chapters 3, 4 and 5 cover the first research question. In **Chapter 3**, a study in India and Nepal defining the normal values for monofilament tests for two additional nerves is described. The results of reliability testing for monofilament and voluntary muscle testing in the six TENLEP centres are given in **Chapter 4**. **Chapter 5** presents a study in which several new portable, battery operated tests are compared to determine if they are useful in early detection of leprosy neuropathy in the field. The second section of this thesis concentrates on study results. **Chapter 6** answers the second research question by presenting the results of the clinical TENLEP trial, aimed to examine whether prednisolone can restore clinical nerve function impairment in leprosy patients. The serious adverse events in the TENLEP trials are addressed in **Chapter 7**. Finally, **Chapter 8** discusses the results of a case-control study in Bangladesh, comparing the diet and food security between newly diagnosed leprosy patients and a control population. In the third and final part, **Chapter 9**, the results are discussed and the research questions are answered.

7. References

1. Monot, M., et al., *On the origin of leprosy*. Science., 2005. 308(5724): p. 1040-2.
2. Brown, S.G., *The history of leprosy*, in Leprosy, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.
3. Pandya, S.S., *Historical background, in IAL textbook of leprosy*, H.K. Kar and B. Kumar, Editors. 2010, Jaypee Brothers Medical Publishers: New Delhi.
4. The International Federation of Anti-Leprosy Associations (ILEP)., *How to Diagnose and Treat Leprosy. Learning Guide.* 2002, London.
5. Nicholls, P.G., et al., *Factors contributing to delay in diagnosis and start of treatment of leprosy: Analysis of help-seeking narratives in northern Bangladesh and in West Bengal, India*. Leprosy Review, 2005. 76(1): p. 35-47.
6. Zhang, F., et al., *Healthcare seeking behaviour and delay in diagnosis of leprosy in a low endemic area of China*. Leprosy Review, 2009. 80(4): p. 416-423.
7. Ridley, D.S., *The bacteriological interpretation of skin smears and biopsies in leprosy*. Transactions of the Royal Society of Tropical Medicine and Hygiene, 1955. 49(5): p. 449-452.
8. Noordeen, S.K., *The epidemiology of leprosy*, in Leprosy, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.
9. Hatta, M., et al., *Distribution and persistence of Mycobacterium leprae nasal carriage among a population in which leprosy is endemic in Indonesia*. Trans R Soc Trop Med Hyg., 1995. 89(4): p. 381-5.
10. Desikan, K.V. and Sreevatsa, *Extended studies on the viability of Mycobacterium leprae outside the human body*. Lepr Rev., 1995. 66(4): p. 287-95.
11. Moet, F.J., et al., *Risk factors for the development of clinical leprosy among contacts, and their relevance for targeted interventions*. Leprosy Review, 2004. 75(4): p. 310-326.
12. Richardus, J.H., et al., *Close contacts with leprosy in newly diagnosed leprosy patients in a high and low endemic area: comparison between Bangladesh and Thailand*. Int J Lepr Other Mycobact Dis., 2005. 73(4): p. 249-57.
13. World Health Organization, *Expert Committee on Leprosy: 7th r*eport. 1998: Geneve.
14. Merle, C.S., S.S. Cunha, and L.C. Rodrigues, *BCG vaccination and leprosy protection: Review of current evidence and status of BCG in leprosy control*. Expert Review of Vaccines, 2010. 9(2): p. 209-222.
15. Setia, M.S., et al., *The role of BCG in prevention of leprosy: A meta-analysis*. Lancet Infectious Diseases, 2006. 6(3): p. 162-170.

16. World Health Organization, *Leprosy Elimination Project: status report 2003*. 2004, WHO: Geneva.

17. Meima, A., J.H. Richardus, and J.D.F. Habbema, *Trends in leprosy case detection worldwide since 1985*. Leprosy Review, 2004. 75(1): p. 19-33.

18. World Health Organization, *Global leprosy update 2014: need for early case detection*, in Weekly Epidemiological record. 2015: Geneve. p. 461-476.

19. Britton, W.J. and D.N.J. Lockwood, *Leprosy*. Lancet, 2004. 363(9416): p. 1209-1219.

20. Thorat, D.M. and P. Sharma, *Epidemiology*, in IAL textbook of leprosy, H.K. Kar and B. Kumar, Editors. 2010, Jaypee Brothers Medical Publishers: New Delhi.

21. Montestruc, E. and R. Berdonneau, 2 *New cases of leprosy in infants in Martinique. Bulletin de la Société de pathologie exotique et de ses filiales*, 1954. 47(6): p. 781-783.

22. Ridley, D.S. and W.H. Jopling, *Classification of leprosy according to immunity. A five-group system*. Int J Lepr Other Mycobact Dis., 1966. 34(3): p. 255-73.

23. Montoya, D. and R.L. Modlin, *Learning from leprosy: insight into the human innate immune response*. Adv Immunol, 2010. 105: p. 1-24.

24. Harboe, M., *The immunology of leprosy*, in Leprosy, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.

25. Mishra, R.S. and J. Kumar, *Classification*, in IAL textbook of leprosy, H.K. Kar and B. Kumar, Editors. 2010, Jaypee Brothers Medical Publishers: New Delhi.

26. Izumi, S., et al., *An epidemiological study on Mycobacterium leprae infection and prevalence of leprosy in endemic villages by molecular biological technique*. Indian J Lepr., 1999. 71(1): p. 37-43.

27. Klatser, P.R., et al., *Detection of Mycobacterium leprae nasal carriers in populations for which leprosy is endemic*. J Clin Microbiol., 1993. 31(11): p. 2947-51.

28. Lavania, M., et al., *Cohort study of the seasonal effect on nasal carriage and the presence of Mycobacterium leprae in an endemic area in the general population*. Clinical Microbiology and Infection, 2013. 19(10): p. 970-974.

29. Godal, T., M. Lofgren, and K. Negassi, *Immune response to M. leprae of healthy leprosy contacts*. Int J Lepr Other Mycobact Dis., 1972. 40(3): p. 243-50.

30. Moet, F.J., et al., *Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomised controlled trial.* BMJ, 2008. 336(7647): p. 761-4.

31. Scollard, D.M., et al., *The continuing challenges of leprosy*. Clin Microbiol Rev, 2006. 19(2): p. 338-81.

32. Gaschignard, J., et al., *Pauci- and Multibacillary Leprosy: Two Distinct, Genetically Neglected Diseases*. PLoS Negl Trop Dis, 2016. 10(5): p. e0004345.

33. Sauer, M.E., et al., *Genetics of leprosy: expected and unexpected developments and perspectives*. Clin Dermatol, 2015. 33(1): p. 99-107.

34. Croft, R.P., et al., *Incidence rates of acute nerve function impairment in leprosy: A prospective cohort analysis after 24 months (The Bangladesh Acute Nerve Damage Study*). Leprosy Review, 2000. 71(1): p. 18-33.

35. Saunderson, P., et al., *The pattern of leprosy-related neuropathy in the AMFES patients in Ethiopia: Definitions, incidence, risk factors and outcome*. Leprosy Review, 2000. 71(3): p. 285-308.

36. Richardus, J.H., et al., *Nerve function impairment in leprosy at diagnosis and at completion of MDT: A retrospective cohort study of 786 patients in Bangladesh*. Leprosy Review, 1996. 67(4): p. 297-305.

37. van Brakel, W.H., I.B. Khawas, and S.B. Lucas, *Reactions in leprosy: An epidemiological study of 386 patients in West Nepal*. Leprosy Review, 1994. 65(3): p. 190-203.

38. Britton, W.J., *The management of leprosy reversal reactions*. Leprosy Review, 1998. 69(3): p. 225-234.

39. Pocaterra, L., et al., *Clinical course of erythema nodosum leprosum: an 11-year cohort study in Hyderabad, India*. Am J Trop Med Hyg., 2006. 74(5): p. 868-79.

40. Pfaltzgraff, R.E. and A. Bryceson, *Clinical leprosy*, in Leprosy, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.

41. Sundar Rao, P.S.S., et al., *Multi-centre, double blind, randomized trial of three steroid regimens in the treatment of type-1 reactions in leprosy.* Leprosy Review, 2006. 77(1): p. 25-33.

42. Scrimshaw, N.S., C.E. Taylor, and J.E. Gordon, *Interactions of nutrition and infection*. The American journal of the medical sciences, 1959. 237(3): p. 367-403.

43. Ferguson, A. and G.E. Griffin, *Nutrition and the immune system*, in Human Nutrition and Dietetics, J.S. Garrow, W.P.T. James, and A. Ralph, Editors. 2000, Elsevier Science Ltd.: London. p. 747-764.

44. Keusch, G.T., *The history of nutrition: malnutrition, infection and immunity*. J Nutr., 2003. 133(1): p. 336S-340S.

45. Scrimshaw, N.S. and J.P. SanGiovanni, *Synergism of nutrition, infection, and immunity: an overview.* Am J Clin Nutr., 1997. 66(2): p. 464S-477S.

46. Edelman, R., *Malnutrition and leprosy--an analytical review*. Lepr India, 1979. 51(3): p. 376-88.

47. Keil, E.G., T*he importance of nutrition in the prevention and cure of leprosy.* International Journal of Leprosy, 1933. 1(4): p. 393-399.

48. Klingmüller, V., *Nahrung als Ursache von Lepra*, in Die Lepra. 1930, Springer-Verlag: Berlin. p. 208-212.

49. van Brakel, W.H., *Peripheral neuropathy in leprosy and its consequences*. Lepr Rev., 2000. 71(Suppl): p. S146-53.

50. van Brakel, W.H., et al., *The INFIR Cohort Study: investigating prediction, detection and pathogenesis of neuropathy and reactions in leprosy. Methods and baseline results of a cohort of multibacillary leprosy patients in north India*. Lepr Rev, 2005. 76(1): p. 14-34.

51. Jiang, J., et al., *A field trial of detection and treatment of nerve function impairment in leprosy - Report from national POD pilot project.* Leprosy Review, 1998. 69(4): p. 367-375.

52. Smith, W.C., et al., *Predicting neuropathy and reactions in leprosy at diagnosis and before incident events-results from the INFIR cohort study*. PLoS Negl Trop Dis, 2009. 3(8): p. e500.

53. Ridley, D.S. and C.K. Job, *The pathology of leprosy*, in Leprosy, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.

54. Rao, P.N. and S.K. Suneetha, *Neuritis: Definition, Clinical manifistations and Proforma to Record Nerve Impairment*, in IAL textbook of leprosy, H.K. Kar and B. Kumar, Editors. 2010, Jaypee Brothers Medical Publishers: New Delhi.

55. Agrawal, A., et al., *Neurological manifestations of Hansen's disease and their management*. Clinical Neurology and Neurosurgery, 2005. 107(6): p. 445-454.

56. Antia, N.H., *The significance of nerve involvement in leprosy*. Plast Reconstr Surg, 1974. 54(1): p. 55-63.

57. Shetty, V.P., N.H. Antia, and J.M. Jacobs, *The pathology of early leprous neuropathy*. Journal of the Neurological Sciences, 1988. 88(1-3): p. 115-131.

58. Shetty, V.P., et al., *Study of evolution of nerve damage in leprosy. Part II--Observations on the index branch of the radial cutaneous nerve in contacts of leprosy*. Lepr India, 1980. 52(1): p. 19-25.

59. McKnight, J., *Clinical testing and prognostic markers for the development of leprosy neuropath*y, in Infectious and Tropical Diseases. 2010, University of London: London.

60. Croft, R.P., et al., *Nerve function impairment in leprosy: design, methodology, and intake status of a prospective cohort study of 2664 new leprosy cases in Bangladesh (The Bangladesh Acute Nerve Damage Study)*. Lepr Rev, 1999. 70(2): p. 140-59.

61. van Brakel, W.H., et al., *The INFIR Cohort Study: assessment of sensory and motor neuropathy in leprosy at baseline.* Lepr Rev, 2005. 76(4): p. 277-95.

62. Croft, R.P., et al., *A clinical prediction rule for nerve function impairment in leprosy patients-revisited after 5 years of follow-up*. Lepr Rev, 2003. 74(1): p. 35-41.

63. Brandsma, J.W. and W.H. van Brakel, *Protocol for motor function assessment in leprosy and related research questions*. Indian J Lepr, 2001. 73(2): p. 145-58.

64. Anderson, A.M. and R.P. Croft, *Reliability of Semmes Weinstein monofilament and ballpoint sensory testing, and voluntary muscle testing in Bangladesh.* Leprosy Review, 1999. 70(3): p. 305-313.

65. Brandsma, J.W., et al., *Intertester reliability of manual muscle strength testing in leprosy patients*. Lepr Rev, 1998. 69(3): p. 257-66.

66. Roberts, A.E., et al., *Ensuring inter-tester reliability of voluntary muscle and monofilament sensory testing in the INFIR Cohort Study*. Lepr Rev, 2007. 78(2): p. 122-30.

67. van Brakel, W.H., et al., *Early diagnosis of neuropathy in leprosy--comparing diagnostic tests in a large prospective study (the INFIR cohort study)*. PLoS Negl Trop Dis, 2008. 2(4): p. e212.

68. World Health Organization. *WHO Model Prescribing Information: Drugs Used in Leprosy*. 1998 [cited 2015 May 7th]; Available from: http://apps.who.int/medicinedocs/en/d/Jh2988e/14.5.html.

69. Shetty, V.P., et al., *The effect of corticosteroids usage on bacterial killing, clearance and nerve damage in leprosy; part 3 - study of two comparable groups of 100 multibacillary (MB) patients each, treated with MDT 1 steroids vs MDT alone, assessed at 6 months post - release from 12 months MDT*. Leprosy Review, 2010. 81(1): p. 41-58.

70. van Brakel, W.H., et al., *The prognostic importance of detecting mild sensory impairment in leprosy: A randomized controlled trial (TRIPOD 2)*. Leprosy Review, 2003. 74(4): p. 300-310.

71. Walker, S.L., et al., *A phase two randomised controlled double blind trial of high dose intravenous Methylprednisolone and oral prednisolone versus intravenous normal saline and oral prednisolone in individuals with leprosy type 1 reactions and/or nerve function impairment.* PLoS Neglected Tropical Diseases, 2011. 5(4).

72. Croft, R.P., et al., *The treatment of acute nerve function impairment in leprosy: results from a prospective cohort study in Bangladesh.* Lepr Rev., 2000. 71(2): p. 154-68.

73. Croft, R.P., et al., *A clinical prediction rule for nerve-function impairment in leprosy patients.* Lancet, 2000. 355(9215): p. 1603-1606.

Chapter 2

Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols

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BMC Neurology 2012, 12:159

Abstract

Introduction

Nerve damage in leprosy often causes disabilities and deformities. Prednisolone is used to treat nerve function impairment (NFI). However, optimal dose and duration of prednisolone treatment has not been established yet. Besides treating existing NFI it would be desirable to prevent NFI. Studies show that before NFI is clinically detectable, nerves often show subclinical damage. Within the 'Treatment of Early Neuropathy in LEProsy' (TENLEP) study two double blind randomized controlled trials (RCT) will be carried out: a trial to establish whether prednisolone treatment of 32 weeks duration is more effective than 20 weeks in restoring nerve function in leprosy patients with clinical NFI (Clinical trial) and a trial to determine whether prednisolone treatment of early subclinical NFI can prevent clinical NFI (Subclinical trial).

Methods

Two RCTs with a follow up of 18 months will be conducted in six centres in Asia. In the Clinical trial leprosy patients with recent (<6 months) clinical NFI, as determined by Monofilament Test and Voluntary Muscle Test, are included. The primary outcomes are the proportion of patients with restored or improved nerve function. In the Subclinical trial leprosy patients with subclinical neuropathy, as determined by Nerve Conduction Studies (NCS) and/or Warm Detection Threshold (WDT), and without any clinical signs of NFI are randomly allocated to a placebo group or treatment group receiving 20 weeks prednisolone. The primary outcome is the proportion of patients developing clinical NFI. Reliability and normative studies are carried out before the start of the trial.

Discussion

This study is the first RCT testing a prednisolone regimen with a duration longer than 24 weeks. Also it is the first RCT assessing the effect of prednisolone in the prevention of clinical NFI in patients with established subclinical neuropathy. The TENLEP study will add to the current understanding of neuropathy due to leprosy and provide insight in the effectiveness of prednisolone on the prevention and recovery of NFI in leprosy patients. In this paper we present the research protocols for both Clinical and Subclinical trials and discuss the possible findings and implications.

Introduction

Damage to peripheral nerves is the main consequence of leprosy and may cause deformities and disabilities in patients. Nerve damage can occur before, during and after multidrug therapy (MDT) and is a result of inflammation in the nerves due to immunological reactions [1]. For many years corticosteroids, mostly prednisolone, have been used to treat nerve function impairment (NFI) in leprosy patients [2]. However, an optimal dose and duration of steroid treatment has yet to be established [3]. In addition, research should focus also on possibilities of timely detection and treatment of early (subclinical) neuropathy in order to prevent NFI and its consequences [4]. The TENLEP study is designed to obtain additional information about prednisolone treatment for preventing and restoring nerve function in people affected by leprosy. Within the TENLEP study two randomized clinical trials will be conducted; one trial focusing on patients with subclinical neuropathy, and the second trial focusing on patients with clinical NFI.

Between 6 and 27% of the 228 474 newly detected leprosy cases in 2010 [5] presented with visible impairment (grade 2 disability) [2,4]. This WHO leprosy disability grading system is the most widely used method to assess impairment in leprosy patients and is generally used for monitoring program quality [6,7]. More accurate assessments for NFI are Voluntary Muscle Testing (VMT) and Monofilament Testing (MFT) or ball point tests [8], which are widely used in clinical practice to assess motor and sensory NFI, respectively. Recently, more sensitive methods have been introduced to detect early, subclinical neuropathy. The INFIR study found that nerve conduction studies (NCS) and Warm Detection Threshold (WDT) are the most effective methods for finding subclinical nerve damage [1]. With these tests subclinical neuropathy can be detected at least 3 months before VMT and MFT can determine the first clinical impairments. Subclinical changes during and following MDT were also found to be predictive of new onset NFI [9].

To examine treatment of clinical NFI several studies have been conducted using prednisolone. In one cohort the WHO recommended prednisolone regimen (starting with 40 mg prednisolone/day, tapered down over 12 weeks [2]) was found not to be successful in the prevention and reversal of NFI in multibacillary (MB) patients treated for reactions or neuropathy [10]. Also a 16-week prednisolone regimen, usual practice nowadays, was found not to be very effective in two randomized controlled trials (RCT) [11,12]. One trial was in patients with Type 1 Reactions and/or NFI, receiving either prednisolone or prednisolone with intravenous methylprednisolone. Close to 50% of the patients required additional prednisolone during or after the treatment period [11]. The second RCT in MB patients with mild sensory impairment did not show a difference in NFI as measured with MFT 12 months after start of prednisolone treatment [12]. Van Veen et al. [3], reviewing RCTs comparing placebo to prednisolone treatment, conclude that studies so far have not provided enough evidence to draw robust conclusions about the long-term effect of corticosteroids on reactions and NFI. However, there is reason to assume that longer steroid treatment might be more beneficial, since one study showed that a 5-month corticosteroid regimen was significantly more effective than a 3-month

regimen [13]. In TENLEP we aim to assess whether prednisolone treatment of 32 weeks duration is more effective than treatment of 20 weeks duration in restoring nerve function in patients with clinical sensory and/or motor NFI of recent onset (<6 months), as detected by VMT and/or MFT.

The effect of prednisolone treatment on patients with subclinical neuropathy to prevent clinical NFI as determined with MFT and/or VMT has not been studied in a RCT before. The objective of the Subclinical trial is therefore to determine whether prednisolone treatment of early subclinical neuropathy, as detected with WDT and NCS, would prevent clinical sensory and/or motor function loss in leprosy patients. This paper presents the protocols for both the Clinical and the Subclinical trial.

Methods

Definitions

General

Neuropathy (peripheral)

Functional impairment and/or structural damage to autonomic, sensory, and motor nerve fibres within the peripheral nervous system.

Nerve function impairment

Sensory, motor or autonomic neuropathy evidenced by clinically detectable reduction in function in sensory, motor and/or autonomic fibres. The 'level' of impairment that is clinically detectable depends on the sensitivity of the testing instruments used. (It does not include abnormality of nerve conduction that is detectable only by electrophysiological means and WDT).

Nerve damage

An imprecise but common term for 'neuropathy', which is also used in relation to trauma. Here it indicates clinical or subclinical damage to a nerve, whether reversible or irreversible.

Neuritis

A condition in which inflammatory cells are found in the nerve, detectable by swelling and/or functional impairment with spontaneous nerve pain and/or nerve tenderness on palpation.

Subclinical neuropathy

Patients have normal values for Voluntary Muscle Testing and Monofilament Testing, but are impaired on Nerve Conduction Studies (NCS) and/or Warm Detection Threshold (WDT).

Entry criteria Clinical trial

At least one nerve with either a VMT score of 4 or less on the 0–5 (modified) MRC scale or with a monofilament threshold increased compared to normal subjects by three or more monofilament levels on any test-site, two levels on one test-site AND at least one level on another test-site, OR one level on three or more test-sites for one nerve. With VMT each nerve is tested with one specific test assessing the strength of a muscle (group) innervated by that nerve. For MFT each nerve is tested on three sites.

Entry criteria Subclinical trial

Any one parameter (Motor Nerve Conduction, Sensory Nerve Conduction, Warm Detection Threshold) abnormal in at least two nerves or any two parameters abnormal in at least one nerve. VMT and MFT values are normal.

Outcome criteria Clinical trial

Restored nerve function

Monofilament Test and/or Voluntary Muscle Test of a nerve are recovered to normal levels (MFT = 0, VMT = 5).

Improved nerve function

At least one nerve shows better results on Monofilament Test and/or Voluntary Muscle Test. The MF thresholds should be reduced by three or more monofilament levels on any site, two levels on one site AND at least one level on another site, OR one level on three or more sites for one nerve. VMT score should be increased by at least 1 point.

Improved Reaction Severity Scale score

When the score on the Reaction Severity Scale [14] decreases by at least 3 points on the sum score or at least 2 points on any individual item in the scale.

Count of nerve function impairments (CNFI)

The sum of MFT and VMT scores (5 being normal) for all nerves tested in the study.

Improved SALSA scale score

The SALSA scale [15] score decreases at least with 1 category in standardized categories of SALSA values as described in SALSA Scale User's Manual (Salsa Scale User's Manual, Version 1.1, July 2010)

Improved Participation scale score

The Participation scale [16] score decreases at least 1 grade on the "Grades of participation restriction" scale (as described on P-scale form).

Outcome criteria Subclinical trial

Clinical motor impairment (MI)

Motor neuropathy resulting in weakness of the muscles innervated by a given nerve. A patient is diagnosed as having clinical motor impairment if the VMT score for a muscle test is 4 or less on the 0–5 (modified) MRC scale. If a score of 4 is found, the test will be repeated by a second assessor.

Clinical sensory impairment (SI)

A patient is diagnosed as having sensory impairment of a nerve if the monofilament threshold is increased by three or more monofilament levels on any site, two levels on one site AND at least one level on another site, OR one level on three or more sites for one nerve.

Subclinical nerve function score (SNFS)

The Subclinical Nerve Function Score (SNFS) will be computed for NC parameters (amplitude and latency) and warm detection threshold, where non-impaired parameter adds 1 point to the overall score.

Improved SNFS

Subclinical Nerve Function Score increased with at least 1 point

Deteriorated SNFS

Subclinical Nerve Function Score decreased with at least 1 point

Design of study

The TENLEP study consists of two multi-centre randomized triple blind controlled trials, both with two treatment arms, to study the effectiveness of prednisolone treatment restoring (Clinical trial) and in preventing (Subclinical trial) clinical nerve function loss.

Six institutions in four different countries participate in this study: Nepal (Lalgadh Hospital and Anandaban Hospital); India (JALMA Institute for Leprosy -Agra and Foundation for Medical Research-Mumbai); Bangladesh (Nilphamari Hospital); and Indonesia (Dr. Soetomo Hospital-Surabaya). Anandaban Hospital and Dr Soetomo Hospital will only take part in the Clinical trial. All collaborative institutions are referral hospitals specialized in the detection and treatment of leprosy. At each institution a Principal Investigator (PI) is responsible for the research at that centre. The overall responsibility lies with the International Coordinator (dr. E. Post), guided by an International Steering Committee.

Participants

In the Clinical trial leprosy patients with clinical NFI will be randomly allocated to either treatment of standard duration (20 weeks) or an interventional treatment of longer duration (32 weeks). Both multibacillary (MB) and paucibacillary (PB) patients diagnosed with clinical sensory and/or motor nerve impairment of less than six months duration are enrolled. For the Subclinical trial leprosy patients with subclinical neuropathy will be randomly divided into an intervention group and a placebo group. Newly registered MB and PB patients without clinical NFI but having subclinical sensory and/or motor neuropathy at diagnosis, or developing this within their first three months of MDT treatment, will be eligible for inclusion in the trial. Patients from the Subclinical trial developing NFI in the first three months of the trial can enter the Clinical trial, but only data of patients that were allocated to the placebo group of the Subclinical trial will be analysed.

Inclusion and exclusion criteria

Patients included in the trials should be between 15 and 60 years of age, must give informed consent and should be free from conditions that may affect the peripheral nervous system, such as diabetes mellitus, and other active underlying diseases for example hypertension, osteoporosis and tuberculosis. Included patients receive deworming treatment with Mebendazol before steroid treatment starts. In both studies patients will be followed-up for 18 months. Excluded will be women pregnant at diagnosis, patients who need steroids for reasons other than recent NFI and patients with a single skin lesion on the trunk as the only sign of leprosy.

Randomization and blinding

In both trials patients will be randomly allocated to one of the two study arms. Randomization tables are provided by the statistician and drugs are labelled accordingly by the manufacturer. The key is held by the International Coordinator, Study Manager and PI of each centre and will be broken after the data analysis is completed or earlier for patients with adverse events, and new or worsening NFI.

Sample size calculation

For the Clinical trial a recovery of 60% is presumed for the standard regimen in the control group [17]. To detect a recovery of 70% in the treatment group, compensating for 20% of loss to follow-up, a total of 720 subjects need to be enrolled.
The INFIR study shows that of the leprosy patients having subclinical neuropathy 16% will eventually develop clinical NFI [1]. For the Subclinical trial we assume a reduction in patients developing clinical NFI by half in the treated group (8%). Anticipating a loss to follow-up of 20% at 18 months, a sample size of 275 subjects per trial arm is required. For both sample size calculations, a one-tailed alternative hypothesis, 80% power and 5% significance is used

Follow-up

Patients will be followed-up for 18 months. Nerve function is monitored by monthly VMT and MFT in both trials and assessments with NCS and WDT takes place at 20 weeks, 12 and 18 months from intake in the Subclinical trial. Participants can be withdrawn from the trials because of medical or clinical reasons or pregnancy. Subjects missing their appointment will be visited at home by research staff within two weeks when contact by cell phone is not possible or did not result in late attendance at the clinic. In cases when only one appointment is missed and medication is not taken for a maximum of one month, treatment will continue from the first package missed. The TENLEP trials started in April 2012, with a planned duration of intake of 1.5 years.

Medication

In both trials the medication provided is prednisolone. Prednisolone will be allocated based on two bodyweight classes. The low weight class (patients below 50 kg), will receive prednisolone treatment based on a body weight of 45 kg. The high weight class (patients 50 kg and more) receives treatment based on a body weight of 60 kg.

Treatment arms

In the Clinical trial patients in the intervention arm receive prednisolone for 32 weeks, in tablets of 5 mg. The control arm will follow a regimen of 20 weeks and receive placebo tablets to keep the number of tablets equal to the treatment group for effective treatment time and duration. The dose in both treatment and control arm starts at 1 mg/kg/day (either 45 or 60 mg/day depending on weight class) and will be tapered down over 32 and 20 weeks, respectively. Figure 1.A shows the dose over time of both arms in the Clinical trial. In contrast to previous trials, the middle range of the prednisolone dose will be maintained at a high level (0.44 mg/kg/day) for a longer period (12 weeks). In the Subclinical trial patients receive either prednisolone or placebo for 20 weeks in tablets of 5 mg. The prednisolone dose starts at 1 mg/kg/day (either 45 or 60 mg/day depending on weight class) and will be tapered down over 20 weeks. Figure 1.B shows the timeline and dosage. The total dosage of prednisolone over 20

weeks will be 2.8 grams for patients under 50 kg body weight, and 3.7 grams for patients over 50 kg body weight.

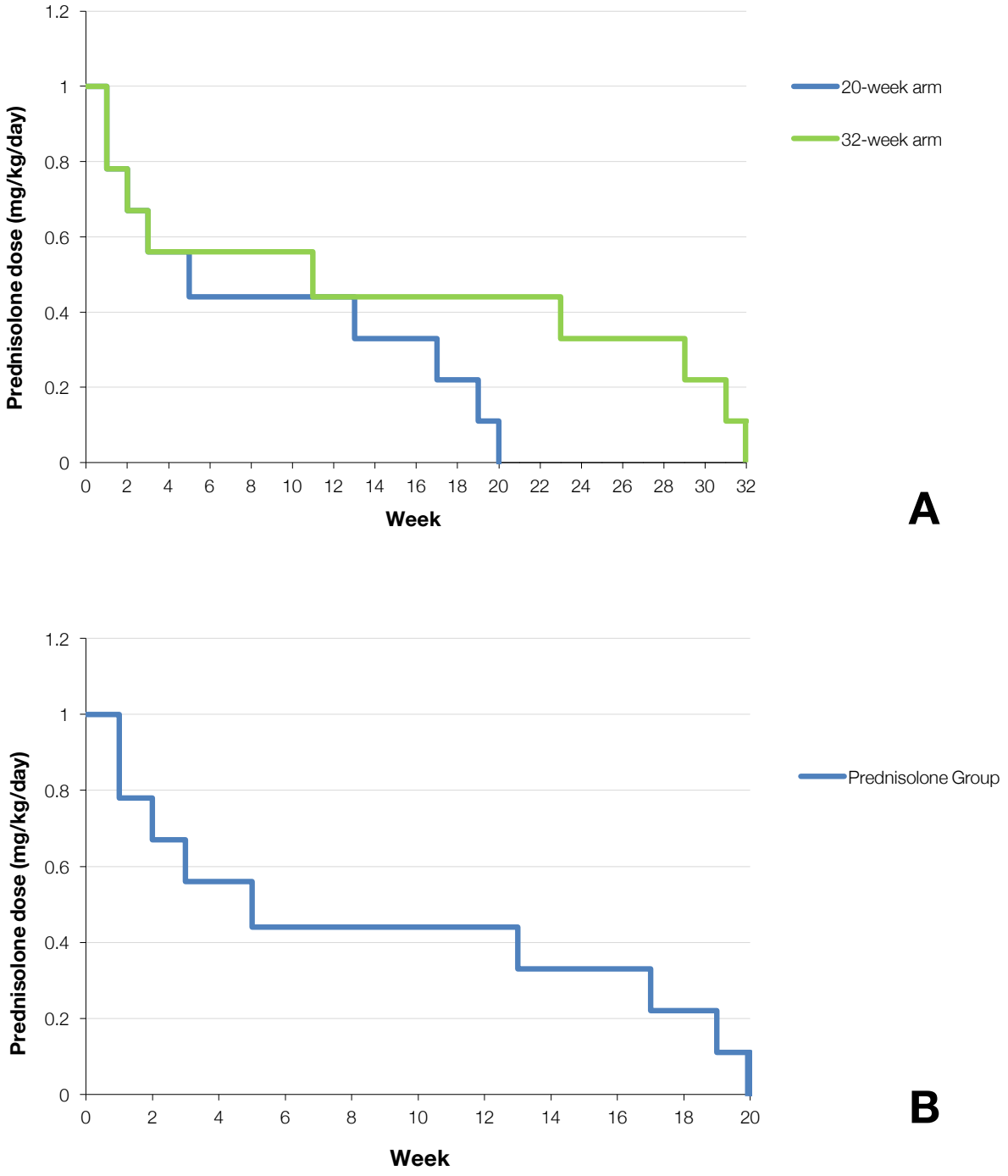

Figure 1 A- Timeline prednisolone dose for patients under 50 kg in the Clinical trial. **Figure 1 B**- Timeline prednisolone dose for patients under 50 kg in the Subclinical trial

Adverse events

Prednisolone has known adverse events, ranging from mild adverse events, such as moon face, fungal infections and acne, to serious adverse events such as peptic ulcer, osteoporosis glaucoma, cataract, psychosis, diabetes and hypertension [18,19]. Serious adverse events have not been encountered frequently in previous trials studying prednisolone for treating NFI in leprosy patients [20], however strict monitoring of adverse events will take place in the TENLEP trials. In case of serious adverse events the PI will break the key and initiate an individualized treatment scheme. In the event of minor side effects additional medication will be prescribed according to normal protocol in the clinic, but the key will not be broken.

Data collection

General assessments

Before intake, the general health of all possible eligible patients will be checked and history will be taken according to the protocol. Additionally, patients will be tested for specific medical conditions related to neuropathy and prednisolone intake such as diabetes mellitus (urine test) and osteoporosis (FRAX). Leprosy status will be categorized using both WHO classification (PB/MB), and Ridley-Jopling classification [21]. The latter will be done on clinical grounds, but a skin biopsy can be taken on voluntary basis for confirmation of the classification by a trained pathologist. For this procedure an additional consent procedure is in place. Of each patient a slit skin smear will be taken for determining the Bacteriological Index (BI).

Clinical nerve function tests

After intake, eligible subjects will be assessed for clinical sensory and motor nerve impairment. The clinical sensory function is tested with monofilaments using a standard set of 5 Semmes-Weinstein monofilaments ranging from blue (200 mg) to pink (300 g) [22]. This test of touch sensibility is based on indenting the skin surface with a series of standard nylon filaments. For each thickness it is recorded whether or not the patient feels the touch, starting with the thinnest filament. For hands the normal threshold is the blue filament and for feet the purple (2 mg) filament; when these filaments are felt by the patient it results in a score of 0. The score increases with 1 for each thicker filament not felt, with a maximum score of 5 in hands and 4 in feet (filament of 300 g not felt) for every site. Each nerve will be tested on 3 sites. The trigeminal nerve is tested by assessing the blink regularity. A patient is diagnosed as having sensory impairment if the monofilament threshold is increased by three or more monofilament levels in one single nerve (over 3 sites).
Motor nerve impairment will be assessed with Voluntary Muscle Testing (VMT) using the 0–5 MRC scale [23,24]. The test is performed by checking the ability of the patient to move a body part to a given position and to hold that position against resistance applied by the tester. A nerve scoring lower than 5 is considered impaired. When a single score of 4 is found this score should be independently confirmed by a second assessor.

Subclinical nerve function tests

When new patients show no abnormal values by VMT and MFT they will be tested further for possible inclusion in the Subclinical trial. For detecting subclinical neuropathy sensory and motor Nerve Conduction Studies (NCS), carried out with the Neurocare 2000® EMG machine (BioTech Ltd, Mumbai), and Warm Detection Threshold (WDT) test, using the TSA II (MEDOC, Israel), will be performed. NCS and WDT will take place in an air-conditioned room at approximately 20-25 °C.

All assessments will be carried out at both sides of the body. Table 1 shows which nerves will be tested and methods used. MFT and VMT will be assessed at start of the trial and subsequently every month in both studies and follow-up periods on all subjects. WDT, Sensory Nerve Conduction (SNC) and Motor Nerve Conduction (MNC) will be carried out at baseline, end of treatment period (20 weeks) and at 12 and 18 months. For the Clinical trial additional information will be obtained using the Reaction Severity Scale (RSS) [25], the Screening of Activity Limitation and Safety Awareness (SALSA) scale [26] and the Participation Scale [27]. All scales will be filled in at baseline, end of treatment period (32 weeks), 12 and 18 months.

Table 1- Nerves assessed by the different methods

Nerve	**VMT**	**MFT**	**MNC**	**SNC**	**WDT**
Trigeminal		blink			
Facial	x				
Ulnar	x	x	x	x	x
Median	x	x	x	x	x
Radial	x	x		x	x
Common peroneal	x		x		
Posterior tibial	x	x	x		x
Sural		x		x	x

Reliability and normative studies

Prior to intake inter-tester reliability studies have taken place for all assessments (MFT, VMT, WDT, NCS) to test and improve comparability of the results and ensure high measurement quality. After reliability studies for NCS and WDT, normative studies were carried out to establish the local normal values for each separate collaborative centre. For each nerve a minimum of 150 normal subjects from surrounding areas were tested, equally spread over both sexes and 3 age categories (15–30, 31–45 and 46–60 years of age). Normal subjects will be screened to ascertain they do not have diabetes or nerve function impairment.

Standard operating procedures and training

For all assessments standard operating procedures (SOP) have been developed. To achieve consistency of the assessments between all research centres, the PI and two main assessors of each centre received training in research protocol procedures and handling equipment. In addition, an online Good Clinical Practice (GCP) course was completed by all PI's.

Outcome measures

The Clinical trial

The primary study outcome is the proportion of patients with restored and improved nerve function (of all nerves) measured by VMT/MFT at 18 months. Secondary outcomes are based on results of the Reaction Severity Scale, SALSA and Participation Scale (Figure 2). Furthermore, a Count of Nerve Function Impairment (CNFI) will be computed to measure the results of treatment on nerve function, with special interest for most commonly affected nerves. The CNFI will consist of the count of nerve function impairments detected by monofilament and VMT testing and will be validated in this trial.

The Subclinical trial

The proportion of patients developing clinical NFI as measured by MFT and VMT is the primary outcome indicator (Figure 2). Secondary outcome measures focus on results of the specific assessments for detection of subclinical neuropathy (WDT, SNC and MNC).

Data analysis

After the data is entered for each centre, data will be checked and cleaned. Regular weekly back-ups will be made for local off-site storage in addition to a monthly upload of data via a web-based repository to the project statistician.

Reliability studies

Inter-rater reliability for categorical outcomes of VMT and MFT are assessed using weighted Kappa statistics. Comparing differences and averages of paired assessments for WDT, SNC and MNC tests are based on Bland-Altman plots. In addition, a mixed model analysis of variance will be used to compute the intraclass correlation coefficient.

Normative studies

To determine normative values for WDT and NCS for each centre, outliers are excluded before limits of normal function are calculated using an approach based on regression analysis taking into account age-related trends. The 97.5^{th} percentile is used to define abnormal function.

Primary outcomes

Analysis of primary outcomes will be done at 20 (Subclinical trial) or 32 weeks (Clinical trial), 12 and 18 months. Possible effects of potential covariates (gender, age, leprosy classification) will be assessed with ANCOVA. All analyses will be carried out using Stata statistical software.

Secondary outcomes

Continuous outcome measures will be analysed with Analysis of (co)variance, assessing one or more covariates. Categorical outcomes may be analysed using chi-squared test or log linear models. For Survival outcomes the log rank test will be used.

Ethics

All national Research Ethics Committees have approved the study protocol, which are for India: Indian Council of Medical Research; Nepal: Nepal Health Research Council (NHRC); Indonesia: Komite Etik Penelitian Kesehatan RSUD Dr. Soetomo Surabaya; Bangladesh: Bangladesh Medical Research Council- National Research Ethics Committee. In addition, all local Research Ethics Committees have given their approval as well. Written consent will be obtained from individual subjects before inclusion and for minors additional consent from their guardians will be sought.

Discussion

This paper describes the protocols of two randomized controlled clinical trials within the TENLEP study. In the Clinical trial the effect of long term prednisolone treatment on the restoration of clinical NFI will be investigated. In the Subclinical trial the efficacy of prednisolone in preventing the development of clinical NFI, in patients with subclinical neuropathy, will be examined.

The optimal dose and duration of prednisolone for treating clinical NFI in leprosy has not been established yet and there is not enough evidence available from randomized controlled clinical trials on the long term effect of prednisolone treatment [3]. The TENLEP study addresses this knowledge gap and will extend the current understanding of prednisolone regimens for the treatment of clinical NFI by comparing a prednisolone treatment of 32 weeks and 20 weeks under controlled circumstances. If a 32 weeks treatment proves to be effective in restoring or improving clinical NFI in leprosy patients, new guidelines can be developed which can significantly improve patient management in leprosy care and especially a positive effect on the prevention of disabilities (POD) can be expected.

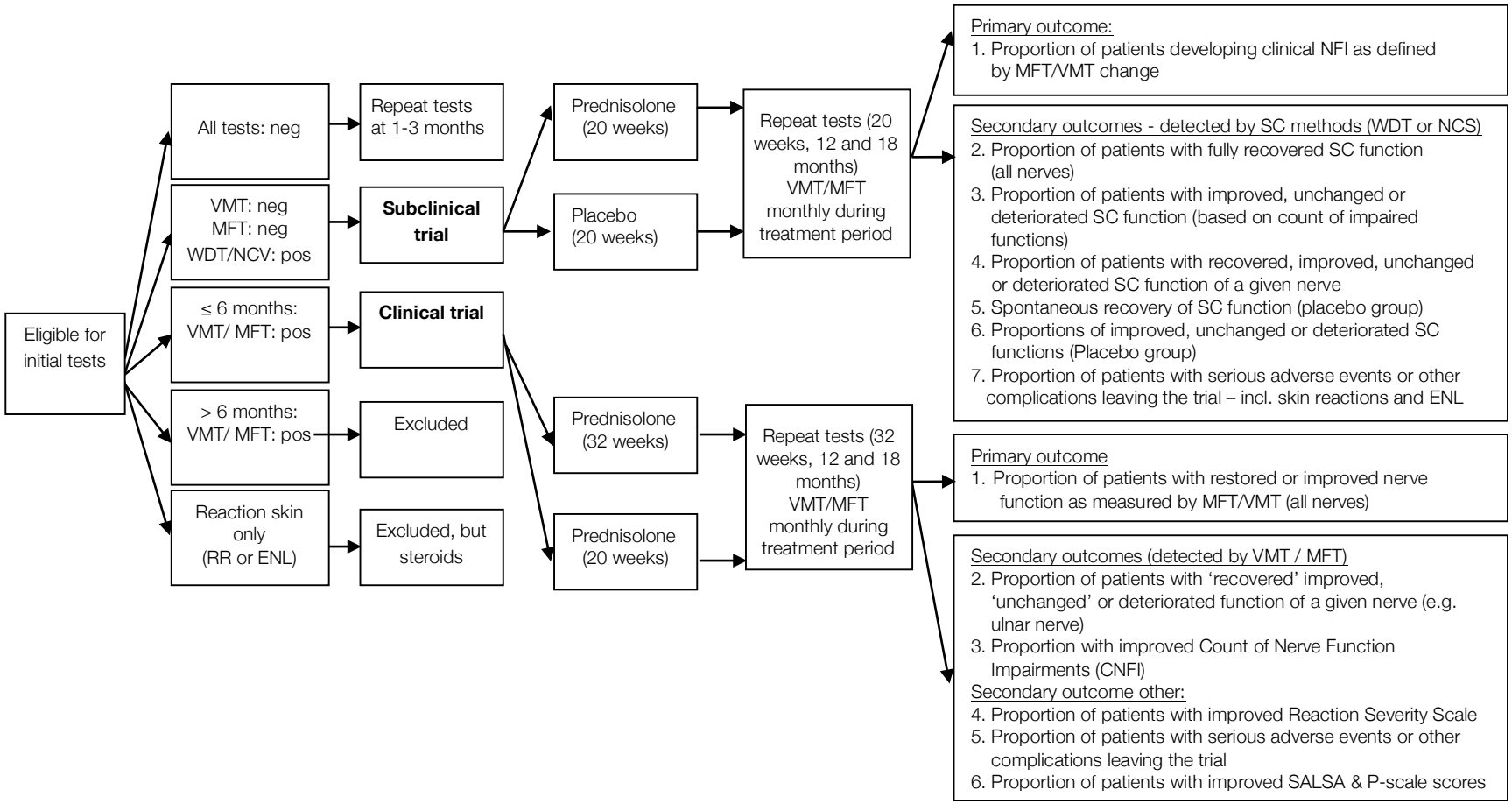

Figure 2- Overview of intake, assessments and outcomes in the Clinical and Subclinical trial

This study will be the first to evaluate prednisolone treatment for the prevention of clinical NFI in people affected by leprosy diagnosed with subclinical neuropathy. The results of the preceding TRIPOD trial provide some insight in possible effects of prednisolone in preventing new NFI [28]. To prevent new NFI and reactions, leprosy patients with and without pre-existing NFI at diagnosis (as determined with VMT and MFT) received a prophylactic low dose of prednisolone (20 mg/day) for four months, tapered down in the last month. Although a reduced incidence of new NFI and reactions was observed at the end of treatment at four months, this was not sustained at one year. More important however, the preventive effect of prednisolone at four months was more than three times higher in patients with no pre-existing NFI [28]. A second study that has some similarities with our Subclinical trial is of that of Capadia et al. (2010), who studied the effect of prednisolone on neuropathy as assessed with NCS. In their study, neuropathy was divided in mild, moderate and severe groups, as percentages deviating from normative values. Their findings suggest that a 12-week prednisolone course is not effective in preventing or reversing nerve damage. However, they found that mildly affected nerves showed higher improvement rates than moderately and severely affected nerves (53%, 21% and 14% of the nerves improved respectively).

The better outcomes of prednisolone treatment and prophylaxis on non-affected and mildly affected nerves from both studies of TRIPOD and Capadia et al. [29] show the importance of early treatment of neuropathy in leprosy patients. With the Subclinical trial we hope to establish that prednisolone treatment can prevent the development of clinical NFI and in this way can prevent disabilities and deformities in newly diagnosed leprosy patients.

If the prednisolone treatment turns out to be effective in the prevention of clinical NFI, the implementation of treatment for patients with evident subclinical neuropathy in clinical practice will be complicated. The methods to detect subclinical neuropathy used in this study (TSA II and Neurocare 2000) will not be available in the field, since the devices are expensive and conditions under which the assessments have to take place, a steady environmental temperature of 20–25 °C, are difficult to realize in tropical climates. Therefore, it is important to search for a cheaper, portable method to detect subclinical neuropathy that can be easily used in field clinics.

However, also without a test to determine subclinical neuropathy the results of this study can be useful to improve treatment guidelines. The current prediction rule allows to distinguish leprosy patients with a high risk for developing NFI [30]. However, at the moment, providing prophylactic prednisolone treatment to this group of patients is considered unethical, as a significant number of patients will take prednisolone unnecessarily. With the information of the TENLEP trials we hope the prediction rule can be refined so administering prophylactic prednisolone is acceptable in certain, well defined groups.

In conclusion, the TENLEP study will add to the current understanding of neuropathy due to leprosy and will provide better insight into the effectiveness of prednisolone treatment in the prevention and recovery of nerve function loss in leprosy patients. If this study shows the effectiveness of prednisolone in the prevention and recovery of NFI it will improve treatment

options and contribute therefore to the prevention of permanent sensory and/or motor nerve function loss in people affected by leprosy and hence prevent disabilities and deformities which will improve the lives of many of these patients.

References

1. van Brakel WH, Nicholls PG, Wilder-Smith EP, Das L, Barkataki P, Lockwood DN: *Early diagnosis of neuropathy in leprosy–comparing diagnostic tests in a large prospective study (the INFIR cohort study)*. PLoS Negl Trop Dis 2008, 2(4):e212.
2. World Health Organization: *WHO expert committee on leprosy.* Volume 874th edition. World Health Organization technical report series; Geneva, WHO 1998:1–43.
3. van Veen NH, Nicholls PG, Smith WC, Richardus JH: *Corticosteroids for treating nerve damage in leprosy.* A Cochrane review. Lepr Rev 2008, 79(4):361–371.
4. van Brakel WH: *Peripheral neuropathy in leprosy and its consequences*. Lepr Rev 2000, 71(Suppl):S146–S153.
5. World Health Organization: *Weekly epidemiological record.* Volume 86th edition. Geneva, WHO, 2011:389–400.
6. Nienhuis WA, van Brakel WH, Butlin CR, van der Werf TS: *Measuring impairment caused by leprosy: inter-tester reliability of the WHO disability grading system*. Lepr Rev 2004, 75(3):221–232.
7. Broekhuis SM, Meima A, Koelewijn LF, Richardus JH, Benbow C, Saunderson PR: *The hand-foot impairment score as a tool for evaluating prevention of disability activities in leprosy: An exploration in patients treated with corticosteroids.* Lepr Rev 2000, 71(3):344–354.
8. Khambati FA, Shetty VP, Ghate SD, Capadia GD, Pai VV, Ganapati R: *The effect of corticosteroid usage on the bacterial killing, clearance and nerve damage in leprosy: A prospective cohort study: Part 1 - Study design and baseline findings of 400 untreated multibacillary patients*. Lepr Rev 2008, 79(2):134–153.
9. Smith WC, Nicholls PG, Das L, Barkataki P, Suneetha S, Suneetha L, Jadhav R, Sundar Rao PS, Wilder-Smith EP, Lockwood DN, et al: *Predicting neuropathy and reactions in leprosy at diagnosis and before incident events-results from the INFIR cohort study*. PLoS Negl Trop Dis 2009, 3(8):e500.
10. Shetty VP, Khambati FA, Ghate SD, Capadia GD, Pai VV, Ganapati R: *The effect of corticosteroids usage on bacterial killing, clearance and nerve damage in leprosy; part 3 - study of two comparable groups of 100 multibacillary (MB) patients each, treated with MDT 1 steroids vs MDT alone, assessed at 6 months post - release from 12 months MDT*. Lepr Rev 2010, 81(1):41–58.
11. Walker SL, Nicholls PG, Dhakal S, Hawksworth RA, Macdonald M, Mahat K, Ruchal S, Hamal S, Hagge DA, Neupane KD, et al: *A phase two randomised controlled double blind trial of high dose intravenous Methylprednisolone and oral prednisolone versus intravenous normal saline and oral prednisolone in individuals with leprosy type 1 reactions and/or nerve function impairment*. PLoS Negl Trop Dis 2011, 5(4): e1041.

12. van Brakel WH, Anderson AM, Withington SG, Croft RP, Nicholls PG, Richardus JH, Smith WCS: *The prognostic importance of detecting mild sensory impairment in leprosy: A randomized controlled trial (TRIPOD 2).* Lepr Rev 2003, 74(4):300–310.

13. Sundar Rao PSS, Sugamaran DST, Richard J, Smith WCS: *Multi-centre, double blind, randomized trial of three steroid regimens in the treatment of type-1 reactions in leprosy*. Lepr Rev 2006, 77(1):25–33.

14. van Brakel WH, Nicholls PG, Lockwood DN, Rao PS, Smith WC: *A scale to assess the severity of leprosy reactions*. Lepr Rev 2007, 78(2):161–164.

15. Screening Activity Limitation and Safety Awareness Users Manual V1.1. http://www.ilep.org.uk/fileadmin/uploads/Documents/Infolep_Documents/Salsa/SALSA_manual_v1.1pdf.pdf.

16. Participation Scale Users Manual v. 6.0. http://www.ilep.org.uk/fileadmin/uploads/Documents/Infolep_Documents/Participation_Scale/Participation_Scale_Users_Manual_v._6.0.pdf.

17. Britton WJ, Lockwood DNJ: *Leprosy*. Lancet 2004, 363(9416):1209–1219.

18. Conn HO, Poynard T: *Corticosteroids and peptic ulcer: Meta-analysis of adverse events during steroid therapy*. J Int Med 1994, 236(6):619–632.

19. Da Silva JAP, Jacobs JWG, Kirwan JR, Boers M, Saag KG, Inês LBS, De Koning EJP, Buttgereit F, Cutolo M, Capell H, et al: *Safety of low dose glucocorticoid treatment in rheumatoid arthritis: Published evidence and prospective trial data*. Ann Rheum Dis 2006, 65(3):285–293.

20. Richardus JH, Withington SG, Anderson AM, Croft RP, Nicholls PG, van Brakel WH, Smith WC: *Adverse events of standardized regimens of corticosteroids for prophylaxis and treatment of nerve function impairment in leprosy: results from the 'TRIPOD' trials*. Lepr Rev 2003, 74(4):319–327.

21. Ridley DS, Jopling WH: *Classification of leprosy according to immunity. A five-group system*. Int J Lepr Mycobact Dis 1966, 34(3):255–273.

22. Bell-Krotoski J: *"Pocket" filaments and specifications for the semmes-weinstein monofilaments*. J Hand Ther 1990, 3:26–31.

23. Brandsma W: *Basic nerve function assessment in leprosy patients*. Lepr Rev 1981, 52(2):161–170.

24. Brandsma JW, van Brakel WH: *Protocol for motor function assessment in leprosy and related research questions.* Indian J Lepr 2001, 73(2):145–158.

25. Walker SL, Nicholls PG, Butlin CR, Nery JAC, Roy HK, Rangel E, Sales AM, Lockwood DNJ: *Development and validation of a severity scale for leprosy type 1 reactions*. PLoS Negl Trop Dis 2008, 2(12): e351.

26. Ebenso J, Fuzikawa P, Melchior H, Wexler R, Piefer A, Min CS, Rajkumar P, Anderson A, Benbow C, Lehman L, Nicholls P, Saunderson P, Velema JP: *The development of a short questionnaire for screening of activity limitation and safety awareness (SALSA) in clients affected by leprosy or diabetes*. Disabil Rehabil 2007, 29(9):689–700.

27. van Brakel WH, Anderson AM, Mutatkar RK, Bakirtzief Z, Nicholls PG, Raju MS, Das-Pattanayak RK: *The participation scale: measuring a key concept in public health.* Disabil Rehabil 2006, 28(4):193–203.

28. Smith WC, Anderson AM, Withington SG, van Brakel WH, Croft RP, Nicholls PG, Richardus JH: *Steroid prophylaxis for prevention of nerve function impairment in leprosy: randomised placebo controlled trial (TRIPOD 1*). BMJ 2004, 328(7454):1459.

29. Capadia GD, Shetty VP, Khambati FA, Ghate SD: *Effect of corticosteroid usage combined with multidrugCa therapy on nerve damage assessed using nerve conduction studies: A prospective cohort study of 365 untreated multibacillary leprosy patients*. J Clin Neurophysiol 2010, 27(1):38–47.

30. Croft RP, Nicholls PG, Steyerberg EW, Richardus JH, Withington SG, Smith WC: *A clinical prediction rule for nerve function impairment in leprosy patients-revisited after 5 years of follow-up*. Lepr Rev 2003, 74(1):35–41.

Chapter 3

Normal threshold values for a monofilament sensory test in sural and radial cutaneous nerves in Indian and Nepali volunteers

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Wim Brandsma
Erik Post
Jan Hendrik Richardus

Leprosy Review (2014) 85, 1–13

Abstract

Introduction

The monofilament test (MFT) is a reliable method to assess sensory nerve function in leprosy and other neuropathies. Assessment of the radial cutaneous and sural nerve in addition to usually tested nerves can help improve diagnosis and monitoring of nerve function impairment (NFI). To enable the detection of impairments in leprosy patients, it is essential to know the monofilament threshold in normal subjects of these two nerves.

Methods

The radial cutaneous, sural, ulnar, median and posterior tibial nerves of 245 volunteers were tested. All nerves were tested at three sites on both left and right body side. Normal monofilament thresholds were calculated per test-site and per nerve.

Results

We assessed 490 radial cutaneous and 482 sural nerves. The normal monofilament was 2-g (Filament Index Number (FIN) 4.31) for the radial cutaneous and 4-g (FIN 4.56) for the sural nerve, although heavy manual labourers demonstrated a threshold of 10-g (FIN 5.07) for the sural nerve. For median and ulnar nerves, the 200-mg (FIN 3.61) filament was confirmed as normal while the 4-g (FIN 4.56) filament was normal for the posterior tibial. Age and occupation have an effect on the mean touch sensitivity but do not affect the normal threshold for radial cutaneous and sural nerves.

Conclusion

The normal thresholds for the radial cutaneous and sural nerves are determined at the 2-g (FIN 4.31) and the 4-g (FIN 4.56) filament respectively. The addition of the radial cutaneous and sural nerve to sensory nerve assessment may improve the diagnosis of patients with impaired sensory nerve functions.

Introduction

In leprosy care, accurate diagnosis and monitoring of nerve function impairment (NFI) are crucial. With early diagnosis and treatment of NFI, severe peripheral nerve impairment, often resulting in disabilities, can be prevented. The main tests used for diagnosis and monitoring of NFI in clinical practice are voluntary muscle tests (VMTs), ball-point tests (BPTs) and monofilament tests (MFTs) on, primarily, hands and feet. In leprosy affected nerves, sensory function loss often precedes motor function loss [1,2]. MFTs are able to detect and monitor sensory nerve function loss accurately, using variation in pressure. They offer more sensitive results than BPTs which generally provide only a yes/no outcome [3]. Monofilament testing is quick, sensitive and reliable and, therefore, a useful method to diagnose and monitor nerve impairment over time [4-6].

Most studies that assess NFI in leprosy patients examine the ulnar, median and posterior tibial nerves because these nerves are responsible for sensation on the hand palms and foot soles, comprising areas of the body most prone to ulceration and burns because of loss of protective sensation. In addition to these three nerves, assessment of the radial cutaneous (innervating a part of the dorsal side of the hand (Figure 1A) and the sural nerves (innervating the lateral side of the foot (Figure 1B) might also prove informative. Impairments of the sensory radial cutaneous and sural nerves are rarely tested in clinical and research settings because these impairments have little functional effect on patients. However, including assessment of the radial cutaneous and sural nerve can yield additional information to improve the diagnosis and monitoring of NFI. The inclusion of the dorsum of the hands and feet in sensory testing procedures has been recommended by Wexler et al. (2007) and Kuipers et al. (1994) because lesions and sensory impairment were located frequently on this part of the hands and feet [7,8]. Furthermore, the ILEP Nerve Function Impairment and Reaction (INFIR) study indicates that the sural and radial cutaneous nerve play an important role in the diagnosis of NFI. In that study, newly diagnosed patients without NFI were followed for two years, during which the sural became impaired in 37.2% of patients tested with MFTs (2), and the radial cutaneous was found to be affected as often as the median nerve (8.8%). Khambati et al. (2009) also reported sensory impairment in 28% and 23% of the sural and radial cutaneous nerves, respectively, in newly diagnosed multibacillary (MB) patients. However, these studies omitted to establish normal values for the MFT thresholds in relation to these two nerves.

In patients who are at risk of NFI, diagnosis of impairment is necessary before reaction treatment can be started. However, impairment can only be determined when the monofilament threshold of normal subjects is known. For MFTs, the normal threshold represents the filament felt by a certain percentage (usually 95 or 97.5%) of normal subjects (not affected by the disease). Studies in different Asian countries determined that the 200-mg (FIN 3.61) filament represents the normal threshold for the palm of the hand (median and ulnar nerves) while the 2-g (FIN 4.31) filament was normal for the plantar side of the forefeet (posterior tibial nerve) [9-11]. These studies provide for the diagnosis of NFI in median, ulnar and posterior tibial nerves in Asian patients. However, before the radial cutaneous and sural nerves can be reliably used in

the diagnosis and follow-up of NFI, the normal values must be known. In this study, we establish normal threshold values for the radial cutaneous and sural nerves in Indian and Nepali volunteers and provide recommendations for clinical practice. Additionally, we assess the median, ulnar and posterior tibial nerves in order to compare our results with previous normative studies.

Methods

Subjects

Subjects were recruited from four leprosy hospitals, two in Nepal and two in India. In each country, one urban and one rural hospital was selected. Visitors accompanying patients, patients from other departments (e.g. dermatology), and hospital staff were asked to participate. Subjects were excluded when they suffered from conditions with neurological implications, such as diabetes mellitus. Nearly all persons invited were willing to participate. A total of 245 Indian and Nepali volunteers, not known to have a history of leprosy, were recruited for MFT assessment. This study to establish normal values, was part of the Treatment of Early Neuropathy in Leprosy (TENLEP) trials, for which ethical approval was obtained from the Indian Council of Medical Research and the Nepal Health Research Council. All subjects were informed about the goal and procedures of the study and verbally agreed to participate.

Data collection

Age, occupation and dominant hand were recorded for all subjects. Prior to analysis, occupation was grouped into three categories: office work (e.g. students, business people and drivers) manual labour (e.g. cleaners, housewives, nurses and tailors) and heavy manual labour (e.g. farmers, labourers and porters). Three age groups were constructed before analyses: 15-30, 30-45 and 45-60. Some subjects were interviewed to determine their smoking behaviour (n=170); and habits of wearing footwear and types of footwear (closed shoes, sandals or a combination) (n=114), and whether and how often they sit cross-legged (n=116).

Monofilaments

The MFT is a touch sensibility test which indents the skin surface with a series of standard nylon filaments of same length but with varying degrees of thickness. A standard “pocket” set of six coloured Semmes-Weinstein monofilaments was used (Sorri-Bauru, São Paulo, Brazil), with bending forces ranging from 70-mg (green, FIN 2.81) to 300-g (pink, FIN 6.65) (Table 1) [12].

Test procedure

According to standard protocol [13], filaments were applied at a straight angle to the skin and with sufficient force to make the filament bend slightly (C shape). The filaments were applied

slowly, the pressure maintained briefly and then removed slowly, taking approximately one second for each step. The subjects were asked to keep their eyes closed during this procedure and to indicate the place where they had felt the touch. Deviation of roughly two centimetres from the exact touch location was considered acceptable when there was adequate response time. The filaments were applied in sequential order from lightest (70-mg) to heaviest (300-g), applying the 70-mg (FIN 2.83) and 200-mg (FIN 3.61) filaments with a maximum of three times per site when the monofilament slipped or was not detected by the subject. The same applied for the 2-g (FIN 4.31) filament used on the feet. In order to keep the subjects focused, efforts were made to keep the test room silent and fans were switched off to minimize noise and the effect of the circulating air on the skin. A pre-test was carried out on all subjects before outcomes were recorded to make sure they understood the test. All data was collected by one trained assessor.

Table 1- Characteristics of the standard pocket set of Semmes-Weinstein monofilaments

Filament	Filament index number	Bending force (grams)
Green	2.83	0.07
Blue	3.61	0.2
Purple	4.31	2
Red	4.56	4
Orange	5.07	10
Pink	6.65	300

Note: The filament index number is calculated from the bending force in milligram: FIN = log (force *10)

Test sites

In addition to the radial cutaneous and sural nerves, the ulnar, median and posterior tibial nerves were tested. For each nerve, three test-sites were assessed on both the left and right side of the body. For the ulnar, median and posterior tibial nerves, the same sites were examined as in the INFIR study [14]. However, we made one exception to the INFIR methodology by assessing the tip of the middle finger as the third point for the median rather than the index finger. The three test-sites for the radial cutaneous and sural nerves were selected as shown in Figure 1. The radial cutaneous nerve innervates a part of the dorsal surface of the hand: the proximal radial site of the index finger and thumb web space. This is shown in grey in Figure 1A [15]. The sural nerve innervates part of the plantar lateral surface of the foot and the lateral aspect of the ankle. The three test-sites can be found on the plantar lateral surface in one straight line about 1.5 cm above the sole of the foot (Figure 1B).

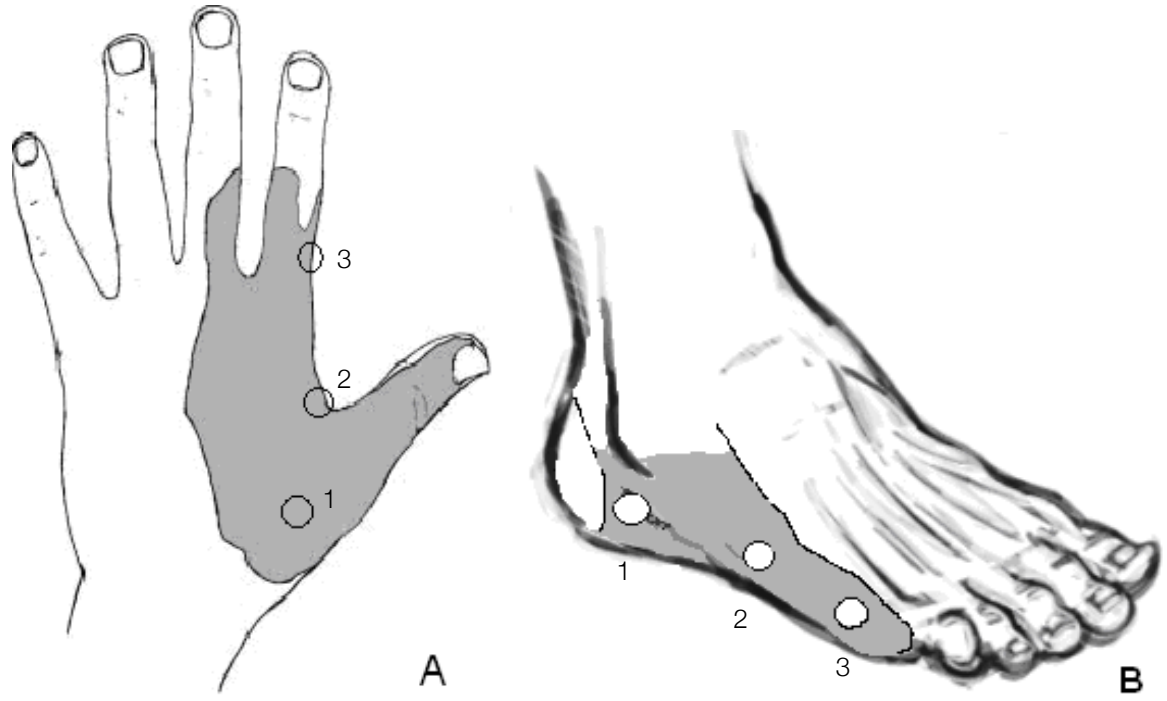

Figure 1 A- Dorsal innervation area of radial cutaneous nerve and the three test-sites. **Figure 1 B**- Innervation area of the sural nerve and the three test-sites

Outcome

For every test-site, the lightest filament detected was recorded, using a scoring method. The lightest filament (70-mg, FIN 2.83) was given a score of 0, and the score increased with one point for each thicker filament felt, with score 1 for 200-mg (FIN 3.61), 2 for 2-g (FIN 4.31), 3 for 4-g (FIN 4.56) and 4 for the heaviest, 10-g, filament (FIN 5.07). A patient is diagnosed with NFI when the overall score of a nerve from the total of the three test-sites is 3 or higher [14,16-18]. Weights and FINs are used to describe the filaments in this study because weights are well understood in the field and FINs are often used in scientific studies [10,19-22].

The normal threshold value for a test-site is defined as the monofilament that was detected by 95% of the volunteers, indicating that subjects only able to detect filaments heavier than the threshold are very likely to have NFI. Figure 2 shows an example in which 2-g, the first filament detected by at least 95% of the subjects, represents the normal threshold value. From the three individual test-sites, the overall normal value for the nerve is derived. The heaviest monofilament detected in any of the three sites determines the normal value for the entire nerve.

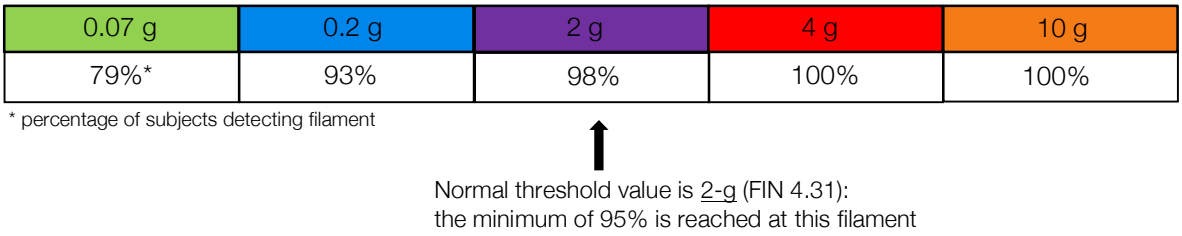

Figure 2- Explanation of the determination of our 95% normal threshold value (an example)

Data analysis

Normal monofilament thresholds were calculated per test-site and per nerve. A McNemar test for correlated proportions was carried out to test whether the percentage of subjects detecting the 200-mg and 2-g filament differed between the right and left hand and foot respectively. When no difference was found, the 95% normal thresholds were calculated for all nerves. Mann-Whitney tests were used to explore the association of sex, country of origin and dominant hand on mean FIN, calculated by averaging the three test-sites per nerve. Kruskal-Wallis tests were carried out to analyse the effect of age and occupational group on the mean FIN. Furthermore, the effect of footwear types and cross-legged sitting on the average filament detected by both nerves in the feet (sural and posterior tibial) was examined in a subgroup of volunteers using Kruskal-Wallis tests. Based on the assumption of independent data, all univariate analyses are performed on the nerves of the right body side only. A p-value of less than 5% was considered statistically significant. All tests were done using SPSS statistical package 19.0.

Results

Radial cutaneous and sural nerves were assessed in 119 men and 126 women. Ulnar, median and posterior tibial nerves were tested in 198 of these persons. The mean age of the subjects was 35.5 years. Table 2 gives an overview of the characteristics of the 245 volunteers.

The weight of the monofilament detected by left and right body side did not significantly differ for any test-site. As a result, normal threshold values were calculated using 490 hands and feet, independent of body side. Normal thresholds, based on the 95% cut-off, are shown in Table 3 for the entire study group and separately for the three age groups because age is known to have a significant effect on touch sensitivity [10,22,23]. For the radial cutaneous and sural nerves, the normal threshold values differed between the three test-sites. In consequence, the heaviest filament was used to determine the normal threshold for the entire nerve.

Table 2- Subjects characteristics of 245 volunteers

		%
Sex (male)		48.6
Age (years)	15-29	34.3
	30-44	40.4
	45-60	25.3
Country	India	38.8
(n = 245)	Nepal	61.2
Smoking	No	81.6
(n = 174)	Yes	18.4
Occupation	Office	41.9
(n =229)	Manual	41.5
	Heavy	16.6
Dominant Hand	Right	95.4
(n = 218)	Left	4.6
Shoe wearing	Sandals	64.0
(n = 114)	Shoes	19.3
	Both	16.7
Cross leg sitting	No	28.4
(n = 116)	Sometimes	28.4
	Often	43.1

This resulted in a normal threshold of 2-g (FIN 4.31) for the radial cutaneous and 4-g (FIN 4.56) for the sural. For both ulnar and median nerves, the 200-mg (FIN 3.61) represents the normal threshold while for the posterior tibial it was the 4-g (FIN 4.56) filament.

Univariate analyses identified age as the only variable that showed significant different mean FIN between the groups across all nerves. The higher the age group, the higher the mean filament weight detected by the subjects (Figure 3). However, comparison of the normal thresholds for the entire study group and the three age groups (Table 3) showed that thresholds are barely affected by age. The youngest group (15-30 years) shows lower thresholds for the radial, sural and posterior tibial; the oldest group (45-60 years) has a higher threshold for the median nerve.

Occupation was significantly associated with the touch sensitivity of all nerves except the radial cutaneous nerve (Table 4). Volunteers undertaking heavy manual labour detected a higher mean size monofilament than those undertaking office work and manual labour, indicating a lower touch sensitivity. Moreover, the heavy manual labour group had a higher normal threshold than the entire study group for sural, ulnar, median and posterior tibial nerves. We also found that for the sural nerve, wearing shoes and never sitting cross-legged resulted in a lower normal threshold, hence a higher touch sensitivity.

Discussion

This study showed that the normal threshold for monofilament testing for the radial cutaneous is the 2-g (FIN 4.31) filament. For the sural nerve, this threshold is the 4-g (FIN 4.56) filament. Furthermore, we established that the 200-mg (FIN 3.61) filament is the normal threshold for the ulnar and median nerve, while the 4-g (FIN 4.56) filament represents the normal threshold for the posterior tibial nerve.

In this study, we confirmed the threshold for the palm of the hand as found in previous normative studies in Asians. The 200-mg (FIN 3.61) filament was identified as normal value for the median and ulnar nerve in two studies in Nepal, using a threshold cut-off of 95% (9) and 97% [11]. A study in Thailand showed that 97% of the normal subjects detected a 3.84 filament (this filament is not used in our study and is placed between 200-mg (FIN 3.61) and 2-g (FIN 4.31)) [10]. Furthermore, these three studies agreed the 2-g (FIN 4.31) filament was the normal threshold for the plantar side of the foot (posterior tibial nerve). This was not confirmed by our findings that the 4-g (FIN 4.56) filament represents the normal threshold for this nerve. It is possible that certain subgroups were overrepresented in previous studies, lowering the thresholds. For example, in the study by Kets et al. (1996), 64% of their subjects were under the age of 30. With this age distribution, a lower threshold is to be expected because we found that the youngest age group (15-29) has a lower threshold for the posterior tibial nerve than the entire group. This might also apply to groups which are left-handed, wearing closed shoes or sit cross-legged sometimes. The earlier studies, however, did not report on these parameters.

The threshold we established for the radial cutaneous was consistent with our expectations, based on studies in Nepal, Thailand and India [19]. We assumed that the touch sensitivity of the dorsum of the hand would be similar to the palmar side of the hand and would lie between 200-mg (FIN 3.61) and 2-g (FIN 4.31), resulting in a threshold of the 2-g (FIN 4.31) filament. The Indian study, testing 57 normal subjects, assessed one test-site for the radial cutaneous nerve, corresponding with our second test-site in the radial cutaneous [19].

However, the cut-off used to determine the normal threshold is not clearly defined in the Indian study. We calculated from their data that the 200-mg (FIN 3.61) filament was detected on the right and left hand by 94.7% and 98.2% respectively. With 95% cut-off used in our study, the normal threshold would then correspond with the 2-g (FIN 4.31) and 200-mg (FIN 3.61) filament respectively.

The difference in threshold between the dorsal and palmar side of the hand may be related to the number of cutaneous nerve endings. The density of tactile receptors can be mapped in detail by a 2-point discrimination test. From this test, it is known that the fingertips and the palm of the hands have a higher density of nerve endings than the dorsum of the hands [24].

Table 3- Monofilament thresholds per test-site and per nerve for all subjects and per age group

Nerves	Radial cutaneous			Sural			Ulnar			Median			Posterior tibial		
Sites*	**R1**	**R2**	**R3**	**S1**	**S2**	**S3**	**U1**	**U2**	**U3**	**M1**	**M2**	**M3**	**P1**	**P2**	**P3**
All subjects															
Result per test-site	4.31	4.31	3.61	4.56	4.56	4.31	3.61	3.61	3.61	3.61	3.61	3.61	4.56	4.56	4.56
Percentage feeling MF threshold	100	99.8	98.4	97.9	99.0	98.5	99.0	98.2	98.0	98.7	96.7	98.2	98.2	99.5	99.0
Result per nerve		**4.31**			**4.56**			**3.61**			**3.61**			**4.56**	
Age groups															
15-29 (n=168)															
Result per test-site	3.61	3.61	3.61	4.31	4.31	4.31	3.61	2.83	2.83	3.61	3.61	2.83	4.31	4.31	4.31
Percentage feeling MF threshold	97.0	98.2	98.8	96.4	98.2	100	100	96.6	97.9	100	100	97.9	95.9	98.6	98.6
Result per nerve		**3.61**			**4.31**			**3.61**			**3.61**			**4.31**	
30-44 (n=198)															
Result per test-site	4.31	4.31	3.61	4.56	4.31	4.31	3.61	3.61	3.61	3.61	3.61	3.61	4.56	4.31	4.31
Percentage feeling MF threshold	100	99.5	97.0	99.5	95.8	99.0	98.0	98.6	98.0	98.6	97.3	98.0	100	95.9	96.6
Result per nerve		**4.31**			**4.56**			**3.61**			**3.61**			**4.56**	
45-60 (n=124)															
Result per test-site	4.31	4.31	3.61	4.56	4.56	4.31	3.61	3.61	3.61	3.61	4.31	3.61	4.56	4.56	4.56
Percentage feeling MF threshold	100	100	100	95.1	95.9	95.9	99.0	95.1	95.1	97.1	100	96.1	95.1	100	98.0
Result per nerve		**4.31**			**4.56**			**3.61**			**4.31**			**4.56**	

*calculated over left and right body side combined

R1= Radial Cutaneous test-site 1, R2= Radial Cutaneous test-site 2, R3= Radial Cutaneous test-site 3, S1= Sural test-site 1, S2= Sural test-site 2, S3= Sural test-site 3, U1= Ulnar test-site 1, U2= Ulnar test-site 2, U3= Ulnar test-site 3, M1= Median test-site 1, M2= Median test-site 2, M3= Median test-site 3, P1= Posterior tibial test-site 1, P2= Posterior tibial test-site 2, P3= Posterior tibial test-site 3

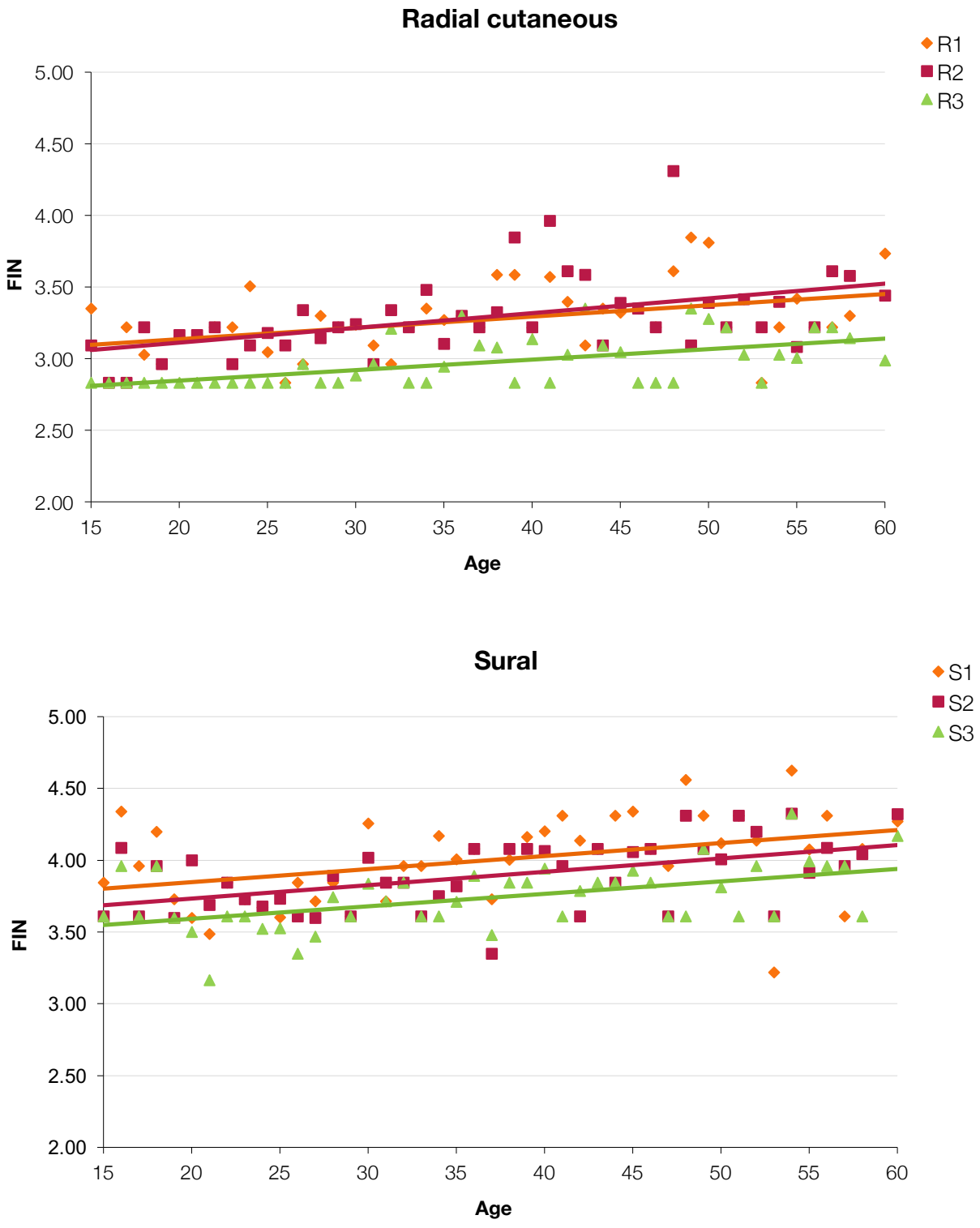

Figure 3- Trend line mean Filament Index Number (FIN) for three test-sites for a radial cutaneous and sural nerves for right body side

The sural threshold established (red, FIN 4.56) was higher than expected. Given that there is no literature on normal thresholds for the sural nerve, we assumed that the lateral border of the foot would have a similar threshold as the plantar side of the foot, determined in previous studies as 2-g (FIN 4.31). Although the threshold was not as expected, our assumption was correct because the threshold for the plantar and the lateral side of the foot were similar.

To determine whether we should implement different thresholds for different groups, we performed univariate analyses and compared the thresholds per group (e.g. males vs. females et cetera). The analyses revealed that age and occupation were important variables associated with touch sensitivity. The decreased sensitivity with increasing age was also reported in other normative studies [10,11,22]. Mitchell and Mitchell (2000) discuss that neural degeneration, reducing the number of touch receptors and slowing down conduction, is the probable cause. In the heavy manual occupational group, normal thresholds were one level higher for the sural, ulnar, median and posterior tibial nerve. A normative study in Thailand found the same pattern with heavy manual labour being associated with higher sensory thresholds [10]. Heavy manual workers, like farmers and labourers, might have thicker callus on hands and feet, leading to higher monofilament thresholds. This would also explain why the radial cutaneous threshold does not differ between the labour groups because the dorsum of the hand is not involved in manual work and is therefore not likely to develop callus. The monofilament threshold for the radial cutaneous and sural nerve, as established in this study, can be used for all persons, with exception of the heavy manual labourer group for whom a higher threshold for the sural nerve applies.

Given the multi-centre, multi-country study design, our findings are generalizable and especially useful for Asian countries where leprosy is often still prevalent and where neuropathies will become more widespread with the rising diabetes prevalence [25]. However, there are some factors that should be taken into account when evaluating our results. First, we mainly assessed close contacts of leprosy patients, such as family and staff of the hospitals. Our volunteers could have been unknowingly infected with leprosy because we did not test for leprosy. Hence, the potential presence of NFI could have resulted in higher thresholds. Second, we had to take into account the habit of this population to take family and friends into the test rooms. As a result, and despite our efforts, it was not always possible to test the volunteer in silence. This could have led to diminished focus and higher thresholds. However, the habit of bringing family will also occur in clinical practice so that our results will be still acceptable to determine the NFI status in leprosy patients. Both factors may have led to an overestimation of the thresholds but overestimation is better than underestimation that might lead to unnecessary treatment. In addition, we intended to correct the outcomes for multiple important variables in a multivariate analysis. However, no reliable tests were available since the data is nested, not independent, not normally distributed and categorical. Finally, even though monofilament testing is an optimal method in clinical practice to quantify NFI, most leprosy programs do not have access to the relatively expensive monofilaments. However, we hope that the rising prevalence of diabetes will stimulate the search for low-cost sources of monofilaments. If this is the case, leprosy clinics will be able to benefit as well.

Table 4- Mean filament index number (FIN) ± SD per nerve for different variable groups for right body side

	Radial cutaneous	Sural	Ulnar	Median	Posterior tibial
Age (n)					
15-29 (84)	3.04[#] ±0.22	3.69[#] ±0.34	2.88 ±0.12	2.90 ±0.14	3.75[#] ±0.42
30-44 (99)	3.19 ±0.32	3.92 ±0.31	2.98 ±0.25	3.00 ±0.27	3.90 ±0.40
45-60 (102)	*3.28 ±0.31	*4.05 ±0.38	*3.02 ±0.30	*3.10[1] ±0.35	*4.06 ±0.45
Sex (n)					
Male (119)	3.19 ±0.33	3.91 ±0.36	2.97 ±0.26	2.99 ±0.29	3.92 ±0.44
Female (126)	3.13 ±0.28	3.84 ±0.37	2.93 ±0.21	2.98 ±0.25	3.84 ±0.43
Dominance (n)					
Right (208)	3.16 ±0.30	3.87 ±0.38	2.95 ±0.23	2.99 ±0.27	3.90 ±0.44
Left (10)	3.19[#] ±0.22	*4.01[2] ±0.30	2.98 ±0.24	2.96[1] ±0.27	*3.72[#] ±0.42
Country (n)					
India (95)	3.16 ±0.33	3.82[#] ±0.39	2.93 ±0.20	2.96 ±0.25	3.82 ±0.49
Nepal (150)	3.16 ±0.28	3.90 ±0.34	2.96 ±0.25	3.00 ±0.28	3.93 ±0.40
Occupation (n)					
Office (96)	3.18 ±0.30	3.79 ±0.36	2.90 ±0.17	2.93 ±0.22	3.80 ±0.40
Manual (95)	3.15 ±0.30	3.89 ±0.35	2.94 ±0.22	3.00 ±0.25	3.92 ±0.42
Heavy manual (38)	3.15 ±0.29	*4.05[2] ±0.41	*3.07[1] ±0.32	*3.09[1] ±0.35	*3.99[2] ±0.51
Smoking (n)					
No (142)	3.11[#] ±0.25	3.82 ±0.38	2.93 ±0.21	2.96 ±0.25	3.85 ±0.44
Yes (32)	3.17 ±0.33	*3.97 ±0.42	2.98 ±0.26	3.02 ±0.29	*4.04 ±0.46
Shoe[Ŧ] (n)					
Sandals (73)		3.90 ±0.33			3.98 ±0.40
Shoes (22)		3.76[#] ±0.29			3.82[#] ±0.33
Both (19)		*4.09 ±0.28			3.99 ±0.39
Cross leg[Ŧ] (n)					
No (33)		3.88[#] ±0.30			3.86 ±0.39
Sometimes (33)		3.74 ±0.34			3.89[#] ±0.43
Often (50)		*4.00 ±0.34			4.04 ±0.35

* a significant ($p<0.05$) difference between groups, [Ŧ]results shown only for nerves in foot, [#]Normal threshold is one FIN lower than the threshold for total group

[1]Normal threshold is 2-g (FIN 4.31) where the total group threshold is 200-mg (FIN 3.61), [2]Normal threshold is 10-g (FIN 5.07) where the total group threshold is 4-g (FIN 4.56)

Similar to large studies on leprosy neuropathy (INFIR, TENLEP), three test-sites per nerve were assessed so that it was possible to use the scoring system described by Anderson [14,16,18]. Despite this, we chose to present our final results per nerve. The monofilament test is a quick and simple field method, and it is desirable to work with a minimal number of threshold values to guarantee its effectiveness.

When the threshold differed between the three test-sites, we decided that the heaviest filament would determine the normal threshold. In those cases that the threshold differed between the test-sites, we could also have chosen alternative methods. For example by taking the filament detected in two out of three test-sites as the final threshold. However, in that case the thresholds would still be the same for the entire study group. When we would have taken the lightest filament of the three test-sites as threshold for the nerve, the threshold for the radial cutaneous and sural nerve would have been one filament lower (200-mg (FIN 3.61) and 2-g (FIN 4.31) respectively). With a lower threshold, NFI will be detected earlier but more 'false positives' would occur. For this reason, we believe that it is better to use a threshold that is on the 'safe side' for the radial cutaneous and the sural nerve, particularly as injuries due to loss of protective sensation are less common on the dorsum of hands and feet.

Conclusions

Taking into account all considerations, we recommend including the assessment of the radial cutaneous and sural nerves for monofilament testing in routine clinical practice. Testing the sensory function of the dorsum of the hand and lateral foot can give additional information on NFI, especially since these two nerves seem to be often impaired and are some of the earliest nerves impaired in leprosy [26]. Additional assessment of the radial cutaneous and sural nerves can improve diagnosis and monitoring of NFI, and may help prevent severe NFI and disabilities in patients with leprosy and neuropathies caused by other diseases (e.g. diabetes). In conclusion, the 2-g (FIN 4.31) filament should be taken as the normal threshold when testing the radial cutaneous nerve while the 4-g (FIN 4.56) filament is the normal threshold for the sural nerve. As an exception, heavy manual labourers have a higher threshold for the sural nerve, namely the 10-g (FIN 5.07) filament.

References

1. Khambati FA, Shetty VP, Ghate SD, Capadia GD. *Sensitivity and specificity of nerve palpation, monofilament testing and voluntary muscle testing in detecting peripheral nerve abnormality, using nerve conduction studies as gold standard; a study in 357 patients*. Lepr Rev. 2009 Mar;80(1):34-50.
2. van Brakel WH, Nicholls PG, Wilder-Smith EP, Das L, Barkataki P, Lockwood DN. *Early diagnosis of neuropathy in leprosy--comparing diagnostic tests in a large prospective study (the INFIR cohort study).* PLoS Negl Trop Dis. 2008;2(4):e212.
3. Koelewijn LF, Meima A, Broekhuis SM, Richardus JH, Mitchell PD, Benbow C, et al. *Sensory testing in leprosy: comparison of ballpoint pen and monofilaments.* Lepr Rev. 2003 Mar;74(1):42-52.
4. Anderson AM, Croft RP. *Reliability of Semmes Weinstein monofilament and ballpoint sensory testing, and voluntary muscle testing in Bangladesh*. Leprosy Review. 1999;70(3):305-13.
5. Naafs B, Dagne T. S*ensory testing: a sensitive method in the follow-up of nerve involvement*. Int J Lepr Other Mycobact Dis. 1977 Oct-Dec;45(4):364-8.
6. van Brakel WH, Khawas IB, Gurung KS, Kets CM, van Leerdam ME, Drever W. *Intra- and inter-tester reliability of sensibility testing in leprosy*. Int J Lepr Other Mycobact Dis. 1996 Sep;64(3):287-98.
7. Kuipers M, Schreuders T. *The predictive value of sensation testing in the development of neuropathic ulceration on the hands of leprosy patients.* Lepr Rev. 1994 Sep;65(3):253-61.
8. Wexler R, Melchior H. *Dorsal sensory impairment in hands and feet of people affected by Hansen's disease in Israel.* Lepr Rev. 2007 Dec;78(4):362-8.
9. Anderson AM, Van Brakel WH. *Age specific normal thresholds for sensibility testing with monofilaments in a Nepali population.* International Journal of Leprosy and Other Mycobacterial Diseases. 1998(66):69A.
10. Birke JA, Brandsma JW, Schreuders TAR, Piefer A. *Sensory testing with monofilaments in Hansen's disease and normal control subjects*. International Journal of Leprosy and Other Mycobacterial Diseases. 2000;68(3):291-8.
11. Kets CM, Van Leerdam ME, van Brakel WH, Devillé W, Bertelsmann FW. *Reference values for touch sensibility thresholds in healthy Nepalese volunteers*. Leprosy Review. 1996;67(1):28-38.
12. Bell-Krotoski J. "*Pocket*" *filaments and specifications for the Semmes-Weinstein monofilaments*. J Hand Ther. 1990;3:26-31.

13. Bell-Krotoski J, Weinstein S, Weinstein C. *Testing sensibility, including touch-pressure, two-point discrimination, point localization, and vibration*. J Hand Ther. 1993 Apr-Jun;6(2):114-23.

14. van Brakel WH, Nicholls PG, Das L, Barkataki P, Suneetha SK, Jadhav RS, et al. *The INFIR Cohort Study: investigating prediction, detection and pathogenesis of neuropathy and reactions in leprosy. Methods and baseline results of a cohort of multibacillary leprosy patients in north India*. Lepr Rev. 2005 Mar;76(1):14-34.

15. Campero M, Serra J, Ochoa JL. *Peripheral projections of sensory fascicles in the human superficial radial nerve.* Brain. 2005;128(4):892-5.

16. Anderson A, Reed N, van Brakel W, Croft RP. *Optimisation of a monofilament test for sensibility for use in a field programme of prevention of impairment.* International Journal of Leprosy and Other Mycobacterial Diseases. 1998(66):69A.

17. van Brakel WH, Shute J, Dixon JA, Arzet H. *Evaluation of sensibility in leprosy--comparison of various clinical methods*. Lepr Rev. 1994 Jun;65(2):106-21.

18. Wagenaar I, Brandsma W, Post E, van Brakel W, Lockwood D, Nicholls P, et al. *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols.* BMC Neurology. 2012 20130118 DCOM- 20130606(1471-2377 (Electronic)).

19. Malaviya GN, Husain S, Girdhar A, Girdhar BK. *Sensory functions in limbs of normal persons and leprosy patients with peripheral trunk damage*. Indian Journal of Leprosy. 1994;66(2):157-64.

20. Jeng C, Michelson J, Mizel M. *Sensory thresholds of normal human feet*. Foot Ankle Int. 2000 Jun;21(6):501-4.

21. McPoil TG, Cornwall MW. *Plantar tactile sensory thresholds in healthy men and women.* Foot. 2006;16(4):192-7.

22. Mitchell PD, Mitchell TN. *The age-dependent deterioration in light touch sensation on the plantar aspect of the foot in a rural community in India: Implications when screening for sensory impairment*. Leprosy Review. 2000;71(2):169-78.

23. van Brakel W. *Peripheral Nerve Function Assessment. Surgical Reconstruction & Rehabilitation in Leprosy and other Neuropathies.* Nepal: Ekta Books; 2004. p. 16-7.

24. Nolan MF. *Two-point discrimination assessment in the upper limbs in young adult men and women.* Phys Ther. 1982;62(7):965-9.

25. Wild S, Roglic G, Green A, Sicree R, King H. Global *Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030*. Diabetes Care. 2004;27(5):1047-53.

26. van Brakel WH, Nicholls PG, Das L, Barkataki P, Maddali P, Lockwood DN, et al. *The INFIR Cohort Study: assessment of sensory and motor neuropathy in leprosy at baseline*. Lepr Rev. 2005 Dec;76(4):277-95.

Chapter 4

Reliability of clinical nerve function assessment in peripheral neuropathies

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Leprosy Review (2014) 85, 29–35

Abstract

Introduction

Sensory and/or motor nerve function impairment as a consequence of neuropathy is often assessed using electro-neurophysiological tests. However, in low resource countries where the required equipment is rarely available, manual muscle strength testing (MMST) and monofilament testing (MFT) offer very reliable alternatives. In six leprosy programmes in four Asian countries, a multi-centre randomised clinical trial (RCT) was carried out to assess the effect of corticosteroids on neuropathy in leprosy-affected people. The sensory and motor nerve function was tested using MMST and MFT, including new test sites for the sural and radial cutaneous nerves (MFT) and the posterior tibial and common peroneal nerves (MMST). The reliability studies of the MMST and MFT tests of the TENLEP (Treatment of Early Neuropathy in LEProsy) trials are presented here.

Methods

Two assessors in each centre independently used the MFT and MMST in 30 leprosy-affected people.

Results

Reliability is good to very good for MFT in nearly all nerves. MMST also shows good to very good agreement, with a few exceptions.

Conclusion

Our study confirms that MMST and MFT can be performed reliably, and that the new tests also have acceptable reliability.

Introduction

There are many neuropathies that may result in sensory and/or motor function impairment that can be assessed and evaluated with practical clinical techniques such as manual muscle strength testing (MMST)[a] and monofilament testing (MFT) [1]. Electro-neurophysiological assessments have an important place in the differential diagnosis and follow up of suspected neurological diseases but are very rarely available in low-resource countries. When clinicians are trained, MMST and MFT could still be a useful clinical adjunct to the electro-neurophysiological assessments. Various studies have reported good reliability of both MMST and MFT [2-8]. Many of these studies have been conducted in leprosy-endemic
countries because of the 'availability' of large numbers of subjects which makes it easier to conduct such studies in a relatively short time. MMST and MFT, however, could also be very useful in assessing neuropathies due to other common diseases such as diabetes, or neuromuscular diseases such as hereditary motor and/or sensory neuropathies.
This study is in part a replicate study of reliability testing. The difference, however, is that this is a multi-centre reliability study that includes sensory and motor tests at sites for which reliability had not yet been determined.
In a randomised clinical trial (RCT), studying the efficacy of corticosteroids in the prevention and treatment of leprosy neuropathy, MMST and MFT were used to screen patients for intake, and for the follow-up of patients for the duration of the trials [9].
TENLEP (Treatment of Early Neuropathy in LEProsy) consists of two related multicentre, multi-country trials. In the Sub-clinical trial, patients with normal monofilament and muscle strength values, but with impaired electrodiagnostic or thermodiagnostic parameters, are enrolled. The outcomes for a group that will receive corticosteroids for 20 weeks will be compared with the outcome for a placebo group. The aim is to assess whether corticosteroids may prevent the onset of clinically detectable nerve function loss.
Patients with impaired nerve function of less than 6 months duration, as confirmed by MMST and MFT, are enrolled in the other arm of the study: the Clinical trial. In this trial, the currently recommended corticosteroid treatment for reaction is compared with an alternative regimen that is 3-4 months longer. The protocols for both trials have been published [9]. As decisions for enrolment and follow-up in both trials are based on the results of MMST and MFT, we felt that these assessments needed to show good reliability in and between the participating study centres. Hence, for both studies, we determined the reliability of MMST and MFT.

[a] VMT (Voluntary Muscle Testing) is the more commonly accepted abbreviation for muscle testing. However, 'voluntary' muscles can also be assessed with dynamometers. To distinguish between the two, the first author prefers to add Manual.

Methods

Following approval by local and national ethical committees, reliability was assessed in the six study centres where TENLEP is implemented: two each in India and Nepal and one centre each in Indonesia and Bangladesh.
The level of experience of selected staff varied between and within centres; therefore all participating staff were trained to perform testing in a standard manner. For this purpose, a Standard Operational Procedure (SOP) manual was developed.

Test-sites and procedures

Table 1 shows nerves in which motor and sensory function are commonly affected and assessed in the diagnosis and follow up of leprosy neuropathy.

Table 1- Overview of nerves and their test-sites for MMST and MFT

Nerve	Muscle strength testing	Sensory testing with monofilaments
Facial	Eye closure	-
Ulnar	Little finger abduction	Little finger/ hypothenar
Median	Thumb abduction	Thumb/ middle finger
Radial (cutaneous)	Wrist extension	Radial site index/ thumb web
Common Peroneal (CP)	Foot dorsiflexion	-
Deep branch CP	Great toe extension	-
Posterior tibial	Great toe plantar flexion	Great toe plantar surface/ forefoot
Sural	-	Lateral border foot

Motor

For each nerve with motor function, tests on one muscle group stimulated by that nerve were performed, using the Medical Research Council (MRC) grades [1]. Motor function tests for facial, ulnar, median, radial cutaneous and common peroneal were carried out as described elsewhere [1]. As a relatively new test we introduced the 'great toe up and down' test to assess the motor function of the terminal branch of the common peroneal nerve, and the posterior tibial nerve respectively. The great toe up test is performed when the patient is sitting with his/her feet flat on the ground. The patient is asked to lift the great toe only and the assessor determines muscle strength, applying pressure on the proximal phalanx. The great toe down test is also called the 'paper grip test' [10]. For this, an MRC score of 5 (strong), 4 (weak) or 0 (paralysed) could be given.

Sensory

For each nerve with a sensory component, three test-sites were used within the innervation area of that nerve. Monofilament testing was performed using five monofilaments: 200 mg, 2 g, 4 g, 10 g and 300 g. For the foot, because of known higher thresholds, the lightest, 200 mg filament, was omitted. Each of three sites was then given a score based on the filament felt, the heaviest monofilament getting the highest score, allowing a maximum total score of 15 for the ulnar, median and radial nerves (4 filaments) and 12 for the foot (3 filaments).
For the ulnar, median and posterior tibial nerves, the same sites were examined as in the INFIR study [11], except for the median nerve where we used the tip of the middle finger instead of the index finger. For the first time, the radial cutaneous and sural nerves were also assessed using three test-sites, which are shown in Figure 1.

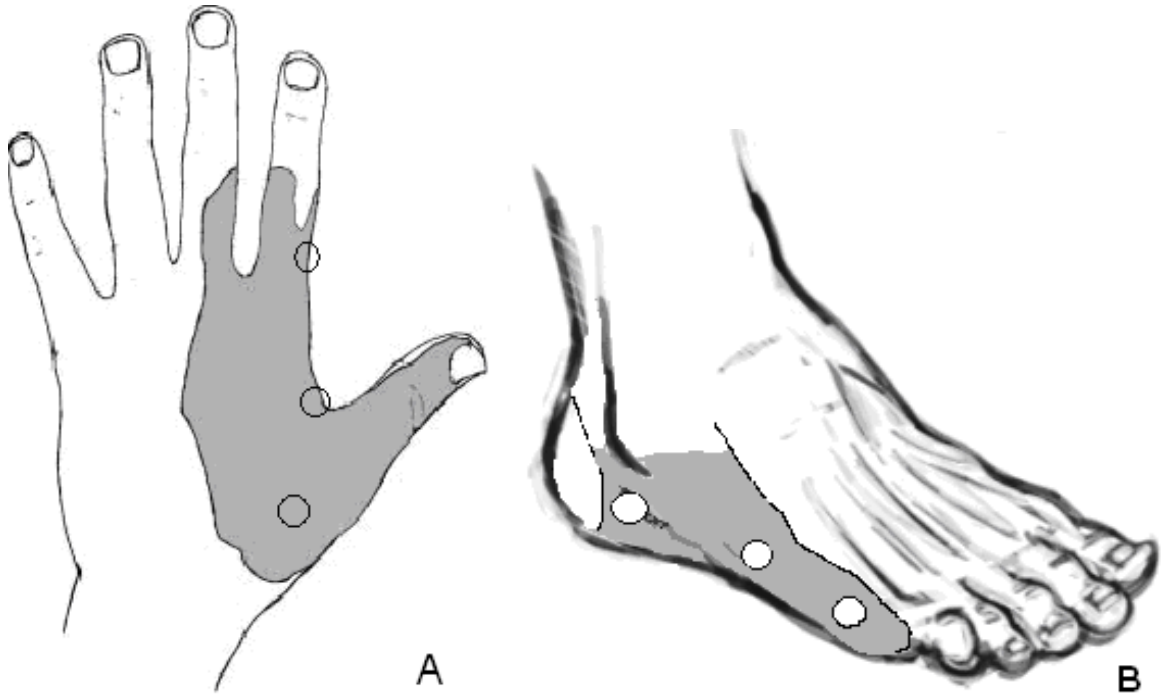

Figure 1- Test-sites for radial cutaneous (hand) and sural nerve (foot) monofilament testing

Reliability testing

To develop expertise, a large number of patients were assessed under supervision of experienced assessors before the reliability study was initiated. Following that period of skill development, both therapists were asked to randomly and independently assess a minimum of 30 subjects, all of whom were leprosy-affected people. The testers were blind to each other's results and the order of subjects was changed randomly between testers. Motor and sensory nerves were assessed bilaterally, effectively doubling the number of tests.
Test results of both assessors were sent to, and analysed by the same statistician (PN).

Weighted Kappa's were used to express reliability: moderate (0.41–0.6), good (0.61–0.8) and very good (more than 0.81).

Results

Reliability for monofilament testing for all nerves was good to very good in all centres, with the exception of one centre where agreement on testing the sensory function of the radial cutaneous nerve was moderate (Table 2).

Table 3 shows that, in general, the muscle strength tests show good to very good reliability. For the few tests that did not show acceptable reliability the two therapists involved in each study centre were both requested to recheck the muscle group where disagreement was apparent, and to come to an agreement on the grading before making a clinical decision.

Table 2- Manual Muscle Strength Test reliability (Weighted Kappa's)

Centre	Eye closure	Ulnar APB	Median	Radial wrist	Lateral popliteal foot	Toe up	Toe down
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	1.000	0.908	0.877	0.662	0.797	0.710	0.471
C	0.600	0.943	0.922	N/A	0.675	0.958	0.918
D	0.575	0.641	0.257	N/A	0.869	0.756	0.688
E	0.615	0.956	0.881	0.851	0.967	0.977	0.943
F	0.191	0.968	0.827	0.705	0.965	0.970	0.991

0.41-0.6= moderate agreement; 0.61-0.8=good; >0.8= very good, N/A: not applicable

Discussion

Our study confirms the findings of other studies that MMST and MFT can be reliably performed [2-8]. The tables show results that reflect the agreements of one pair of testers in each centre. As intake to TENLEP was initially slow, additional staff also had to be trained to enable the involvement of more clinics. Intertester reliability of new staff was also determined prior to intake. The tables only give results of the initial pairs of testers. Testers were encouraged to repeat MMST and VMT for a sample of subjects at regular intervals to maintain standards in the execution and interpretation of tests.

The testing of the sural and radial cutaneous nerves for sensibility with monofilaments was a new development. A related study in leprosy neuropathy had shown good reliability but only

one test site was used for these two nerves whereas we tested three sites for all nerves in our study [6]. We decided to give equal value to sensory nerve function and decided on three sites for these two nerves that could be affected.

Table 3- Monofilament test reliability (Weighted Kappa's)

Centre	Ulnar	Median	Radial cutaneous	Posterior tibial	Sural
A	0.981	0.986	0.986	0.992	0.988
B	0.948	0.945	0.932	0.982	0.960
C	0.776	0.877	0.815	0.863	0.869
D	0.799	0.807	0.601	0.731	0.834
E	0.938	0.901	0.960	0.919	0.930
F	0.987	0.986	0.989	0.993	0.993

0.41-0.6= moderate agreement; 0.61-0.8=good; >0.8= very good

New tests that were introduced for grading motor nerve function were great toe down and up for the posterior tibial and deep common peroneal nerve (terminal motor branch), respectively [9]. These tests also showed good to very good reliability in all but one study centre. These tests are, as yet, not routinely used in leprosy neuropathy but should be considered clinically as important. Isolated weakness of great toe flexion and/or extension, with normal foot dorsiflexion-eversion indicates nerve function impairment at ankle level or dorsal site of the foot. Paralysis of the intrinsic foot muscles may contribute to the onset of tissue breakdown in the presence of loss of protective sensation, which is often the case in leprosy neuropathy. The great toe down test is the only test that can reliably test the motor function of the posterior tibial nerve, a nerve often involved in leprosy and diabetic neuropathy. Weakness in great toe flexion will indicate impaired posterior tibial function at ankle level involving the intrinsic foot muscles. Toe flexion will still be possible because the extrinsic toe muscles are rarely involved in leprosy neuropathy (i.e. impairment of the posterior tibial nerve at knee level). This test was validated by Win et al., asking control subjects to perform the test with and without an anesthetised posterior tibial nerve [10].

Low reliability coefficients were recorded for eye closure (i.e. facial nerve/orbicularis oculi) in some centres. In cases where isolated facial nerve impairment was suspected at intake and during the trial and follow up, the advice given was that testers should 'double check' and agree on a grade. Differences in reliability between centres were seen, mainly for MMST, most likely as a consequence of differences in levels of experience. One centre even scored a perfect 1.0 for muscle testing. This was the only centre in which both testers had lengthy experience with muscle grading and may illustrate the point that correct practice makes perfect. Table 3 does not specifically mention isolated muscles with their official anatomical names. The table

indicates that movements are tested rather than individual muscles, which is what happens in most MMST tests [1,4-6]. Testing muscles in 'isolation' is, with rare exceptions, not possible. In most muscle tests there is synergistic action of multiple muscles. Most of the studies of reliability of MMST and MFT have been conducted with leprosy patients. This is probably because in many leprosy hospitals and their out-patient departments there are enough patients with neuropathy to be able to conduct a reliability study in a short time. We suggest, however, that for many other neuropathies and neuromuscular diseases these simple clinical tests of MMST and MFT could also be used, although it would require sufficient practice by clinicians using a standard protocol to achieve a reliable assessment. It is our opinion that where neurophysiological assessments such as nerve conduction studies and warmth detection thresholds, in other neurological conditions, are frequently performed alone, MMST and MFT could be of complementary value.

Conclusion

Manual muscle strength testing and monofilament testing can be reliably performed by experienced testers using a standard protocol. These tests can be usefully applied in diagnosis and follow up of neuromuscular diseases, neuropathies and suspected nerve lacerations and repairs, either as stand-alone procedures or as complementary procedures to electro-neurophysiological and warmth detection assessments.

References

1. Brandsma JW. *Basic nerve function assessment in leprosy patients*. Lepr Rev, 1981; 52: 161–170.
2. Bell-Krotoski JA, Tomacik E. *The repeatability of testing with Semmes-Weinstein monofilaments.* J Hand Surg, 1987; 12A: 155–161.
3. Birke JA, Brandsma JW, Schreuders TAR et al. *Sensory testing with monofilaments in Hansen's disease and normal control subjects*. Int J Lepr, 2000; 68: 291–298.
4. Brandsma JW, Schreuders TAR, Birke JA et al. *Manual muscle strength testing. Intra- and interobserver reliability of the intrinsic muscles of the hand*. J Hand Ther, 1995; 8: 185–190.
5. Brandsma JW, Brakel van WH, Anderson AM et al. *Intertester reliability of manual muscle strength testing in leprosy patients*. Lepr Rev, 1998; 69: 257–266.
6. Roberts AE, Nicholls PG, Maddali P, van Brakel WH. *Ensuring inter-tester reliability of voluntary muscle and monofilament sensory testing in the INFIR cohort study*. Lepr Rev, 2007; 78: 122–130.
7. Wexler R, Melchior H. *Dorsal sensory impairment in hands and feet of people affected by Hansen's disease in Israel*. Lepr Rev, 2007; 78: 362–368.
8. Anderson AM, Croft RP. *Reliability of Semmes Weinstein monofilament and ballpoint testing in Bangladesh.* Lepr Rev, 1999; 70: 305–313.
9. Wagenaar I, Brandsma JW. *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: The TENLEP Study Protocols*. BMC Neurology, 2012; 12–59. www.biomedcentral.com/1471-2377/12/159.
10. Win de MM, Theuvenet WJ, Roche PW et al. *The paper grip test for screening intrinsic muscle paralysis in the foot in leprosy patients*. Int J Lepr Mycobact Dis, 2002; 70: 16–24.
11. van Brakel WH, Nicholls PG, Das L, *The INFIR et al. Cohort Study: investigating prediction, detection and pathogenesis of neuropathy and reactions in leprosy. Methods and baseline results of a cohort of Multibacillary leprosy patients in north India*. Lepr Rev, 2005; 76: 14–34.

Chapter 5

Early detection of neuropathy in leprosy: A comparison of five tests for field settings

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Abstract

Background

Early detection and treatment of neuropathy in leprosy is important in the prevention of disabilities. The INFIR study showed that 12 weeks before nerve function became abnormal with monofilament tests (MFT), Nerve Conduction Studies (NCS) and Warm Detection Thresholds (WDT) were already affected. These two methods are promising for early detection of neuropathy, however, they require climate-controlled rooms and highly trained staff, are very expensive, and are therefore not practical to use under field conditions. Our aim was to find and field-test alternative test methods and compare their sensitivity and specificity to detect neuropathy at an early stage.

Methods

Through a literature search we identified five alternative devices that appeared simpler, more affordable, portable and/or battery operated: the Neuropad®, Vibratip™, NC-Stat®DPNCheck™, NeuroQuick and the Thermal Sensibility Tester (TST), assessing respectively sweat function, vibration sensation, nerve conduction, cold sensation and warm sensation. In leprosy patients in Bangladesh, the posterior tibial and sural nerves that tested normal for MFT and voluntary muscle test (VMT) were assessed with the reference standard tests NCS and WDT. The alternative devices were then tested on 94 nerves with abnormal WDT and/or NCS results and on 94 unaffected nerves. Sensitivity and specificity were the main outcomes.

Results

The NeuroQuick and the TST on the sural nerve showed the highest sensitivity and specificity (>80%), followed by the Neuropad®, NC-Stat®DPNCheck™ and Vibratip™. On the posterior tibial nerve the NeuroQuick and the TST also showed better sensitivity.

Conclusions

The NeuroQuick and TST are good candidates for further field testing for reliability and reproducibility, and the costs and options for further development and production should be studied.

Introduction

Leprosy is a major cause of peripheral neuropathy in low resource countries, affecting sensory, motor and autonomic nerve function. Complications of neuropathy are sensory loss and muscle weakness. Earlier studies found that 10% to 55% of new leprosy cases presented at diagnosis with one or both of these clinical symptoms [1-4], and up to a fourth of the patients developed neuropathy during or after treatment [5]. When neuropathy remains untreated or is treated too late it can lead to disabilities. Worldwide over three million people are living with disabilities due to leprosy [6]. In the prevention of disabilities, timely detection and treatment of neuropathy is essential.

Sensory nerve function impairment (NFI) is often the first symptom of leprosy neuropathy. Detection of sensory NFI is typically done with monofilament tests (MFT) or ballpoint tests, and motor function is assessed with voluntary muscle tests (VMT). It is assumed that when sensory impairment is clinically detectable, quite some damage has already been done to the nerves, the so-called subclinical neuropathy [7]. Methods to detect neuropathy in such early stages were studied in the INFIR (ILEP Nerve Function Impairment and Reactions) study [4]. To find the most sensitive methods, changes in the nerves of leprosy patients were monitored over time with multiple methods assessing different modalities of neuropathy. Nerve conduction studies (NCS) were found to be affected most frequently, followed by warm detection thresholds (WDT). These two methods were able to detect abnormalities in the nerves up to twelve weeks before MFT became abnormal. NCS assesses the large Aβ-fibres, responsible for perception of vibration, touch and pressure. The thermal thresholds test small myelinated Aδ- and unmyelinated C-fibres, which mediate pain, warm- and cold sensation and are responsible for the autonomic function –e.g. sweating [8-10].

Even though NCS and WDT are very important techniques in the detection of early neuropathy, the devices are not optimal for use in the field of leprosy endemic areas. They are costly, require well trained staff and need stable environmental (temperature) conditions and stable electricity supply. There is a need for cheap, easy-to-use, sensitive and reliable screening tools to detect early neuropathy. Our aim is to find potential alternative tests methods that can detect early neuropathy in leprosy patients and to compare the sensitivity and specificity of these methods when assessed in field conditions, using NCS or WDT as reference standard test.

Methods

A literature search was conducted to identify simple, portable devices used for the diagnosis of early neuropathy, regardless of the pathology. We covered the Embase, Medline and Cochrane databases and used the search engine Google, using search terms related to 'neuropathy', 'diagnosis', 'device' and 'simple'.
This resulted in list of possible eligible devices (Appendix Table A). A final selection of devices was made based on the following requirements: costs less than €1500; availability; easiness of use- i.e. no extensive training required; and practicality and suitability for field conditions: small portable, battery operated devices providing direct results without the need for additional (computer) analyses. We also intended to select methods assessing different modalities of neuropathy.

Selected alternative devices

The final selection of devices consisted of the Neuropad® (TrigoCare International), Vibratip™ (McCallan Medical Limited), NC-Stat®DPNCheck™ (NeuroMetrix, Inc.), NeuroQuick (Schweers) and the Thermal Sensibility Tester (TST) (World Health Organization).

The *Neuropad* is a patch designed to test the autonomic function of the diabetic foot by assessing sweat production. The colour of the patch changes from blue to pink due to a chemical reaction between the complex salt anhydrous cobalt-II-chloride and sweat on the skin. The main outcome is whether or not the patch turned completely pink after ten minutes, and the total time to complete colour change is recorded as well. The Neuropad is applied on the great toe or on the plantar surface of the foot between the first and second metatarsal head.

The *Vibratip* is a pocket-sized device to test vibration sensation. This disposable, battery operated device produces a stimulus of 128 Hz, comparable with a tuning fork, and is activated by pinching. The main outcome is whether or not vibration is felt.

The automated *NC-Stat DPNCheck* evaluates sensory nerve conduction of the sural nerve. This device offers an alternative to standard NCS and is hand-held, battery operated, fast and user-friendly, as general health care providers can handle it with minimal training. The sural nerve is stimulated with a 100 mA current, and the signal is detected orthodromically by a biosensor at 92.2 mm from the simulation probes. Conduction velocity and amplitude are the main outcomes.

The portable *NeuroQuick* device tests cold sensation using an air flow produced by the integrated fan. The adjustable fan speed can be increased until the patient perceives the air flow. The outcome, on a 0-9 scale, is compared to a normal threshold to define whether the sensory nerve function is impaired.

The *Thermal Sensibility Tester* has the size of a pen and assesses warm sensation. It was originally designed to test sensation in leprosy skin lesions [11]. Both ends of the pen have a

little metal disc, one side adjusts to room temperature and one becomes warm when switched on, with a temperature between 45° and 60°C depending on environmental temperature [12]. The main outcome is whether or not the difference between the warm and normal side can be felt.

Patients and controls

Leprosy patients who had normal MFT and VMT results for the posterior tibial and sural nerves were included when they had abnormalities in at least one sural or posterior tibial nerve with NCS and/or WDT test. Patients were excluded from the study when they suffered from other diseases that may affect the nerve function, e.g. diabetes. Patients under the age of 18 or over 70 years were excluded as well. Patients on prednisolone treatment were excluded from Neuropad testing, as a rare side effect of this drug is hyperhidrosis. To assess the specificity of the devices we also needed to test nerves with normal NCS and/or WDT. When one of the body sides of the included patients had normal nerve conduction and/or WDT test results for the sural or posterior tibial nerve, this nerve was taken as control. At the end of the study, we also had to include some leprosy patients with only normal NCS and/or WDT results as control subject in order to acquire the calculated number of normal nerves.

Test procedure

The study took place in the Danish Bangladesh Leprosy Mission hospital at the Rural Health Program (RHP) department in Nilphamari, which is run by The Leprosy Mission International Bangladesh. Patients with normal VMT and MFT for sural and posterior tibial nerves were sent to Nilphamari RHP from the nearby field clinics. There, the subjects were assessed again with MFT and VMT, according to a previous described protocol [13]. In short, a standard set of Semmes-Weinstein monofilaments was used to assess three test-sites for each nerve, the 2g filament representing the normal threshold for the foot. For motor function assessment, the 0-5 Medical Research Council (MRC) scale was used [14]. An MFT score under 3 and a VMT score of 5 were considered normal.

Reference standard test

When MFT and VMT were normal, the reference standard tests, sensory NCS and WDT tests were carried out. Sensory NCS were performed for the sural nerve only, because sensory NCS for the posterior tibial nerve is difficult and therefore we considered it unreliable. Sural NCS were recorded antidromically at 14 cm from the standard stimulation site. The test was done with the Neurocare 2000W EMG machine (BioTech Ltd, Mumbai), and a nerve was considered affected when either amplitude or velocity was impaired. The WDT test was carried out using TSA II (MEDOC, Israel) for both sural and posterior tibial nerves, on respectively the mid-lateral border of the foot and the plantar aspect of the great toe. Both reference standard tests were done bilaterally, in an air-conditioned room by two experienced assessors who showed good inter-tester reliability (intraclass correlation coefficient of sural SNC, Velocity: 0.89; Amplitude: 0.99). The outcomes of NCS and WDT were compared to age-adjusted normal values that had been

determined earlier for the TENLEP trial at Nilphamari Rural Health Program (using the 97.5th percentile) [13]. However, for the posterior tibial nerve, WDT cut-off levels were set at the maximum temperature reached with the TSA, i.e. 50°C. Applying this cut-off, no patient was considered abnormal. Therefore, we redefined the abnormal threshold by using the 95th percentile from the normative studies of TENLEP, instead of the originally used 97.5th percentile.

Alternative devices

Four out of five tests were performed for the sural nerve, which was chosen because this nerve is the most commonly affected in leprosy [15,16] and because the NC-stat was specifically designed to assess the sural nerve. The Neuropad test was carried out on the posterior tibial nerve, since this test has been validated for the sole of the foot only. We tested the other two small nerve fibre tests -the NeuroQuick and the TST- on the posterior tibial nerve as well (Table 1). Not all patients were tested with all devices, for two reasons. First, only the nerve(s) identified as abnormal by one of the reference standard tests and their contralateral normal side were assessed with the alternative devices. Second, only the alternative devices that assessed the same type of nerve fibre - small or large - as the abnormal reference standard test was tested (Table 1). For example, in a patient with abnormal sensory NCS for one sural nerve we tested both sural nerves with NC-Stat DPNCheck and Vibratip. The tests with the alternative devices were carried out on the same day as the reference standard tests, by one trained assessor who was blinded to the NCS and WDT results. The tests took place outside, without fan or air-conditioning to mimic field circumstances, and were done in a random order following a randomization table created in Excel. Before the testing started, the assessor thoroughly explained and demonstrated the test procedures to the patient.

The Vibratip, NeuroQuick and TST assessed the sural nerve at the mid-lateral border of the foot. With the Vibratip, the skin was touched twice, each time for approximately one second, and randomly once with vibration and once without vibration. After the second touch, the patient was asked during which of the two touches he felt the vibration. The test was repeated two more times. When the answer was correct at least two out of three times, the patient's vibration sensation was recorded as normal [17]. The outcome of the Vibratip tests was compared to NCS. A similar procedure was followed for the Thermal Sensibility Tester: the patient's skin was touched twice for about three seconds, randomly once with the warm side and once with the 'normal side'. After the second touch, the patient was asked which of the two touches was perceived as warm. The test was repeated two more times. When the answer was correct at least two out of three times, the patient's warmth sensation was recorded as normal. The outcome of the Thermal Sensibility Tester was compared to WDT. The NeuroQuick test was started at fan speed level 0 and the device was then held for 5 seconds at a distance of 23 cm from the foot. This exact distance is shown by two crossing laser beams. When the patient did not perceive the air flow, the fan speed was increased by one level and held above the foot for another 5 seconds. This process was repeated until the airflow was perceived by the patient. The total procedure was repeated two more times and the average of three measurements was the outcome of the test. This outcome was compared to the cut-off levels

Table 1- Modality, nerve type and reference standard test for the alternative devices

Test	Modality	Nerve	Nerve fibre	Reference standard test
NC-Stat	Conduction	Sural	Large fibre (Aβ)	NCS sural
Vibratip	Vibration	Sural	Large fibre (Aβ)	NCS sural
NeuroQuick	Cold sensation	Sural and PT	Small fibre (Aδ)	WDT sural/ PT
TST	Warm sensation	Sural and PT	Small fibre (C)	WDT sural/ PT
Neuropad	Sweat function	PT	Small fibre (C)	WDT PT

PT= posterior tibial nerve

of the normative test to determine whether it was abnormal. The reference test used for comparison was WDT.

For the test with the NC-Stat DPNCheck, the patient was asked to place his lower leg on a chair in a position that the muscles were relaxed [18]. The skin was cleaned with alcohol, a biosensor was inserted in the device and gel was applied on the stimulating probes. The sural nerves were stimulated for 10-15 seconds, just posterior to the lateral malleolus. Both velocity and amplitude were read directly from the device, and were automatically corrected for skin temperature. The manufacturer suggests four severity categories: normal (velocity >40 m/s and amplitude >4 μV), mild (velocity <40 m/s, but amplitude >4 μV), moderate (amplitude 1-4 μV), and severe (amplitude<1 μV). The outcome of the DPNCheck was compared to NCS. The Neuropad, NeuroQuick and TST were assessed on the plantar side of the great toe. Similar procedures as described above were followed for NeuroQuick and TST. The Neuropad was applied after the patient had acclimatised for ten minutes with bare feet. For each Neuropad, a stopwatch was started immediately after application of the pad. The total time to complete colour change from blue to pink was recorded as outcome, or if the change was not complete, the colour of the patch after ten minutes was noted (blue/pink or blue). The outcome of the Neuropad test was compared to WDT. After all the tests had been finished, we asked the patient which of the five tests he valued the best and the worst and for what reasons. We also asked the assessor's opinion on the practicality of the tests and we looked at the costs.

Ethics

This is a sub-study of the TENLEP trial, for which ethical approval was given by the Bangladesh Medical Research Council (BMRC/NREC/2010-2013/533). An informed written consent was obtained from all participants.

Analyses

Sample size calculations were done for an expected sensitivity of 0.60 and a confidence interval width of 0.20. This resulted in 92 nerves per test. A similar number of normal nerves should be tested to determine the specificity at 0.60.

A normative study was carried out for the NeuroQuick to define the cut-off for abnormality. We tested the posterior tibial and sural nerves of 50 healthy males and 50 healthy females between 18 and 60 years of age. The NeuroQuick test procedure was followed as described above. As the outcomes for left and right body side did not differ significantly, the average of both body sides was used to calculate the normal thresholds. The correlation of NeuroQuick outcome with sex and age was tested with Spearman's rho. Only age was significantly related with the NeuroQuick outcome for both nerves (sural: $r= 0.64$, $p<0.001$; posterior tibial: $r=0.57$, $p<0.001$). We therefore used a regression equation to calculate an age-related cut-off for abnormality. For the sural nerve, the NeuroQuick result was considered abnormal when larger than 0.02*Age+3.4, and for posterior tibial when larger than 0.01*Age+3.3.

As primary outcome for our study, the sensitivity and specificity of each alternative device were calculated against the appropriate reference standard test –NCS or WDT- with their 95% confidence intervals. Also the positive and negative likelihood ratios and area under the curve (AUC) were calculated for each alternative device. A p-value < 0.05 was considered to be statistically significant.

Results

We enrolled 209 patients, and examined a total of 95 abnormal and 89 normal sural nerves with NCS, 94 abnormal and 90 normal sural nerves with WDT, and 75 abnormal and 115 normal posterior tibial nerves with WDT. The majority of patients had PB leprosy (67%) and were still on MDT (55%). On average leprosy was diagnosed 11 months earlier. The patients' demographic and clinical characteristics are presented in Table 2.

All patients tested with the Vibratip were able to detect the vibration for all three touches, whether it was tested on an abnormal or normal nerve. For results on abnormal and normal of TST and NQ see Table 3. Abnormal nerve conduction was indicated by the NC-Stat DPNCheck in 14% of the sural nerves. One nerve was mildly affected, 18 moderately affected and seven severely affected. The colour of the Neuropad had changed completely after ten minutes in 90 cases, and in 77 cases some blue was still visible. None of the pads remained completely blue. Mean time until complete colour change was 7.5 minutes, ranging from 2 to 10 minutes. Excluding the people without complete colour change, the average time to complete colour changes was 4.9 minutes.

The sensitivity, specificity, positive and negative likelihood ratios of all alternative devices are shown in Table 3. For the sural nerve, the NeuroQuick had the highest sensitivity and specificity (both 86%), closely followed by the TST (respectively 83% and 82%). The two devices assessing large fibre neuropathy in the sural nerves, the Vibratip and NC-Stat DPNCheck, had poorer outcomes. The sensitivity of the Vibratip was 0%, and 100% specific. For the posterior tibial nerve, the NeuroQuick showed the highest sensitivity as well (93%), but a lower specificity

Table 2- General characteristics of the patients (n=209)

Characteristics		
Sex (% male)		57%
Age (mean)		35,3
Height (cm)		157
Weight (kg)		51
Time since diagnosis (months, mean)		11
RFT (%)		46%
RJ Classification (%)	TT	2%
	BT	89%
	BB	1%
	BL	4%
	LL	4%
	PN	1%
WHO classification (% PB)		67%
Smoking status (%)	Never	67%
	Current	26%
	Former	7%

RFT: Released from anti-leprosy treatment; RJ: Ridley-Jopling; WHO: World Health Organisation

than the TST. The sensitivity and specificity of the Neuropad were average (56% and 60%). The highest positive likely hood ratio was seen in the NeuroQuick for the sural nerve: 6.0 (3.6-10.0).

Patients' and assessor's preferences

Sixty-eight patients were tested with at least four out of the five tests. More than half (53%) indicated a preference for the TST, mainly because they found it easy to feel. The second favourite test was the Neuropad (25%), also because it was easy as it does not require any response from the patient. The Vibratip and the NeuroQuick followed with 13% and 9%. When asked which test was least preferred, 54% answered the NC-Stat DPNCheck. The main reason was that it was too painful. The second least preferred test was the NeuroQuick (24%), since it was too difficult and third the Neuropad (22%), because it was seen as time consuming.

The assessor indicated he preferred the TST and Neuropad, because they are easy to carry out. He pointed out that for the patient the TST and the Vibratip are the best, since the warmth and vibration are easily detected. Finally, the assessor reported that the TST and Vibratip are the fastest tests to perform.

Table 3- Outcomes of the alternative devices when compared to the reference standard test

Alternative devices		**Reference standard test**						
		Abnormal	Normal	Sensitivity	Specificity	AUC	PLR	NLR
Sural NCS (n)		*95*	*89*					
NC-Stat DPNCheck	Abnormal	15	11	16%	88%	0.52	1.3	1.0
	Normal	80	78	(9-25)	(79-94)	(0.43-0.60)	(0.6-2.6)	(0.9-1.1)
Vibratip	Abnormal	0	0	0%	100%	0.50	-	1.0
	Normal	95	89	(0-4)	(96-100)	(0.41-0.58)		(1.0-1.0)
Sural WDT (n)		*94*	*90*					
TST	Abnormal	78	16	83%	82%	0.83	4.7	0.2
	Normal	16	74	(74-90)	(73-89)	(0.76-0.89)	(3.0-7.4)	(0.1-0.3)
NeuroQuick	Abnormal	81	13	86%	86%	0.86	6.0	0.2
	Normal	13	77	(78-92)	(77-92)	(0.80-0.92)	(3.6-10.0)	(0.1-0.3)
Posterior tibial WDT (n)		*75*	*115*					
Neuropad	Abnormal	42	35	56%	61%	0.59	1.5	0.7
	Normal	33	57	(44-67)	(51-72)	(0.51-0.67)	(1.1-2.1)	(0.5-1.0)
	Missing		23					
TST	Abnormal	60	50	83%	57%	0.70	1.9	0.3
	Normal	12	65	(72-91)	(47-66)	(0.63-0.76)	(1.5-2.4)	(0.2-0.5)
NeuroQuick	Abnormal	67	68	93%	41%	0.67	1.6	0.2
	Normal	5	47	(85-98)	(32-50)	(0.60-0.74)	(1.3-1.9)	(0.1-0.4)

(95% confidence interval); NCS: Nerve Conduction Studies; TST: Thermal Sensibility Tester; WDT: Warm Detection Thresholds; AUC: area under the curve; PLR: positive likelihood ratio; NLR: negative likelihood ratio

Costs

We have not performed a cost-effectiveness analysis, though we would like to present the costs of the devices (Table 4). The retail price for Neuropads for clinics is $11 per test, containing two pads. The Vibratip is a disposable device and can be used thousands of times [19]. The list price for the NC-Stat DPNCheck is the highest of the alternative devices in our study, $1000 for the device and another $20 per sensor, which can be used to assess both left and right sural nerve in one patient. The TST and NeuroQuick are not available for purchasing, and therefore the prices are unknown. A paper published in 1989 describes that the cost of a TST was about $35 [12].

Table 4- Overview of the costs of the alternative devices

Device	Price per unit	Additional costs
Neuropad	$11 *	-
Thermal Sensibility Tester	- $35 in 1980's	2x AA battery
NeuroQuick	- Not available on the market	2x AA battery
Vibratip	$9* + packaging costs	-
NC-Stat DPNCheck	$1,000	$20 per sensor

*Prices were calculated from other currencies at 25/2/2016. The original price for Neuropad was €10 and Vibratip was £6.50

Discussion

It is important to detect leprosy neuropathy at an early stage, as it can progress to nerve function impairment and subsequently may lead to disabilities. In the INFIR study, 20-50% of the newly diagnosed leprosy patients either had subclinical neuropathy at diagnosis or developed this during follow up [20]. Of the patients who have subclinical neuropathy, around 16% developed clinical NFI in the INFIR study. In TENLEP, preliminary data show that this occurred in 8% of the subclinical patients. And although treatment of subclinical neuropathy has not been successful [21,22], knowing the subclinical status of a patient's nerves is actually very valuable. Because in the patients that do develop clinical NFI, treatment with prednisolone can be immediately started. In this study we aimed to compare simple methods for detecting early leprosy neuropathy in field settings. We found that the NeuroQuick and TST are promising screening methods, especially for the sural nerve, since they show high sensitivity and specificity and are positively perceived by both patients and assessor.

Both the NeuroQuick and the TST examine small fibre function. The INFIR study found however that there was variation between the patients in the first affected modality and type of nerve fibre -large or small [23]. First of all, this indicates that the processes and patterns of neuropathy are different for each individual. Second, this has consequences for the assessment of early neuropathy. Adding a second test method that assesses large fibre function is recommended. Unfortunately, the two devices in our study testing large fibres, the NC-stat DPNCheck and the Vibratip, are not suitable for early detection of leprosy neuropathy. So far NCS are the only reliable option for detection of early large fibre neuropathy.

None of the alternative devices included in our study were new. The TST was used in leprosy to assess sensitivity of leprosy skin lesions [11]; the Neuropad, NeuroQuick, Vibratip and NC-stat DPNCheck have been used before for diagnosis of diabetes neuropathy. Studies in diabetes patients showed generally good sensitivity and specificity for the detection of neuropathy [17,24-29]. Although both small and large fibres are involved in diabetes neuropathy and in

leprosy neuropathy, diabetes neuropathy is often symmetrical [30] and the order of affected modalities is somewhat different between the two diseases. In diabetes for example, cold perception was found to be the most sensitive thermal test [31], where in leprosy warm detection was more sensitive [20]. Furthermore, the tests in diabetes patients have been performed in a completely different setting and environment, namely western hospital settings. Therefore, it was necessary to test the devices in a leprosy endemic country with a subtropical climate, in a field setting with high temperatures and humidity. It is interesting to see that the TST, designed for testing in high environmental temperatures, showed good results. The temperature of the warm-end of the TST adapts to the ambient temperature, when between 15°C and 45°C. A graph with the correlation is depicted on the device.

The accuracy of the reference standard test, to which the new tests are compared, is an important point to take into consideration. When the reference standard test is not 100% accurate, this has an effect on the sensitivity and specificity estimates [32]. NCS have been compared with nerve biopsies and were found to be accurate [33]. In addition, NCS are more reliable because they are independent of the patient's response and therefore objective. The WDT test, on the other hand, has never been compared to a gold standard in leprosy neuropathy. Furthermore, it requires a response from the subject and is therefore less reliable. Perkins et al. describe that variances of 50% and higher can occur in thermal thresholds testing. However, since we use very high cut-off levels for normality (97.5%), we are confident that the sural nerves tested abnormal in our study are in fact abnormal. This is less the case for the WDT of the posterior tibial though. We could not rely on the upper thresholds for the posterior tibial nerve, since they mainly lay outside the measurement range of the TSA (50°C). Therefore, we used a lower cut-off by taking the 95th percentile, but even with that method it was very difficult to include abnormal nerves.

We made an effort to select the most promising devices for this study. The appendix contains a table presenting the reasons for inclusion and exclusion of the different options. Since in leprosy sensory function generally is affected before motor function we did not include any assessments for motor nerves. Several other tests might be worthwhile to look at in future studies. We intended to include the NerveCheck (PhiMed Europe), which is a portable device that assesses four modalities: cold, warm, pain and vibration sensation [34]. Unfortunately, at the time of our study no NerveCheck device was available for field testing. Second, it might be interesting to look at skin wrinkling as an autonomic function test. Although the duration of the test takes long - 30 minutes - the results are quite promising in diabetes neuropathy [35,36].

In conclusion, based on our results the Neuropad, NCstat and Vibratip do not qualify for further testing for detection of early leprosy neuropathy. For both the NeuroQuick and TST, however, we recommend that three different aspects should be further studied. First, the options for further development and possible production on a larger scale should be examined. Second, repeatability and reproducibility should be determined for these two tests, and preferably assessed in different populations as well. Finally, additional testing of the accuracy on the hands

can give more information on the usability of the two tests in detecting early leprosy neuropathy in field settings, as upper extremity neuropathy is common as well.

References

1. Croft RP, Nicholls P, Anderson AM, van Brakel WH, Smith WC, Richardus JH. *Effect of prophylactic corticosteroids on the incidence of reactions in newly diagnosed multibacillary leprosy patients.* Int J Lepr Other Mycobact Dis 1999; 67(1): 75-7.
2. Croft RP, Nicholls PG, Richardus JH, Smith WCS. *Incidence rates of acute nerve function impairment in leprosy: A prospective cohort analysis after 24 months (The Bangladesh Acute Nerve Damage Study)*. Leprosy Review 2000; 71(1): 18-33.
3. Saunderson P. *The pattern of leprosy-related neuropathy in the AMFES patients in Ethiopia: definitions, incidence, risk factors and outcome*. Leprosy Review 2000; 71(3): 285-308.
4. van Brakel WH, Nicholls PG, Das L, et al. *The INFIR Cohort Study: investigating prediction, detection and pathogenesis of neuropathy and reactions in leprosy. Methods and baseline results of a cohort of multibacillary leprosy patients in north India*. Lepr Rev 2005; 76(1): 14-34.
5. Smith WC, Nicholls PG, Das L, et al. *Predicting neuropathy and reactions in leprosy at diagnosis and before incident events-results from the INFIR cohort study.* PLoS Negl Trop Dis 2009; 3(8): e500.
6. World Health Organization. *Enhance Global Strategy for Further Reducing the Disease Burden Due to Leprosy*. New Delhi: WHO, 2009.
7. Pearson JMH, Ross WF. *Nerve involvement in leprosy. Pathology, differential diagnosis and principles of management.* Leprosy Review 1975; 46(3): 199-212.
8. Ziegler D, Mayer P, Wiefels K, Gries FA. *Assessment of small and large fiber function in long-term type 1 (insulin-dependent) diabetic patients with and without painful neuropathy*. Pain 1988; 34(1): 1-10.
9. Konietzny F, Hensel H. *The dynamic response of warm units in human skin nerves.* Pflugers Arch 1977; 370(1): 111-4.
10. Hensel H, Andres KH, von During M. *Structure and function of cold receptors.* Pflugers Arch 1974; 352(1): 1-10.
11. Schreuders T, Kuipers M. *Thermal sensibility tester. Can it be used to find early nerve damage in leprosy?* Leprosy review 1992; 63(3): 294.
12. Srinivasan H, Stumpe B. *Leprosy diagnosis: A device for testing the thermal sensibility of skin lesions in the field*. Bulletin of the World Health Organization 1989; 67(6): 635-41.
13. Wagenaar I, Brandsma W, Post E, et al. *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols.* BMC Neurology 2012; (1471-2377 (Electronic)).

14. O'Brien MD. *Aids to the examination of the peripheral nervous system*: Elsevier Health Sciences; 2010.

15. van Brakel WH, Nicholls PG, Das L, et al. *The INFIR Cohort Study: assessment of sensory and motor neuropathy in leprosy at baseline*. Lepr Rev 2005; 76(4): 277-95.

16. Jardim MR, Vital R, Hacker MA, et al. *Leprosy neuropathy evaluated by NCS is independent of the patient's infectious state*. Clinical Neurology and Neurosurgery 2015; 131: 5-10.

17. Ziegler D, Siekierka-Kleiser E, Meyer B, Schweers M. *Validation of a novel screening device (NeuroQuick) for quantitative assessment of small nerve fiber dysfunction as an early feature of diabetic polyneuropathy.* Diabetes Care 2005; 28(5): 1169-74.

18. NeuroMetrix. *NC-stat DPNCheck User Manua*l. 2013. http://www.dpncheck.com/resources/Resources/nc-stat_dpncheck_user_manual_pn2203282_rev_g.pdf (accessed 24-02 2016).

19. Horsfield I, Levy A. *VibraTip® durability in clinical practice: How long does it last?* British Journal of Diabetes and Vascular Disease 2013; 13(2): 91-5.

20. van Brakel WH, Nicholls PG, Wilder-Smith EP, Das L, Barkataki P, Lockwood DNJ. *Early diagnosis of neuropathy in leprosy - Comparing diagnostic tests in a large prospective study (the INFIR Cohort Study)*. PLoS Neglected Tropical Diseases 2008; 2(4).

21. Brandsma JW, Post EP, Wagenaar IM. *Prednisolone treatment of subclinical neuropathy in leprosy patients* (unpublished, in preparation). 2016.

22. Smith WCS, Anderson AM, Withington SG, et al. *Steroid prophylaxis for prevention of nerve function impairment in leprosy: Randomised placebo controlled trial (TRIPOD 1)*. British Medical Journal 2004; 328(7454): 1459-62.

23. McKnight CJ. *Clinical testing and prognostic markers for the development of leprosy neuropathy:* London School of Hygiene & Tropical Medicine; 2010.

24. Papanas N, Giassakis G, Papatheodorou K, et al. *Sensitivity and specificity of a new indicator test (Neuropad) for the diagnosis of peripheral neuropathy in type 2 diabetes patients: a comparison with clinical examination and nerve conduction study*. Journal of Diabetes and its Complications 2007; 21(6): 353-8.

25. Bowling FL, Abbott CA, Harris WE, Atanasov S, Malik RA, Boulton AJM. *A pocket-sized disposable device for testing the integrity of sensation in the outpatient setting*. Diabetic Medicine 2012; 29(12): 1550-2.

26. Bracewell N, Game F, Jeffcoate W, Scammell BE. *Clinical evaluation of a new device in the assessment of peripheral sensory neuropathy in diabetes*. Diabetic Medicine 2012; 29(12): 1553-5.

27. Poulose S, Cheriyan E, Poulose A, Cheriyan R, Vadakkanezath B, Ziemer P. *Usefulness of the NC-stat DPNCheck nerve conduction test in a community pharmacy as an*

educational tool for patients with diabetes. Canadian Pharmacists Journal 2015; 148(1): 17-20.

28. Lee JA, Halpern EM, Lovblom LE, Yeung E, Bril V, Perkins BA. *Reliability and validity of a point-of-care sural nerve conduction device for identification of diabetic neuropathy*. PLoS ONE 2014; 9(1).
29. Tsapas A, Liakos A, Paschos P, et al. *A simple plaster for screening for diabetic neuropathy: A diagnostic test accuracy systematic review and meta-analysis*. Metabolism: Clinical and Experimental 2014; 63(4): 584-92.
30. Bansal V, Kalita J, Misra UK. *Diabetic neuropathy*. Postgraduate Medical Journal 2006; 82(964): 95-100.
31. Ziegler D, Mayer P, Gries FA. *Evaluation of thermal, pain, and vibration sensation thresholds in newly diagnosed Type 1 diabetic patients*. Journal of Neurology Neurosurgery and Psychiatry 1988; 51(11): 1420-4.
32. Valenstein PN. *Evaluating diagnostic tests with imperfect standards*. Am J Clin Pathol 1990; 93(2): 252-8.
33. Shetty VP, Mehta LN, Antia NH, Irani PF. *Teased fibre study of early nerve lesions in leprosy and in contacts, with electrophysiological correlates*. Journal of neurology, neurosurgery, and psychiatry 1977; 40(7): 708-11.
34. Ponirakis G, Odriozola MN, Odriozola S, et al. *NerveCheck: An inexpensive quantitative sensory testing device for patients with diabetic neuropathy.* Diabetes Research and Clinical Practice 2016; 113: 101-7.
35. Ng KWP, Ong JJ, Nyein TDN, et al. *EMLA-induced skin wrinkling for the detection of diabetic neuropathy*. Frontiers in Neurology 2013; 4 SEP).
36. Teoh HL, Chow A, Wilder-Smith EP. *Skin wrinkling for diagnosing small fibre neuropathy: Comparison with epidermal nerve density and sympathetic skin response*. Journal of Neurology, Neurosurgery and Psychiatry 2008; 79(7): 835-7.

Appendix

Table A- Results literature search and selection

Included tests	Modality	Reason selected
NeuroQuick	Cold sensation	Portable, battery operated
Neuropad	Sweat function	Easy, direct results, no training
NC-Stat DPNCheck	Nerve conduction	Portable, battery operated
Vibratip	Vibration	Portable, battery operated
Thermal Sensibility Tester	Warm sensation	Portable, battery operated
Excluded tests	**Modality**	**Reason not selected**
NerveCheck	Warm, cold, pain and vibration sensation	Portable, battery operated, not available at the moment of this study
Tiptherm	Cold sensation	Weakest performance compared with other tests 17
Neurometer	Nerve conduction	Expensive
Biothesiometer	Vibration	Expensive
Neurotip	Touch sensation	Low expected sensitivity, comparable to MFT
Neuropen	Pain sensation	Pain sensation loss is at a later stage than touch sensation loss 37,38
Sudoscan	Sweat function	Expensive
EzScan	Sweat function	Expensive
Bumps	Touch sensation	Low sensitivity, comparable to MFT
LDI-flare	Axon reflex–induced flare after heating skin (Doppler Imager)	Not portable, power needed
NervePace	Nerve conduction	Assesses motor latencies, while sensory conduction is more often affected in leprosy neuropathy
Neurosentinel	Nerve conduction	Similar to DPNCheck, only assessed Median nerve, which is less often affected in leprosy neuropathy
Thermotropic liquid Chrystal strip assessment	Skin temperature	Works when only one body side is affected (need to be compared to normal side)

Chapter 6

Effectiveness of 32 versus 20 weeks of prednisolone in leprosy patients with recent nerve function impairment: A randomized controlled trial

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Abstract

Background

While prednisolone is commonly used to treat recent nerve function impairment (NFI) in leprosy patients, the optimal treatment duration has not yet been established. We evaluated whether a 32-week prednisolone course is more effective than a 20-week course in restoring and improving nerve function.

Methods

In this multi-centre, triple-blind, randomized controlled trial, leprosy patients who had recently developed clinical NFI (<6 months) were allocated to a prednisolone treatment regimen of either 20 weeks or 32 weeks. Prednisolone was started at either 45 or 60 mg/day, depending on the patient's body weight, and was then tapered. Throughout follow up, motor NFI was assessed by voluntary muscle testing and sensory NFI by monofilament testing. The primary outcome was the proportion of patients with improved or restored nerve function at week 78, tested with a Chi-Square test in an intention-to-treat analysis. As secondary outcomes, we analysed improvements between baseline and week 78 on the Reaction Severity Scale, the SALSA Scale and the Participation Scale. Serious Adverse Events and the need for additional prednisolone treatment were monitored and reported.

Results

We included 868 patients in the study, 429 in the 20-week arm and 439 in the 32-week arm. At 78 weeks, the proportion of patients with improved or restored nerve function did not differ significantly between the groups: 78.1% in the 20-week arm and 77.5% in the 32-week arm ($p=0.821$). Nor were there any differences in secondary outcomes, except for a significant higher proportion of Serious Adverse Events in the longer treatment arm.

Conclusion

In our study, a 20-week course of prednisolone was as effective as a 32-week course in improving and restoring recent clinical NFI in leprosy patients. Twenty weeks is therefore the preferred treatment duration.

Introduction

Leprosy is an infectious disease caused by *Mycobacterium leprae*. Since the introduction of antibiotic multidrug treatment (MDT) in the 1980's, the number of leprosy diagnoses has decreased dramatically and the disease was even declared eliminated[a] at a global level in the year 2000. Nevertheless, in 2014 a total of 213 000 new leprosy patients were diagnosed worldwide [1].

The main complication of leprosy is neuropathy, which often causes sensory and motor nerve function impairment (NFI). Untreated NFI can result in deformities of the hands and feet, and may also affect the eyes. NFI can develop before MDT has started, but it can also arise during MDT and even several years after leprosy treatment has been completed [2,3]. The risk of developing new NFI within two years of starting MDT can be as high as 65% [4].

To prevent disabilities and deformities in leprosy patients, it is very important to detect and treat neuropathy early. Neuropathy is commonly treated with prednisolone [5]. Although ideally prednisolone therapy is adjusted to individual needs and response, this is not always feasible in field clinics, which often lack the treatment expertise of referral centres [6]. In these situations, the WHO recommends a standardized prednisolone treatment for 12 weeks [7].

Even though observational studies suggest that prednisolone can improve nerve function in 60-70% of nerves [2, 8-10], this effect has never been established in randomized controlled trials [11]. There are indications, however, that a longer treatment duration may be more effective than the WHO standardized treatment. In an RCT in India, type 1 reaction (T1R) patients on a 20-week course required less additional prednisolone than patients on a 12-week course [12]. Further research is needed to establish the optimal prednisolone regimen specifically for leprosy patients with NFI.

For this reason, we set up the study entitled "Treatment of Early Neuropathy in Leprosy" (TENLEP), aimed at determining whether a longer period of prednisolone treatment gives better results in the prevention of permanent NFI. In the Clinical trial, we evaluated whether a 32-week prednisolone course is more effective than a 20-week course in restoring and improving recent clinical neuropathy (<6 months) [13].

[a] Elimination is defined as a prevalence of less than 1 case per 10 000 inhabitants

Methods

The TENLEP study was a multicentre, triple blind parallel-group clinical trial, conducted in six referral centres in India, Nepal, Bangladesh and Indonesia. A detailed description of the TENLEP study can be found in the study protocol paper [13].

Patients

Leprosy patients between 15 and 60 years of age with any recent peripheral NFI - onset less than 6 months ago - were eligible for the trial. NFI was established with voluntary muscle testing (VMT) and/or monofilament testing (MFT). Patients were excluded if they were pregnant, already receiving prednisolone treatment, suffered from other conditions that may affect the peripheral nervous system, or presented with a single skin lesion on the trunk as the only sign of leprosy. The sample size was calculated to be able to detect 'restored or improved' nerve function in 70% of the intervention group, compared to an assumed proportion of 60% in the control group. This one-tailed hypothesis, using 80% power, 5% significance and allowing for 20% loss to follow-up, lead to a sample size of 720 patients. During the trial period, however, the loss to follow-up turned out to be higher than 20%. We therefore extended the inclusion period to reach the calculated sample size.

Treatment

Prednisolone dose started at 45 mg/day for patients with low weight (≤50 kg) and at 60 mg/day for patients with high weight (>50 kg). The prednisolone dose was then slowly tapered during the treatment period [13], maintaining a plateau of 0.4-0.5mg/kg for 20 weeks in the 32-week arm. To check the chemical composition of the prednisolone and placebo tablets, a random selection of packages from both treatment groups was evaluated at the start of the trial by the manufacturer (Rubicon), and an independent Indian laboratory (Medibios Laboratories). After 20 months the composition was checked again by the Royal Dutch Society of Pharmacists (KNMP). Treatment adherence was checked every month either verbally or by checking the medication package of the previous month.

Randomization and blinding

Patients were randomly allocated to either 20 or 32 weeks of oral prednisolone, using a separate computer-generated blocked randomization sequence for each centre and weight group. This sequence was prepared by the project's statistician (PN), and the manufacturer labelled the trial drug packages accordingly. Every new patient received the next available numbered package. Patients and research staff were kept unaware of the assigned treatment group. Additionally, the statistician was kept blinded until all data analyses were performed. The key to treatment allocation was only broken if a patient had serious adverse events (SAE) or required individualized treatment for a reaction or worsening NFI.

Assessments

In each centre, monofilament testing and voluntary muscle testing were carried out by two trained assessors, except in the Indonesian centre where ten assessors have performed the tests. Reliability studies at baseline generally showed good inter-tester reliability [14]. For every patient the sensory function of six nerves and the motor function of seven nerves were assessed on both left and right body side, adding up to a total of 26 assessments. Sensory function was tested on three test-sites for each nerve, using a standard set of Semmes-Weinstein monofilaments, with the 200 mg filament representing the normal threshold for the hand, and the 2g filament for the foot. For motor function assessment, the 0-5 Medical Research Council (MRC) scale was used [15]. The exact test methods and -sites are described in the protocol paper [13]. When the total monofilament score for a nerve was 3 or more, the sensory nerve function was considered impaired. A motor nerve scoring less than 5 on the Medical Research Council scale was also regarded as impaired. Follow-up assessments for VMT and MFT were carried out monthly during the treatment period (up to week 32), and at week 52 and 78. At baseline and the end of the study a Screening of Activity Limitation and Safety Awareness (SALSA) scale [16] and a Participation (P) scale [17] were completed for each patient. In addition, reaction severity was monitored with a Reaction Severity Scale (RSS) [18] at baseline, week 32, 52 and 78. When a patient did not show up for their follow-up appointment, a telephone call or in some cases a home visit was made in an effort to get the patient visit the clinic for assessments. When indicated in advance, a patient was allowed to miss one assessment, and the medication was provided for eight weeks.
When a patient's nerve function had deteriorated during the trial treatment period -the first 32 weeks-, the trial treatment was stopped and the patient received a tailored prednisolone treatment. Patients who developed new NFI or who experienced NFI worsening after the 32-week treatment period were also given additional prednisolone, as indicated by a medical doctor. When determining whether this additional treatment was needed, the definition of deteriorated MFT used was: an increase of 6 points or more on the score per nerve since the last assessment, or an increase of 3 points or more on the score per nerve that was confirmed on the consecutive assessment. VMT deterioration was defined as: a reduction in VMT score by two or more points or a reduction of 1 point on two consecutive assessments. Patients who developed serious adverse events (SAE) were taken off trial treatment as well, and their complications were treated. Patients who received additional prednisolone or developed SAE were still followed up in the trial, and data were collected at 78 weeks.

Primary and secondary outcomes

The primary study outcome was the proportion of patients with restored or improved nerve function (of all nerves) measured by MFT and/or VMT at 78 weeks. We established for each nerve whether it had been restored (normal function), improved (but not restored), unchanged or deteriorated. For the primary outcome, the MFT score was considered improved when the total score per nerve had decreased with at least three points. Deterioration was defined as an increase of at least three points in MFT score. For VMT the score was considered improved or

deteriorated when increased or decreased by one point on the MRC scale. Since the primary outcome was at patient level, we calculated a composite score by subtracting the number of deteriorated nerves from the number of restored and improved nerves. A patient's nerve function was considered completely restored when all nerves had returned back to normal function. Improved nerve function was defined as a positive composite score, i.e. more restored and improved nerves than deteriorated and unchanged nerves.
For the secondary outcomes we looked at six variables. First, we compared the proportion of restored, improved, unchanged, deteriorated and fully impaired patients between the groups at week 78 for each specific nerve -e.g. ulnar. This was done for all 13 nerves. Second, a count of impairments was calculated per patient by giving each impaired nerve a score of 1, and adding the scores up for all nerves, leading to a maximum possible score of 26. The scores were compared between the intervention and control groups, and the change over time was assessed. Third, change in reaction severity was monitored with the RSS. The patient was considered improved when the score reduced with at least 3 points on the sum score or at least 2 points on any individual item of the scale. Fourth, the improvement in SALSA-scale and P-scale scores between baseline and week 78 was evaluated. For the SALSA-scale and P-scale, a patient was considered improved when classified in a better category, e.g. extreme, severe, moderate, and mild. Fifth, we compared the difference in proportion of patients with Serious Adverse Events (SAE) between the intervention and control group. Last, we looked at the proportion of patients needing additional prednisolone and analysed whether there was a difference in timing, dose and duration of the provided additional prednisolone treatment.

Data analysis

Data were entered at each centre in an Access Database and then combined to be analysed in Stata. Data were analysed according to the modified intention-to-treat principle: data of all randomized patients who matched the inclusion criteria were analysed for week 78, whether they had finished treatment or not, including all patients lost to follow-up. To handle missing data of patients lost to follow-up, the last observation carried forward method was used. For patients who received additional prednisolone, the assessment recorded at the time when additional was first prescribed was carried forward. Only nerves with new impairments (<6 months) were included in the analyses. The primary and secondary outcomes were analysed using a Chi-Square test. A difference between treatment groups was considered significant when the p-value was < 0.05.

Ethics

Written informed consent was taken from all patients. Ethical permission was obtained in-country. In India from the Indian Council of Medical Research and the Ethics Committee of the Foundation for Medical Research, Mumbai; in Nepal from the Nepal Health Research Council (NHRC); in Indonesia from the Komite Etik Penelitian Kesehatan RSUD Dr. Soetomo Surabaya; in Bangladesh from the Bangladesh Medical Research Council- National Research Ethics

Committee. The trial was registered in the Netherlands Trial Register (NTR2300) and in the Clinical Trials registry India (CTRI/2011/09/002022 and 23).

Results

Participants

A total of 875 leprosy patients were enrolled in the trial, of whom 432 were randomized to the 20-week arm and 443 to the 32-week. Patients were recruited between February 2012 and October 2013, and the last follow-up data were collected in July 2015. The flow diagram in Figure 1 illustrates the number of patients followed up and the reasons for drop out. Seven of the randomized patients were excluded in the final analyses: one patient who had missing baseline MFT assessments and six patients who did not meet the inclusion criteria of having recent NFI. The number included in the analyses reported here was therefore 868.

Baseline data

Baseline demographic and clinical characteristics of the patients are shown in Table 1. Differences in demographic and clinical characteristics did not reach statistical significance between the groups. The most important characteristics are also presented per centre in Table 2. At baseline, sensory function was more often impaired than motor function: for both groups the median number of nerves with impaired sensory function was 3, ranging from 0-12, while the median number of nerves with impaired motor function was 1 (0-13). The proportions of sensory and motor impairment per nerve are shown in Figure 2.

Number analysed

Of the 868 patients enrolled in the study, the trial treatment period of 32 weeks was completed by 281 (65.5%) in the 20-week arm and 293 (66.7%) in the 32-week arm. Complete follow-up data until week 78 were collected for 230 (82%) of the patients of the 20-week arm and 219 (75%) patients of the 32-week arm. At week 78, follow-up data were also collected for 233 additional patients who did not complete trial treatment due to new NFI, new or recurrent reactions, SAEs or patients who were temporarily lost to follow-up. For the intention-to-treat analyses, the primary and secondary outcomes were analysed using the data of all patients who met the inclusion criteria (n=868). A separate per protocol analysis was carried out including only patients who had completed treatment (n=574).

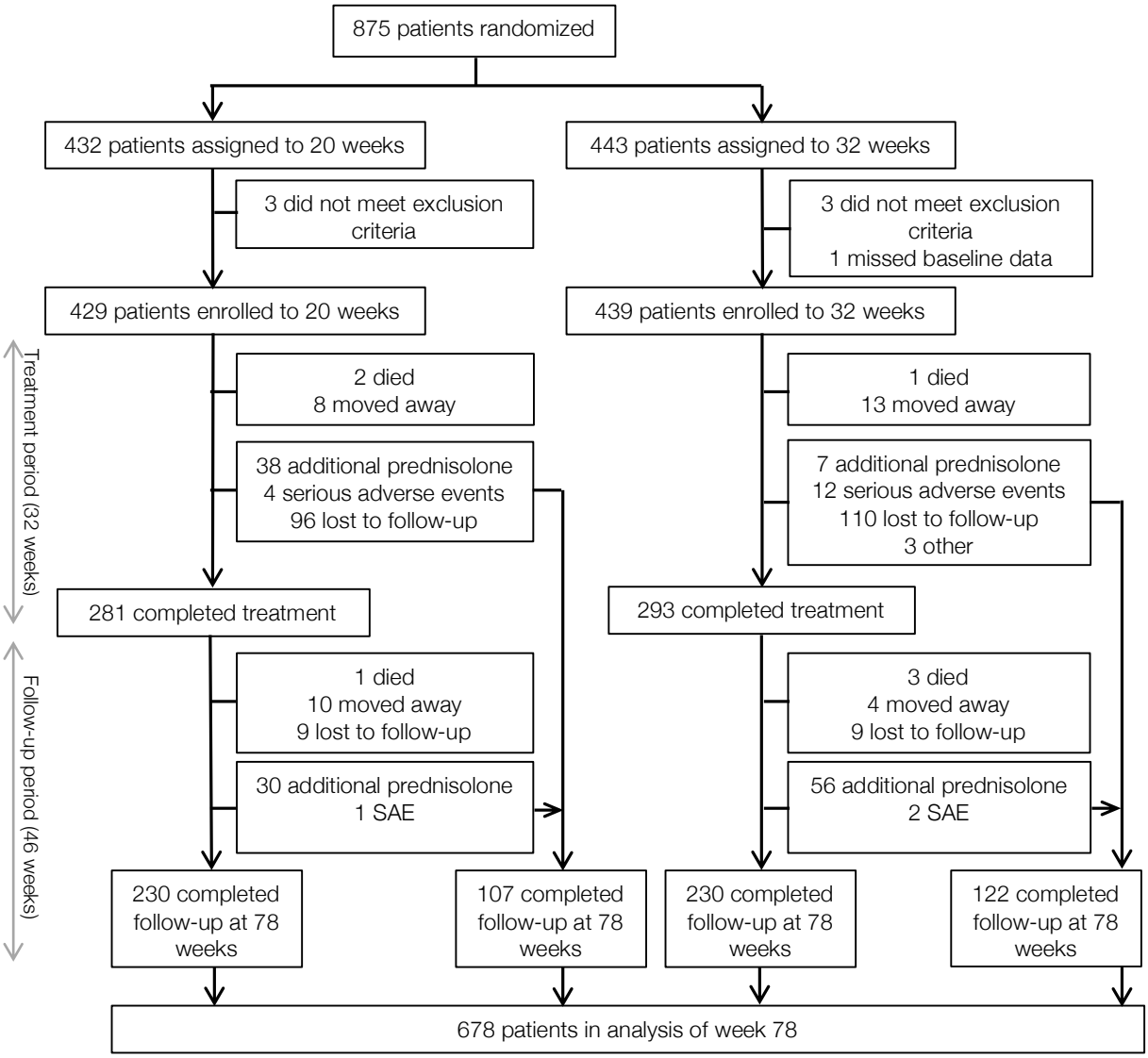

Figure 1- Flow diagram of number of patients enrolled, randomized, treated and followed-up

Table 1- Baseline demographics and clinical characteristics of the 868 patients

	20 weeks (n= 429)		32 weeks (n= 439)	
Gender (female)	101	(23.4%)	90	(20.4%)
Age (mean ±SD)	34.7	(12.1)	34.3	(12.1)
Literate (%)	241	(56.5%)	247	(56.3%)
MB/PB (% MB)	339	(79.2%)	357	(81.0%)
RJ classification				
TT	9	(2.1%)	20	(4.6%)
BT	212	(49.4%)	203	(46.2%)
BB	61	(14.2%)	55	(12.5%)
BL	67	(15.6%)	70	(16.0%)
LL	40	(9.3%)	51	(11.6%)
PN	40	(9.3%)	40	(9.1%)
Average smear (mean ±SD)	0.8	(1.4)	0.9	(1.5)
Disability grade				
0	171	(39.9%)	165	(37.6%)
1	158	(36.8%)	176	(40.1%)
2	100	(23.3%)	98	(22.3%)
T1R				
Mild	66	(15.4%)	59	(13.4%)
Severe	14	(3.3%)	24	(5.5%)
T2R				
Mild	11	(2.6%)	14	(3.2%)
Severe	2	(0.5%)	6	(1.4%)

MB: Multibacillary; PB: Paucibacillary; RJ: Ridley-Jopling; T1R: Type 1 Reaction; T2R: Type 2 Reaction

Table 2- Intake per research centre

Centre	Number of patients	Gender (% female)	Mean age	Low weight group (%)	20-week arm (%)	Area
Anandaban hospital *Nepal*	57	26%	36.7	37%	47%	Kathmandu
Foundation for Medical Research *India*	82	15%	31.8	34%	49%	Mumbai
JALMA *India*	230	17%	31.5	53%	50%	Agra and surroundings
Lalgadh hospital *Nepal*	166	28%	34.3	45%	50%	Rural south-east Nepal
Nilphamari *Bangladesh*	232	22%	39.1	51%	50%	Rural north-west Bangladesh
Dr. Soetomo hospital *Indonesia*	101	25%	31.9	46%	48%	Surabaya and Madura

Primary outcome

An overview of the primary and secondary outcomes and their definitions are presented in Table 3. The proportion of patients with restored or improved nerve function at week 78 was similar in both groups: 78.1% in the 20-week arm and 77.5% in the 32-week arm (p=0.821). At week 32 and week 52, this outcome was not significantly different between the two groups either. Figure 3 shows the proportion of patients in each category of improvement. In the 20-week arm, more patients showed completely restored nerve function than in the 32-week arm, 23.5% against 18.7%. The per-protocol analysis, leaving out treatment non-compliers, resulted in an overall slightly higher proportion of patients with improved and restored nerves. However, again no significant difference was found between the groups: 81.9% in the 20-week arm and 81.7% in the 32-week arm (p=0.960). The percentage of patients with full restored nerve function in this specific group is higher in the 20-week arm than in the 32-week arm, 26.4% against 20.5%.

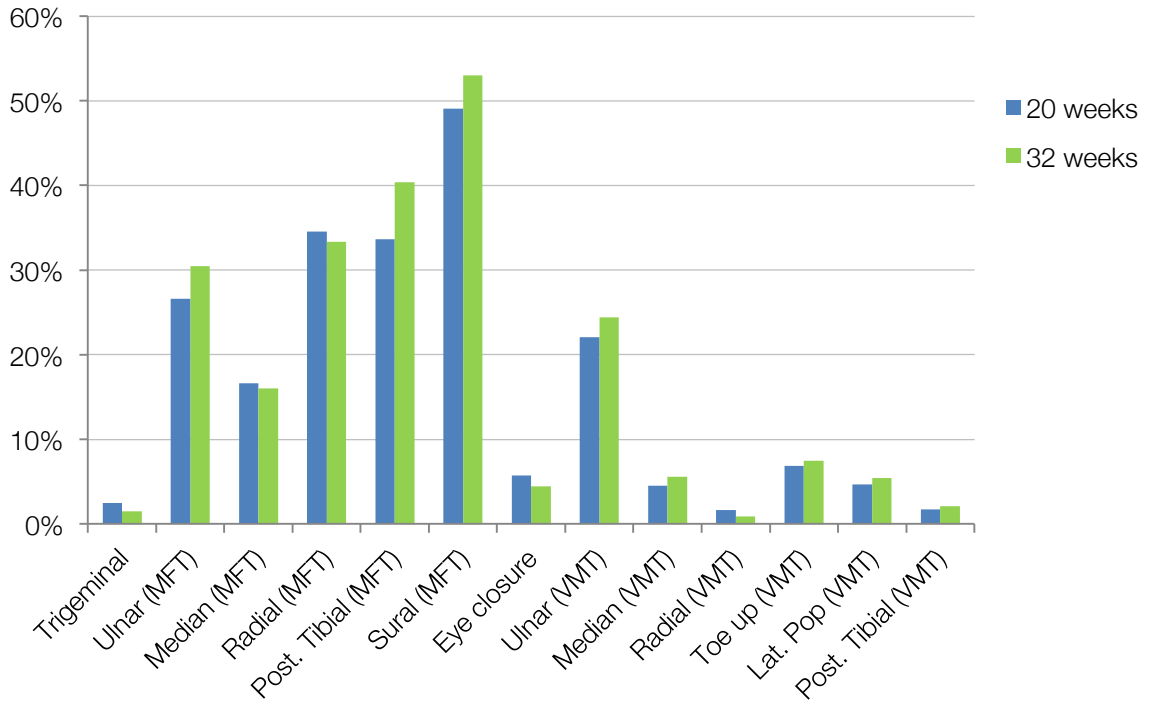

Figure 2- Baseline impairments for sensory and motor nerves

Secondary outcomes

Table 4 presents the outcomes per nerve, for six sensory and seven motor nerves. The proportion of improved and restored motor nerves was overall higher than for sensory nerves. The motor function of median, lateral popliteal, radial and trigeminal nerves improved most between baseline and week 78. Although the last three nerves were involved in only small number of patients. The sensory function of the radial, median and ulnar nerves had the highest proportion of improvement and restoration. The average count of impairments at baseline, week 32, week 52 and week 78 is shown in Table 5. 75.7% of the patients in the 20-week arm showed an improvement in count of impairment, compared to 70.8% in the 32-week arm (p=0.149). Table 5 also presents the results of the Reaction Severity scale, the SALSA scale and the P-scale for every assessment point. The proportion of patients with improved in Reaction Severity Scale score at week 78 was higher in the 32-week arm (70%) than in the 20-week arm (65%), but this difference was not statistically significant (p=0.09). After 78 weeks, also the day-to-day situation for patients improved: the scores of both SALSA and P-scale improved between baseline and week 78, but did not differ between the treatment groups at either point in time. The absolute change over time also did not differ significantly (p=0.638 for SALSA and p=0.543 for P-scale).

Table 3- Overview of primary and secondary outcomes

	Outcome	Definitions
Primary outcome	Proportion of patients with restored or improved nerve function	Using a composite score. Restored: all nerves back to normal function Improved: more restored and/or improved nerves than unchanged and/or deteriorated nerves Unchanged: same number of nerves impaired Deteriorated: more deteriorated nerves than improved and/or restored nerves
Secondary outcomes	Proportion of restored, improved, unchanged, deteriorated and fully impaired nerves for each specific nerve – e.g. ulnar nerve	For six sensory nerves and seven motor nerves
	Count of impairments	Per patient 26 nerves were assessed. Each impaired nerve adds 1 point to the total score
	Improvement Reaction Severity Scale	Improvement: reduction in RSS of 3 or more points on the total score, or a reduction of 2 or more points on any individual item of the scale
	Improvement in SALSA-scale and P-scale	Improvement: classified in a better category (extreme, severe, moderate, mild, none)
	Proportion of patients with Serious Adverse Events	
	Proportion of patients needing additional steroids for treatment of reaction or worsening NFI	

Additional prednisolone, to treat new or deteriorating NFI and reactions, was required in 68 (16%) patients in the 20-week arm and 65 (15%) patients in the 32-week arm. This difference was not statistically significant. Interestingly, the majority (38/68) of these patients in the 20-week arm needed additional prednisolone between week 21 and week 32, while in the 32-week arm additional prednisolone was given mostly between week 33 and 52 (56/65). The additional prednisolone given before week 32 was in 78% of the cases because of a reaction- with or without accompanying worsening NFI-, while the additional prednisolone given after week 32 was more often for deteriorating NFI without reaction (55% of the cases needing additional prednisone did not have other reaction symptoms). Figure 4 shows the survival time until the first course of additional prednisolone. The dose and duration of additional prednisolone treatment was not significantly different between the two groups.

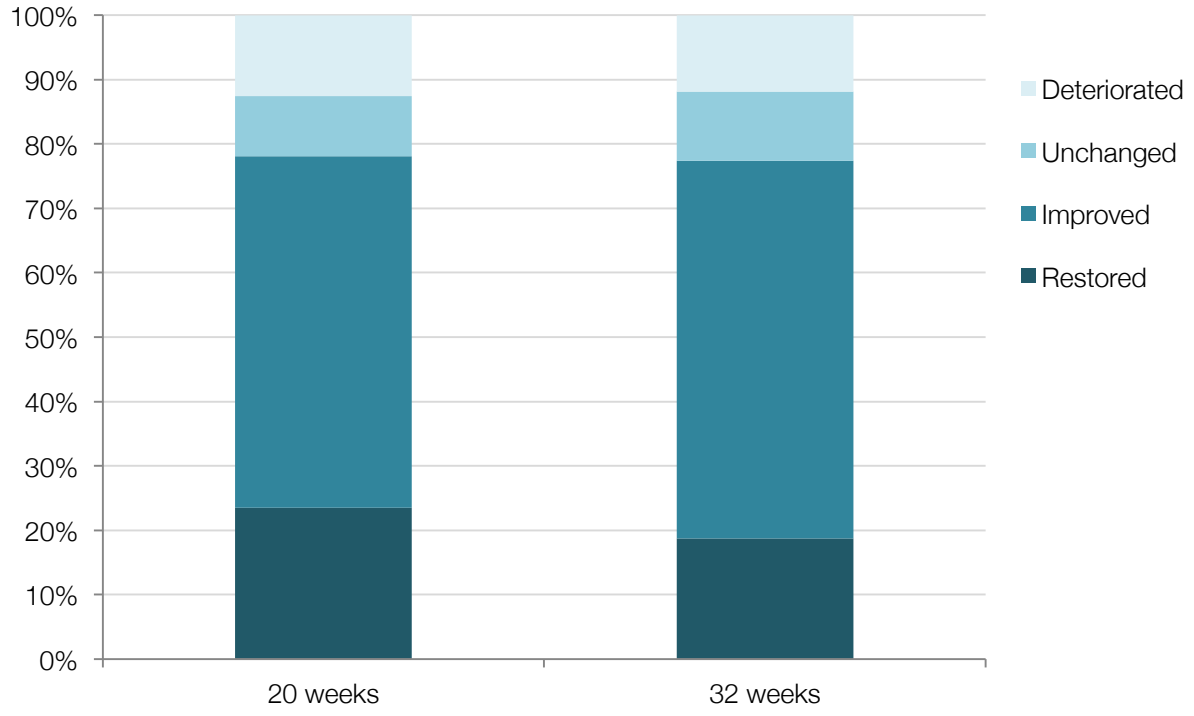

Figure 3- Percentage of patients with restored, improved, unchanged and deteriorated nerve function impairment at week 78 as primary outcome

Adverse events

There were seven deaths, 3 in the 20-week arm and 4 in the 32-week arm, but none was related to the prednisolone treatment. Serious adverse events occurred in both groups, but were more often reported in the intervention group: 12 cases (2.7%) in the 32-week arm and 4 cases (0.9%) in the 20-week arm [19]. This difference was statistically significant (p=0.04). The main reported SAE's were hypertension (6), diabetes (6), and peptic ulcer (2). Minor side effects were reported in 66% in the intervention group and 68% in the control group. Detailed results will be reported separately.

Additional analyses

We used logistic regression to assess the association between clinical and demographic data recorded at baseline and the primary outcome of improved or restored nerve function. The characteristics that showed a significant correlation with the primary outcome were weight group and BMI, gender, WHO disability grade and Eye Hand Foot (EHF) score. A patient with a higher BMI had higher odds on improved or restored nerve function at 78 weeks (OR= 1.09 (1.04-1.14)). The high weight group (≥50 kg) had a 1.43 (1.09-1.86) higher probability of a positive primary outcome than the low weight group. Furthermore, women were 1.46 (1.06-2.10) times more likely to have a restored or improved nerve function than men. The subgroup analysis also showed that patients having a grade 1 or 2 impairment at baseline were less likely

Table 4- Outcomes per nerve at week 78

	Arm		**Restored**	**Improved**	**Unchanged**	**Deteriorated**
Sensory		n	%	%	%	%
Trigeminal	20 weeks	22	59.1	-	36.4	4.5
	32 weeks	16	75.0	-	6.3	18.8
Ulnar	20 weeks	330	43.0	23.3	13.6	20.0
	32 weeks	380	39.7	26.6	12.6	21.1
Median	20 weeks	260	46.5	18.5	13.1	21.9
	32 weeks	283	37.1	26.5	12.0	24.4
Radial	20 weeks	218	46.3	26.6	11.0	16.1
	32 weeks	224	42.9	23.2	12.1	21.9
Posterior tibial*	20 weeks	394	35.0	22.6	20.3	22.1
	32 weeks	448	31.0	30.4	20.8	17.9
Sural*	20 weeks	459	25.3	30.1	24.2	20.5
	32 weeks	520	25.6	28.1	33.3	13.1
Motor		n	%	%	%	%
Eye closure	20 weeks	83	50.6	18.1	21.7	9.6
	32 weeks	71	59.2	18.3	12.7	9.9
Ulnar	20 weeks	322	39.1	18.3	26.7	15.8
	32 weeks	334	33.5	21.0	31.7	13.8
Median	20 weeks	132	52.3	3.8	17.4	26.5
	32 weeks	134	53.7	8.2	20.9	17.2
Radial	20 weeks	37	70.3	2.7	5.4	21.6
	32 weeks	40	62.5	2.5	12.5	22.5
Lateral	20 weeks	81	61.7	11.1	17.3	9.9
	32 weeks	89	57.3	7.9	22.5	12.3
Posterior tibial up	20 weeks	172	52.3	10.5	22.1	15.1
	32 weeks	162	48.1	9.3	30.2	12.3
Posterior tibial down	20 weeks	188	51.1	1.6	25.5	21.8
	32 weeks	202	55.0	1.5	29.2	14.4

*Difference between arms is statistically significant

Table 5-Secondary outcomes: count of impairment, severity scale, salsa scale, p-scale

	n	20 weeks	n	32 weeks	Test statistic
Count of impairments					
Baseline sensory	429	3.55 (2.98)	439	3.74 (2.91)	NS
Baseline motor	429	2.11 (2.40)	439	2.16 (2.29)	NS
Total count	429	5.66 (4.48)	439	5.90 (4.27)	NS
Week 78 sensory	338	2.33 (2.80)	343	2.63 (3.03)	NS
Week 78 motor	338	1.15 (1.88)	343	1.35 (1.90)	$p<0.05$
Total count	338	3.48 (4.22)	343	3.98 (4.38)	NS
Difference baseline-week 78	338	2.11 (3.73)	343	1.68 (3.34)	NS
Reaction Severity Scale					
Baseline	426	7.26 (9.15)	438	7.22 (6.79)	NS
Week 32	425	4.21 (6.09)	437	4.16 (5.66)	NS
Week 52	427	3.93 (5.81)	437	4.59 (6.18)	NS
Week 78	422	3.97 (5.96)	432	4.50 (6.16)	NS
Difference baseline-week 78	420	3.35 (7.47)	432	2.75 (4.62)	NS
SALSA scale					
Baseline	402	27.77 (10.26)	412	27.08 (9.03)	NS
Week 78	269	23.49 (7.93)	272	23.92 (8.41)	NS
Difference baseline-week 78	269	1.62 (8.96)	272	1.37 (8.74)	NS
P-Scale					
Baseline	402	13.02 (12.79)	412	11.57 (11.74)	NS
Week 78	269	9.41 (9.87)	273	9.43 (10.21)	NS
Difference baseline-week 78	269	0.04 (15.42)	273	-1.38 (13.27)	NS

(Mean (SD)); NS: not significant

to have restored or improved nerve function at week 78 (OR Grade 1: 0.73 (0.54-0.98) and OR Grade 2: 0.19 (0.12-0.28)). A higher EHF score also reduced the odds of a good outcome (OR: 0.73 (0.67-0.80). Neither WHO nor Ridley-Jopling classification had influence on improvement of the nerve function, nor did skin smear, nor the presence or absence of severe Type 1 or Type 2 reaction at baseline.

Discussion

Our study demonstrated that a 32-week course of prednisolone is not more effective than a 20-week course in restoring and improving recent clinical nerve function impairment in leprosy patients. This result is not in accordance with our hypothesis that a longer prednisolone regimen would be more beneficial. This was based on the results of Rao et al., who found that T1R patients on a 20-week prednisolone course required significantly less additional prednisolone than patients receiving a 12-week course. The theory underlying this hypothesis was that a longer prednisolone treatment suppresses the immune system long enough for the antigenic load to sufficiently decrease [20,21]. Nevertheless, in both treatment arms in our study, a large group of patients showed improvement and recovery of nerve function, indicating that 20-week prednisolone treatment is sufficient for most patients. Nerve function improved in around 78% of the patients, and 24-30% of these patients recovered completely. Unfortunately, there was also a group of patients that had a reaction or had deteriorating NFI, for which additional prednisolone was still required. Notably, the proportion of patients needing additional prednisolone was similar in both groups (15% and 16%). The total duration and dose of the additional prednisolone treatment did not differ between the groups. However, we did see a difference in timing of the need for additional prednisolone. New NFI and reactions were primarily reported in the weeks after prednisolone treatment had ended, thus after 20 weeks and 32 weeks respectively for the control and intervention arm. A similar rebound effect was observed in the TRIPOD study and in the study of Rao et al. [12,22]. The longer prednisolone treatment in our study seems to merely postpone the immune response in some patients.

The group of patients needing additional prednisolone differed at baseline from the group that did not need extra prednisolone. This former group had significantly more MB patients -mainly BL and LL-, had higher average skin smear, had a higher EHF score, had T1R and T2R more often at baseline and showed more often signs of neuritis and reactions, i.e. inflamed and raised skin lesions, peripheral oedema, nerve pain and tenderness. An even longer prednisolone course than assessed in this study might be beneficial for these patients, as it seems that the immune response against dead *M. leprae* bacilli is too persistent to be permanently suppressed by a 32-week prednisolone course [23]. New studies with even longer prednisolone treatments are needed in an attempt to find the best treatment for recent NFI in this specific group of patients.

The mechanisms behind nerve damage in leprosy are generally not well understood, because it is difficult to study. Nerve biopsies can give some insight in the pathophysiology of neuropathy, but are limited to a single point in time and do not provide information about the onset and further development of impairment [20]. Several mechanisms are described in literature though. The first mechanism is demyelination of the nerves. In cell cultures, demyelination has been shown to occur when *M. leprae* invades the Schwann cells [24-26]. This process can take place in an early stage, when the immune system has not been activated yet. After the immune system gets involved, demyelination can also be induced by cytokines [27-31]. The second mechanisms, shown in Schwann cell cultures, is the direct attack and damaging of Schwann cells by T-cells [32-34], destroying the nerves. The last mechanism of nerve damage in leprosy is mechanical. The high pressure in the nerve due to oedema, a characteristic sign of inflammation, can lead to axonal loss. The pressure can also close blood vessels and cause ischemia [20,35]. Damaged nerves can regenerate when the inflammation is under control. However, the duration of the inflammation may influence how well the nerve can regrow [36]. In addition, scar formation is often seen around the damaged nerves, hindering regeneration [35]. Prednisolone can reduce the inflammation, and therefore reduce oedema and pressure, and stop the killing of Schwann cells. In addition, prednisolone reduces scar formation [37], giving the damaged nerves time and space to regenerate.

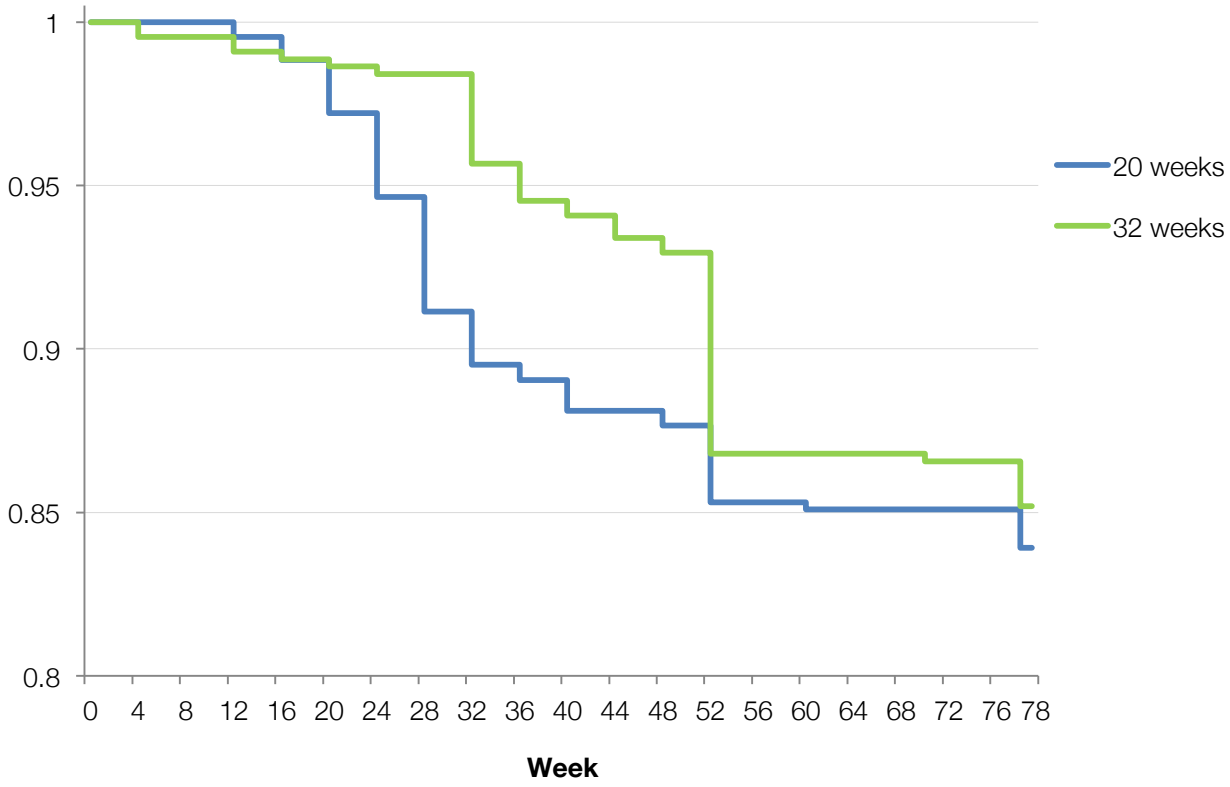

Figure 4- Survival until first course of additional prednisolone for all cases (n=868)

Observational studies have shown that prednisolone improves nerve function [2,8-10]. And even though prednisolone is generally considered an important drug for improving NFI, it is not known to what extent prednisolone is responsible for the reported improvements in these observational studies. Its effectiveness has never been confirmed in placebo controlled randomized controlled trials, such as the TRIPOD studies [22,38]. Additionally, the high proportion of patients experiencing spontaneous nerve function improvement should be considered [22,39,40]. Previous studies have found that 33%-75% of impairments improved spontaneously, depending of the severity of NFI and the type of nerve [39,41]. Even in patients with old NFI, receiving a placebo, 51% showed improvement [40]. A placebo controlled RCT could be an important next step to investigate the actual effect of prednisolone on the treatment of recent NFI (without reaction). Alternatives for prednisolone should be studied as well, however, so far no treatments have been proven effective. Azathioprine and cyclosporine are immunosuppressants used to treat reactions, but their effect is limited [42,43]. These drugs have not been studied in the treatment of NFI by itself. Nerve decompression, a surgical method to relieve the pressure on the nerves, is used to improve neuropathy in leprosy. Evidence from controlled randomized on the effectiveness of this method trials is lacking as well [44].

The strongest point of our study is the multi-centre, multi-country set up. We included patients from rural and urban areas, PB and MB patients, and patients with or without reaction symptoms. With some confidence we can therefore generalize our results. In addition, our RCT was unique in maintaining a middle plateau for a longer time with a relatively high dose of prednisolone. We are also the first to assess three points for the sural and radial cutaneous nerves for MFT. Adding the sural and radial cutaneous nerves to the Composite Nerve Score increased its reliability. One limitation in this study was the high loss to follow-up. The percentage of patients lost to follow-up exceeded the Evidence Based Medicine cut off of 20%, leading to a loss of validity [45]. At 78 weeks, we collected data of 72% of the patients in the 20-week arm and 78% of the patients in the 32-week arm; including patients who did not finish trial treatment because of SAE, the need for additional prednisolone or temporary lost-to-follow up. However, we tried to limit the effects of the high loss to follow up. First, we prolonged the intake period to increase the number of patients in the trial, so we could reach the required power. Second, we analysed our data according to the intention to treat principle, using the last observation carried forward approach. This should limit the bias related to the non-random loss of patients. The disadvantage of an intention-to-treat analyses is that patients who do not finish treatment are included as well. Their results will dilute the outcome, but this situation is similar to what would have happened in non-controlled conditions in a field clinic. Consequently, it is likely that our outcome is conservative and therefore the actual improving effect of prednisolone on NFI might be even larger. A second limitation is that the duration of NFI was self-reported. Patients were included in the study when the duration of NFI was maximum 6 months. However, one can wonder whether estimation of duration of impairments by the patients themselves is reliable. When the delay in prednisolone treatment is too long (>6 months), the damage to the nerve is considered to be irreversible. From previous studies [5,46] it seems that NFI of shorter duration has better chances on improvement. It could therefore be that in some

patients nerves do not improve because the actual duration of NFI is much longer than the self-reported duration. Unfortunately, there is no reliable method to establish the duration of NFI.

In conclusion, we hypothesized that the 32-week prednisolone treatment would be more beneficial in improving nerve function than the 20-week treatment. The results of this study show that this is not the case; the 32-week and 20-week regimens were equally effective. In absence of published RCT data on shorter duration - 16-weeks would be a good candidate- in a similar patient group, we conclude that a 20-week course is sufficient to improve nerve function in 78% of patients with recent NFI. Future studies should focus on the group of patients who do not seem to benefit from prednisolone treatment of this duration. To help better treat this group, it is important to unravel the pathophysiology of nerve function impairment and to study the actual effect of prednisolone on immune function as it relates to NFI. Understanding the mechanisms behind NFI can lead to alternative, more effective solutions for the treatment of NFI and the prevention of irreversible impairments and subsequent disabilities.

References

1. World Health Organization, *Weekly Epidemiological Record.* 2014. 89(36): p. 389-400.
2. Saunderson, P., *The pattern of leprosy-related neuropathy in the AMFES patients in Ethiopia: definitions, incidence, risk factors and outcome*. Leprosy Review, 2000. 71(3): p. 285-308.
3. Rosenberg, N.R., W.R. Faber, and M. Vermeulen, *Unexplained delayed nerve impairment in leprosy after treatment.* Leprosy Review, 2003. 74(4): p. 357-365.
4. Croft, R.P., et al., *A clinical prediction rule for nerve function impairment in leprosy patients-revisited after 5 years of follow-up*. Lepr Rev, 2003. 74(1): p. 35-41.
5. Naafs, B., Bangkok Workshop on Leprosy Research. *Treatment of reactions and nerve damage.* International journal of leprosy and other mycobacterial diseases : official organ of the International Leprosy Association, 1996. 64(4 Suppl): p. S21-28.
6. Britton, W.J., *The management of leprosy reversal reactions*. Leprosy Review, 1998. 69(3): p. 225-234.
7. World Health Organization, WHO Expert Committee on Leprosy- *Seventh report*, in World Health Organization technical report series No. 874. 1998: Geneva.
8. Britton, W.J. and D.N.J. Lockwood, *Leprosy.* Lancet, 2004. 363(9416): p. 1209-1219.
9. Croft, R.P., J.H. Richardus, and W.C.S. Smith, *Field treatment of acute nerve function impairment in leprosy using a standardized corticosteroid regimen - First year's experience with 100 patients*. Leprosy Review, 1997. 68(4): p. 316-325.
10. Schreuder, P.A.M., *The occurrence of reactions and impairments in leprosy: Experience in the leprosy control program of three provinces in northeastern Thailand, 1978-1995. III. Neural and other impairments*. International Journal of Leprosy, 1998. 66(2): p. 170-181.
11. van Veen, N.H., et al., *Corticosteroids for treating nerve damage in leprosy.* Cochrane Database Syst Rev, 2007(2): p. CD005491.
12. Sundar Rao, P.S.S., et al., *Multi-centre, double blind, randomized trial of three steroid regimens in the treatment of type-1 reactions in leprosy*. Leprosy Review, 2006. 77(1): p. 25-33.
13. Wagenaar, I., et al., *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols.* BMC Neurology, 2012(1471-2377 (Electronic)).
14. Brandsma, J.W., et al., *Reliability of clinical nerve function assessment in peripheral neuropathies.* Leprosy Review, 2014. 85(1): p. 29-35.
15. O'Brien, M.D., *Aids to the examination of the peripheral nervous system*. The Guarantors of Brain. Vol. 5th edition. 2010: Elsevier Health Sciences.

16. The SALSA Collaborative Study Group. *Screening Activity Limitation and Safety Awareness Users Manual* V1.1. 2010 [cited 2012 September 28th]; Available from: http://www.ilep.org.uk/fileadmin/uploads/Documents/Infolep_Documents/Salsa/SALSA_manual_v1.1pdf.pdf.

17. van Brakel, W.H. *Participation Scale Users Manual* v. 6.0 2010 [cited 2012 September 28th]; Available from: http://www.ilep.org.uk/fileadmin/uploads/Documents/Infolep_Documents/Participation_Scale/Participation_Scale_Users_Manual_v._6.0.pdf.

18. Walker, S.L., et al., *Development and validation of a severity scale for leprosy type 1 reactions*. PLoS Neglected Tropical Diseases, 2008. 2(12).

19. Post E., W.I., Brandsma W., Nicholls P., Richardus J.H., *Prednisolone Adverse Events in the Treatment of Leprosy Neuropathy*. In preparation, 2016.

20. Scollard, D.M., R.W. Truman, and G.J. Ebenezer, *Mechanisms of nerve injury in leprosy*. Clinics in Dermatology, 2015. 33(1): p. 46-54.

21. Lockwood, D.N.J., *Steroids in leprosy type 1 (reversal) reactions: Mechanisms of action and effectiveness*. Leprosy Review, 2000. 71(SUPPL.): p. S111-S114.

22. van Brakel, W.H., et al., *The prognostic importance of detecting mild sensory impairment in leprosy: A randomized controlled trial (TRIPOD 2)*. Leprosy Review, 2003. 74(4): p. 300-310.

23. Shetty, V.P., et al., *Persistence of Mycobacterium leprae in the peripheral nerve as compared to the skin of multidrug-treated leprosy patients*. Leprosy Review, 1992. 63(4): p. 329-336.

24. Oliveira, R.B., et al., *Expression of toll-like receptor 2 on human schwann cells: A mechanism of nerve damage in leprosy.* Infection and Immunity, 2003. 71(3): p. 1427-1433.

25. Rambukkana, A., et al., *Contact-dependent demyelination by Mycobacterium leprae in the absence of immune cells.* Science, 2002. 296(5569): p. 927-31.

26. Tapinos, N., M. Ohnishi, and A. Rambukkana, *ErbB2 receptor tyrosine kinase signaling mediates early demyelination induced by leprosy bacilli.* Nature Medicine, 2006. 12(8): p. 961-966.

27. Constantin, G., et al., *Induction of adhesion molecules on human Schwann cells by proinflammatory cytokines, an immunofluorescence study*. Journal of the Neurological Sciences, 1999. 170(2): p. 124-130.

28. Fujioka, T., D.L. Kolson, and A.M. Rostami, *Chemokines and peripheral nerve demyelination*. Journal of NeuroVirology, 1999. 5(1): p. 27-31.

29. Andrade, P.R., et al., *Inflammatory Cytokines Are Involved in Focal Demyelination in Leprosy Neuritis.* J Neuropathol Exp Neurol, 2016.

30. Shetty, V.P., *Pathomechanisms of Nerve Damage*, in IAL Textbook of Leprosy, H. Kumar and B. Kumar, Editors. 2010, Jaypee Brothers Medical Publishers (P) Ltd.: New Delhi.

31. Khanolkar-Young, S., et al., *Tumor necrosis factor-alpha (TNF-α) synthesis is associated with the skin and peripheral nerve pathology of leprosy reversal reactions.* Clinical and Experimental Immunology, 1995. 99(2): p. 196-202.

32. Lockwood, D.N. and P.R. Saunderson, *Nerve damage in leprosy: A continuing challenge to scientists, clinicians and service providers.* International Health, 2012. 4(2): p. 77-85.

33. Ford, A.L., W.J. Britton, and P.J. Armati, *Schwann cells are able to present exogenous mycobacterial hsp70 to antigen-specific T lymphocytes*. Journal of Neuroimmunology, 1993. 43(1-2): p. 151-160.

34. Steinhoff, U., et al., *Mycobacterium leprae renders Schwann cells and mononuclear phagocytes susceptible or resistant to killer cells*. Infection and Immunity, 1991. 59(2): p. 684-688.

35. Job, C.K., *Pathology and pathogenesis of leprous neuritis; a preventable and treatable complication*. International Journal of Leprosy and Other Mycobacterial Diseases, 2001. 69(2 SUPPL.): p. S19-S29.

36. Burnett, M.G. and E.L. Zager, *Pathophysiology of peripheral nerve injury: a brief review.* Neurosurg Focus, 2004. 16(5): p. E1.

37. Naafs, B., *Treatment of reactions and nerve damage.* International Journal of Leprosy, 1996. 64(4 SUPPL.): p. S21-S28.

38. van Veen, N.H., et al., *Corticosteroids for treating nerve damage in leprosy*. A Cochrane review. Lepr Rev, 2008. 79(4): p. 361-71.

39. Croft, R.P., et al., *The treatment of acute nerve function impairment in leprosy: Results from a prospective cohort study in Bangladesh.* Leprosy Review, 2000. 71(2): p. 154-168.

40. Richardus, J.H., et al., *Treatment with corticosteroids of long-standing nerve function impairment in leprosy: a randomized controlled trial (TRIPOD 3)*. Lepr Rev, 2003. 74(4): p. 311-8.

41. Smith, W.C., et al., *Steroid prophylaxis for prevention of nerve function impairment in leprosy: randomised placebo controlled trial (TRIPOD 1)*. BMJ, 2004. 328(7454): p. 1459.

42. Lambert, S.M., et al., *Comparison of Efficacy and Safety of Ciclosporin to Prednisolone in the Treatment of Erythema Nodosum Leprosum: Two Randomised, Double Blind, Controlled Pilot Studies in Ethiopia.* PLoS Neglected Tropical Diseases, 2016. 10(2).

43. Lambert, S.M., et al., *A Randomized Controlled Double Blind Trial of Ciclosporin versus Prednisolone in the Management of Leprosy Patients with New Type 1 Reaction, in Ethiopia*. PLoS Negl Trop Dis, 2016. 10(4): p. e0004502.

44. van Veen, N.H., et al., *Decompressive surgery for treating nerve damage in leprosy*. Cochrane Database Syst Rev, 2009(1): p. CD006983.

45. Oxford Centre for Evidence-based Medicine. *Levels of Evidence*. 2009 [cited 2016 4th of April]; Available from: http://www.cebm.net/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/.

46. Becx-Bleumink, M., D. Berhe, and W. Mannetje, *The management of nerve damage in the leprosy control services*. Lepr Rev, 1990. 61(1): p. 1-11.

Chapter 7

Prednisolone adverse events in the treatment of leprosy neuropathy

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Abstract

Introduction

Corticosteroids (prednisolone) are the mainstay in the treatment of leprosy reactions, including neuropathy. However, these drugs have adverse effects for which patients need to be carefully monitored.

Methods

In a double-blind randomized controlled trial (RCT), treatment effectiveness of a 20-week prednisolone regimen was compared with a longer and more intensive 32-week course to assess whether the latter would be more effective to restore nerve function. In a parallel double-blind RCT, patients with subclinical neuropathy received either a course of 20 weeks prednisolone or placebo to assess if the former could prevent the development of clinical neuropathy. In both trials, the occurrence of serious adverse events (SAE) was an important secondary outcome. In both trials, patients were assigned to a prednisolone starting dose according to weight: high weight (≥50 kg) started at 60mg and low weight (<50 kg) started at 45mg.

Results

In the Clinical trial, 868 patients enrolled, of whom 16 (1.8%) developed a serious adverse event (SAE). There were twelve SAEs in the longer treatment arm (n=439, event rate of 2.7%), and four in the shorter arm (n=429, event rate of 0.9%) (p=0.041). In the Subclinical neuropathy trial 4/364 (1.1%) developed an SAE, of which one in the placebo arm. In both trials, minor adverse events were very common and varied greatly in occurrence of event and between centres.

Discussion/conclusion

When searching for the optimum dose and duration of prednisolone in the treatment and prevention of neuropathy in leprosy patients, one has to weigh possible advantages of prednisolone treatment against the possible serious adverse events. Our trials have not shown significant benefits of a longer prednisolone treatment for clinical neuropathy than 20 weeks, nor the effectiveness of 20 weeks of prednisolone in the prevention of clinical neuropathy in case of subclinical neuropathy. At the same time, our trials did show a significantly increased percentage of SAE in the longer treatment arm, be it with an overall low event rate.

Introduction

Leprosy is a disease of skin and nerves. Leprosy neuropathy is a major complication that may lead to permanent disability when untreated. Nerve function impairment (NFI) might be prevented if neuropathy is diagnosed in time and the patient is given a course of an immunosuppressive drug such as prednisolone [1-4].
The World Health Organization has recommended regimens for the treatment of neuropathy taking into account age (child/adult) and type of 'reactional' neuropathy, typically a 12-weeks regimen [5]. In case of a type 1 reaction, ILEP advises a 12-weeks regimen for PB cases, and a 24-weeks regimen for MB cases. For type 2 reaction, this is a short course of 6 weeks [6].
Many studies have shown both benefits and limitations of treating leprosy neuropathy with corticosteroids using different treatment regimens, with variations in dose and duration [1-3,7]. These studies have shown that treatment durations longer than proposed in the WHO guideline are more effective.
Outside the field of leprosy, the adverse events of corticosteroids have been well documented for the treatment of diverse pathologies [8].
A recently concluded randomized, double-blind clinical trial compared the effectiveness of a longer, 32 weeks course of prednisolone with the commonly accepted treatment regimens of 20 weeks in improving and restoring clinically established recent sensory and/or motor loss [9].
In a parallel randomised, double-blind and placebo-controlled trial, prednisolone was given for 20 weeks to patients who had only subclinical nerve impairment, as assessed by nerve conduction studies (NCS) and/or Warmth Detection Thresholds (WDT) [10-12]. The aim of the study was to determine whether prednisolone treatment would prevent clinical function loss in leprosy patients as established by Voluntary Muscle Testing (VMT) and Monofilament Testing (MFT). These two trials are known as the TENLEP trials (Treatment of Early Neuropathy in LEProsy) [13]. This study reports on possible steroid induced serious and minor adverse events in the two TENLEP trials.

Methods

The trials took place in six centres in four leprosy endemic countries: India (2), Nepal (2), Bangladesh and Indonesia. Eligible patients were assessed with Voluntary Muscle Tests (VMT), Monofilament Tests (MFT), Nerve Conduction Studies (NCS), and Warmth Detection Tests (WDT). Reliability and normative studies were carried out prior to screening for possible inclusion. Patients, between the ages of 15 and 60, were eligible for enrolment, irrespective of the leprosy classification. More detailed information regarding in- and exclusion criteria can be found in the protocol article [13]. Patients were monitored for 78 weeks, whereby assessments were carried out monthly until completion of the intervention period at 20 or 32 weeks, followed by assessments at week 52 and week 78.

Table 1- The types of adverse events as recorded in this study

Serious Adverse Events (SAE)	Minor Adverse Events (MAE)
Peptic ulcer	Gastric pain
Diabetes	Moon face
Tuberculosis	Acne
Hypertension	Fungal infection (local)
Psychosis	Weight gain
Glaucoma	Other
Cataract	
Corneal ulcer	
Worm infestation	
Other	

In both trials the medication provided is prednisolone. Adverse events were recorded as listed in Table 1. The option 'other' adverse events was included to leave room for the clinicians to report for example extremely rare adverse events.

In the Clinical trial , patients in the treatment arm received prednisolone for 32 weeks, in tablets of 5 mg [13]. The control arm followed a regimen of 20 weeks and received placebo tablets to keep the number of tablets equal to that in the treatment group. The dose in both the intervention and control arm started at 1 mg/kg/day (either 45 or 60 mg/day depending on body weight being under or above 50kg respectively) and was tapered down over 32 and 20 weeks, respectively (see Figure 1). Contrary to previous trials, the middle range of the prednisolone dose was maintained at a high level (0.44 mg/kg/day) for a longer period (12 weeks).
In the Subclinical trial, patients received either prednisolone or placebo for 20 weeks in tablets of 5 mg [13]. The prednisolone dose started at 1 mg/kg/day (either 45 or 60 mg/day depending on weight class), and was be tapered down over 20 weeks. Figure 1 shows the timeline and dosage. The total dosage of prednisolone over 20 weeks will be 2.8 grams for patients under 50 kg body weight, and 3.7 grams for patients over 50 kg body weight.
Prior to enrolment, patients were screened for underlying morbidities such as diabetes and osteoporosis. Patients with these diagnoses were not enrolled in the trial. All patients were de-wormed before starting the trial medication.

A distinction was made between minor and serious adverse events. Adverse events were only counted once, even when they occurred multiple times in one patient or were recorded on multiple visits. When minor adverse events were noticed these were treated and the patient continued in the trial. When serious adverse events were noticed or leprosy 'reactions' or other complications occurred, patients were taken off trial treatment and continued on individualised treatment.

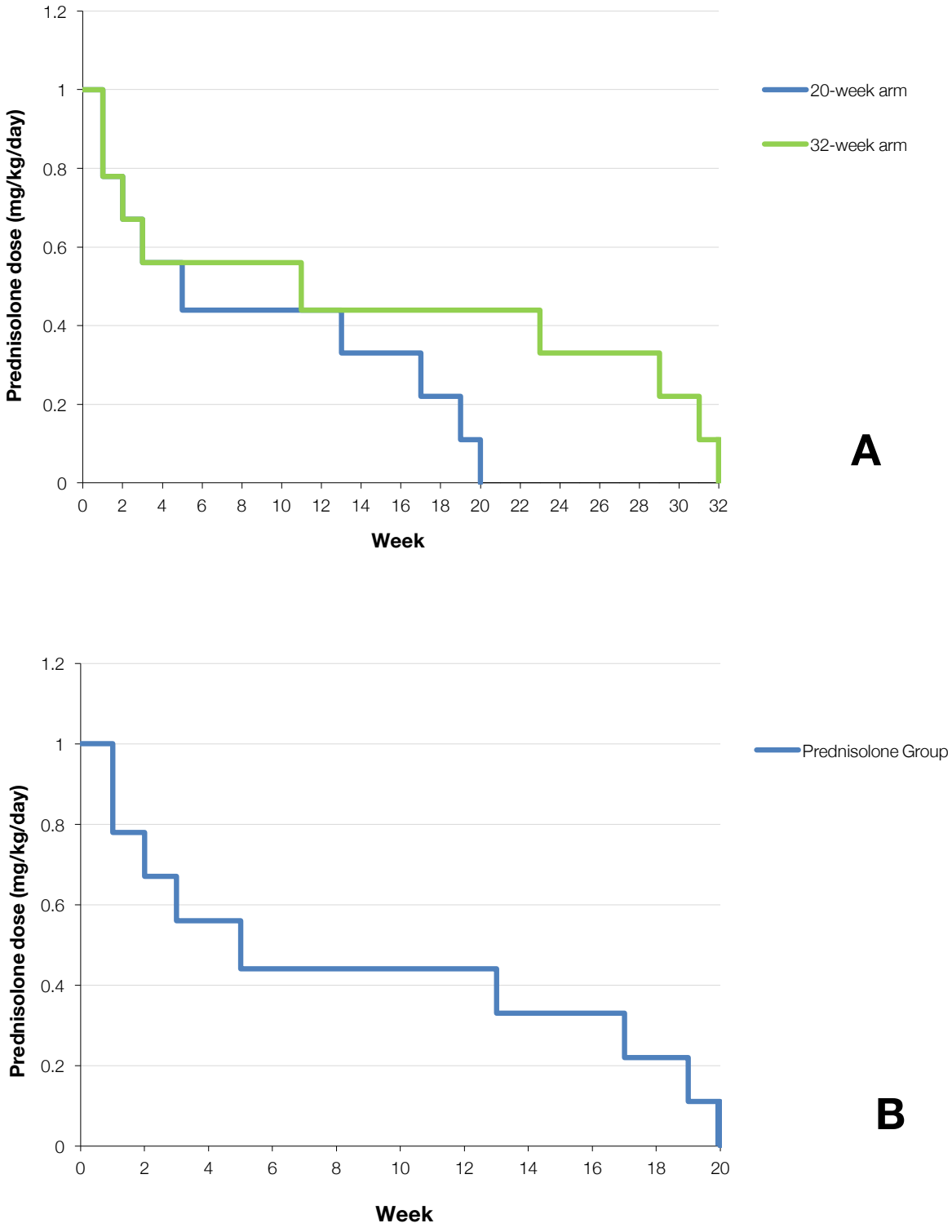

Figure 1 A- Timeline prednisolone dose for patients under 50 kg in the Clinical trial. **Figure 1 B**- Timeline prednisolone dose for patients under 50 kg in the Subclinical trial

Data from the participating centres were send to PN and were analysed with Stata.
Informed written consent was obtained from each patient, who could withdraw from the treatment at any time. In India, ethics approval was obtained from the Indian Council of Medical Research and the Ethics Committee of the Foundation for Medical Research, Mumbai; in Nepal from the Nepal Health Research Council (NHRC); in Indonesia from the Komite Etik Penelitian Kesehatan RSUD Dr. Soetomo Surabaya; in Bangladesh from the Bangladesh Medical Research Council - National Research Ethics Committee. The trial was registered in the Netherlands Trial Register (NTR2300) and in the Clinical Trials registry India (CTRI/2011/09/002022 and 23).

Results

A total of 868 patients were enrolled in the Clinical trial and 364 patients in the Subclinical trial. In both trials there were no statistically significant differences in demographics nor assignment to the treatment arms [9,14]. We refer to these papers for primary outcomes and detailed information regarding classification and 'flow' of patients through the trial phase.

Table 2 summarises the serious- and minor adverse events from both trials. In the Subclinical trial, with a prednisolone (20 weeks) and a placebo arm, only four SAEs were noticed: three patients with hypertension and one patient with TB, the latter who had been assigned to the placebo arm. All SAEs in the Subclinical trial were reported by one centre, and all were in the high weight group.

In the Clinical trial, with two treatment arms 16 SAE were recorded. Table 3 shows the SAEs per centre and trial arm. It is noticeable that not all possible SAEs, as listed in Table 1, did occur in our cohort. In addition, it can be seen that there is considerable variation between the centres in the number of SAEs reported. All SAEs were reported within the period that prednisolone was given. More SAEs were reported from the longer treatment arm, namely 12 of the 16 ($p=0.041$). Of these 16, 11 were in the high weight group ($p=0.378$).

The minor adverse events are reported in Tables 4 and 5. In both trials, minor adverse events were very common: 49% of the patients in the Subclinical trial and 67% of the patients in the Clinical trial showed one or more MAEs. MAEs varied greatly in occurrence of event and between centres, e.g. in the Subclinical trial: moon face (2-68%), and acne (8-61%). Minor events were also noticed in the placebo arm, e.g. moon face: 0-53% (Table 4).

The same great variation in numbers of minor adverse events between the centres is also noticeable for the Clinical trial (Table 5). In both trials and all arms differences could be seen between the high and low weight groups, however, the differences were not clearly in one direction. The high weight group in the Subclinical trial 44% had an MAE and in the Clinical trial 66%. In the low weigh groups, this was 56% and 69%, respectively.

Table 2- Relative risk, confidence intervals and p-values of adverse events in both trials

Clinical Trial	**20-week arm n=429**	**32-week arm n=439**	**RR**	**CI**	**p-value†**
Serious Adverse Events	4	12	2.9	(0.95-9.02)	0.041*
Minor Adverse Events	284	300	1.0	(0.94- 1.13)	0.275
Subclinical Trial	**20-week arm n=181**	**Placebo n=182**	**RR**	**CI**	**p-value†**
Serious Adverse Events	3	1	3.0	(0.32-28.73)	0.309
Minor Adverse Events	102	75	1.4	(1.10-1.70)	0.003

RR: relative risk; CI: confidence interval; †One- sided p-value; * statistically significant difference

Discussion

The TENLEP trials were conducted to a) assess the effectiveness of a relatively longer course of prednisolone than normally practiced in the treatment of recent NFI and b) to assess if a 20-week course of prednisolone would reduce the incidence of clinical NFI in patients with subclinical neuropathy [13]. The results of these trials are reported elsewhere [9,14]. An important secondary outcome was the occurrence of adverse events due to prednisolone. Do hypothesised benefits outweigh the possible increase in incidence of prednisolone related serious adverse events?
The Clinical trial did not show a significant beneficial effect of 32 weeks prednisolone in the treatment of NFI compared to the 'standard' 20-week course. Likewise, the treatment in the Subclinical trial was not significantly more effective than a placebo in the prevention of NFI.

Our studies have shown that serious adverse events do occur, but that the overall event rate of SAE was very low. A longer duration of prednisolone (32 weeks), however, did significantly increase the event rate of SAEs. If our trials had shown significant benefits of the longer treatment, a longer course could be recommended considering the small numbers of SAE.

It should be noted that patients in our Clinical trial not only received prednisolone for a longer duration then commonly practiced in leprosy, the total amount of prednisolone was higher as well. This was not only because of the longer duration, but also because we maintained a 'middle-medium plateau' dose for a relative longer time before tapering down (Figure 1). The plateau dose remained above the 'critical' dose of 25mg as recommended by Naafs except for the last three weeks [1].

Table 3- Serious adverse events in the Clinical trial

	20-week arm					32-week arm				
Centre	**A**	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
Peptic ulcer				1†			1#			
Diabetes				1#	1#			1#	3†##	
Tuberculosis										
Hypertension				1#		1#		2†#	2††	
Psychosis								1#	1#	
Glaucoma										
Cataract										
Corneal ulcer										
Worm infestation										
Other										

Centre F did not report any SAE; † In low weight group, #In high weight group

Despite the somewhat higher dose used in our trials, SAE event rates were quite similar to those in the TRIPOD trials, at less than 3% [15]. All patients developing SAE did so during the trial treatment period of 32 weeks. In both trials, when patients had deteriorating nerve function or reactions, they received additional prednisolone. None of those patients developed SAE.

In one of the centres, many patients were recalled for an additional physical examination after the 72 weeks follow up period, but within 120 weeks after enrolment in the trial. An additional three patients were diagnosed with cataract and two with hypertension. Hypertension can have multiple causes and is not likely to be related to the use of prednisone many months after the use of prednisolone.

Regarding cataract, there seems to be a relationship between prednisolone treatment and a specific type of cataract, sub-capsular. This specific type was diagnosed in two of the patients. The aetiology of steroid induced posterior subcapsular cataracts is explained by James [16]. One study in lepromatous leprosy patients in India found that cataract develops in 3% per person year and 7% per person year in lepromatous patients over 40 years of age. There was, however, no significant association with oral steroids [17]. Leprosy patients are 'at risk' because of the generally long duration of the disease and often present subclinical intra-ocular inflammation [2,17]. Further prospective studies are recommended to investigate the late occurrence of cataract and its possible relation to prednisolone.

Minor adverse events have been reported in another placebo controlled trial, and similar to our study, the MAEs were present in the placebo arm, though less frequent [15]. It appears, therefore, that some of the minor adverse events that can be attributed to prednisolone also

Table 4- Minor Adverse Events in Subclinical trial per treatment arm for each centre

	n (total)	n (20-week)	MAE 20-week n		MAE Placebo n		MAE Total n	
Moon face								
A	61	30	1	(3%)	0	(0%)	1	(2%)
B	136	68	56	(82%)	36	(53%)	92	(68%)
C	48	24	4	(17%)	0	(0%)	4	(8%)
D	118	59	3	(5%)	1	(2%)	4	(3%)
Acne								
A	61	30	3	(10%)	2	(6%)	5	(8%)
B	136	68	52	(76%)	31	(46%)	83	(61%)
C	48	24	5	(21%)	1	(4%)	6	(13%)
D	118	59	7	(12%)	3	(5%)	10	(8%)
Fungal infection								
A	61	30	1	(3%)	2	(6%)	3	(5%)
B	136	68	8	(12%)	4	(6%)	12	(9%)
C	48	24	1	(4%)	2	(8%)	3	(6%)
D	118	59	3	(5%)	1	(2%)	4	(3%)
Gastric pain								
A	61	30	0	(0%)	0	(0%)	0	(0%)
B	136	68	7	(10%)	4	(6%)	11	(8%)
C	48	24	0	(0%)	0	(0%)	0	(0%)
D	118	59	19	(32%)	21	(36%)	40	(34%)

happen due to other causes, co-incidentally. Some, like moon face, depend on subjective clinical judgement. MAEs can be treated or will resolve when prednisolone treatment is stopped. However, they can be aesthetically unacceptable and therefore need careful explanation [18].

This study had some limitations to take into consideration. First, even though the trial was double-blind, experienced leprosy physicians and field-staff may suspect that patients are on the prednisolone arm through for example the appearance of moon-face features, or acne, which happens in many patients [3]. This knowledge could influence the clinical observation, besides alertness and conscientiousness, in the recording of minor adverse events. However, this seems to be contradicted by the finding that minor adverse event were also noticed in the placebo group, confirming findings by others [15]. Second, in both arms of the Clinical trial there were a considerable proportion of patients who were lost to follow-up. This was seen in both the 20-week (96/429 (22.4%) and the 32-week (110/439= 25.1%) prednisolone arms. One possible reason for loosing patients to follow-up is the occurrence of side effects of the treatment, and therefore the number of SAE could potentially have been higher than reported [8,19].

Obvious differences are seen in the incidence of minor adverse events between the project sites. It should be born in mind that the studies took place in six hospitals in four countries with structurally differing leprosy programs. Difference in access to services and illness behaviour, changes in staff and commitment may be an explanation for diversity in therapy adherence and reporting and noticing of, especially, minor adverse events. In addition, one could wonder whether these variations could be attributed to ethnic differences [20].

One 'beneficial' adverse event that is known but little researched is the dependency on prednisolone [21]. Prednisolone supposedly may cause euphoria, which may result in patients requesting the drug, even purchasing prednisolone themselves. Fardet mentions many different mental consequences [18], not systematically recorded in our study, but euphoria is not listed. This effect was not studied in our cohorts either.

The search for alternative, more effective drugs continues, but none has been shown to be superior yet to prednisolone in terms of better outcomes and fewer adverse events [22,23]. Hence, corticosteroids remain 'first choice' in neuropathy and 'reactional' phases in leprosy whether skin, nerve, or both are involved. More research is needed, though, into the effect of prednisolone in the prevention and treatment of NFI and often concomitant other signs of leprosy reactions, not only in delineation of leprosy- and patient specific characteristics that determine response but also in alternative drug regimens.

The TENLEP studies are two of very few studies that have looked systematically in randomized trials into the adverse events of corticosteroids in the treatment of leprosy neuropathy. Outside leprosy, adverse events due to corticosteroids have been extensively reviewed by Fardet for many other clinical conditions [8,18]. Because of its completeness and clinical grouping, the classification of adverse events as developed by Fardet seems most suitable to follow in future leprosy clinical projects using steroids or other immune-suppressive drugs [8,24].

Though it is important to have a protocol, a 'guiding' (guideline) document, for the treatment of nerve function impairment, ideally treatment for reaction/NFI should be patient-centred. This is not always practical under field conditions with integrated services and declining leprosy expertise. Nevertheless, ideally, ".. *treatment should be tailored to the individual patient. ... response carefully assessed and adapted to changing circumstances, immediately if necessary*" [1].

When searching for the optimum dose and duration of prednisolone in the treatment and prevention of neuropathy in leprosy patients, one has to weigh possible advantages of prednisolone treatment against the possible serious adverse events. The documented advantages of prednisolone for the treatment of recent nerve function impairment, to a reported 70-80% improvement [7], outweigh the disadvantages of SAEs, especially with the low rates in our study.

Table 5- Minor Adverse Events in Clinical trial per treatment arm for each centre

	n (total)	n (20-week)	MAE 20-week n		MAE 32-week n		MAE Total n	
Moon face								
A	82	40	3	(8%)	1	(2%)	4	(5%)
B	230	115	99	(86%)	97	(84%)	196	(84%)
C	166	83	12	(14%)	11	(13%)	23	(14%)
D	232	116	24	(21%)	31	(27%)	55	(24%)
E	101	48	13	(27%)	15	(28%)	28	(28%)
F	57	27	23	(85%)	29	(97%)	52	(91%)
Acne								
A	82	40	7	(18%)	6	(14%)	13	(16%)
B	230	115	98	(85%)	97	(84%)	195	(85%)
C	166	83	14	(17%)	15	(18%)	29	(17%)
D	232	116	15	(13%)	22	(19%)	37	(16%)
E	101	48	20	(42%)	15	(28%)	35	(35%)
F	57	27	7	(26%)	8	(27%)	15	(26%)
Fungal infection								
A	82	40	0	(0%)	0	(0%)	0	(0%)
B	230	115	11	(10%)	15	(13%)	26	(11%)
C	166	83	4	(5%)	11	(13%)	15	(9%)
D	232	116	24	(21%)	28	(24%)	52	(22%)
E	101	48	13	(27%)	20	(38%)	33	(33%)
F	57	27	6	(22%)	6	(20%)	12	(21%)
Gastric pain								
A	82	40	0	(0%)	0	(0%)	0	(0%)
B	230	115	19	(17%)	14	(12%)	33	(14%)
C	166	83	0	(0%)	0	(0%)	0	(0%)
D	232	116	54	(47%)	51	(44%)	105	(45%)
E	101	48	16	(33%)	15	(28%)	31	(31%)
F	57	27	7	(26%)	11	(37%)	18	(32%)

References

1. Naafs, B., J.M. Pearson, and H.W. Wheate, *Reversal reaction: the prevention of permanent nerve damage. Comparison of short and long-term steroid treatment*. Int J Lepr Other Mycobact Dis, 1979. 47(1): p. 7-12.
2. Rao, P.S., et al., *Multi-centre, double blind, randomized trial of three steroid regimens in the treatment of type-1 reactions in leprosy*. Lepr Rev, 2006. 77(1): p. 25-33.
3. Sugumaran, D.S., *Steroid therapy for paralytic deformities in leprosy*. Int J Lepr Other Mycobact Dis, 1997. 65(3): p. 337-44.
4. Croft, R.P., et al., *The treatment of acute nerve function impairment in leprosy: results from a prospective cohort study in Bangladesh.* Lepr Rev, 2000. 71(2): p. 154-68.
5. World Health Organisation, *WHO Model Prescribing Information: Drugs Used in Leprosy.* 1998. p. 28.
6. ILEP, *How to Recognise and Manage Leprosy Reactions*. 2002.
7. van Veen, N.H., et al., *Corticosteroids for treating nerve damage in leprosy*. Cochrane Database Syst Rev, 2016(5): p. Cd005491.
8. Fardet, L., et al., *Corticosteroid-induced adverse events in adults: frequency, screening and prevention.* Drug Saf, 2007. 30(10): p. 861-81.
9. Wagenaar I., P.E., Brandsma J.W., E*ffectiveness of 32 versus 20 weeks of prednisolone in leprosy patients with recent nerve function impairment: a randomized controlled trial.* In prepration, 2016.
10. McKnight, J., *Reference values for nerve function assessments among a study population in northern India - ii: Thermal sensation thresholds.* Neurology Asia, 2010. 15(1): p. 27-38.
11. McKnight, J., *Reference values for nerve function assessments among a study population in northern India - iii: Sensory and motor nerve conduction.* Neurology Asia, 2010. 15(1): p. 39-54.
12. van Brakel, W.H., et al., *Early diagnosis of neuropathy in leprosy--comparing diagnostic tests in a large prospective study (the INFIR cohort study*). PLoS Negl Trop Dis, 2008. 2(4): p. e212.
13. Wagenaar, I., et al., *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols.* BMC Neurol, 2012. 12: p. 159.
14. Post E., W.I., Brandsma J.W., *A 5-month prophylactic prednisolone course is not effective in preventing clinical neuropathy in leprosy patients with subclinical neuropathy.* In prepration, 2016.

15. Richardus, J.H., et al., *Adverse events of standardized regimens of corticosteroids for prophylaxis and treatment of nerve function impairment in leprosy: results from the 'TRIPOD' trials*. Lepr Rev, 2003. 74(4): p. 319-27.

16. James, E.R., *The etiology of steroid cataract.* J Ocul Pharmacol Ther, 2007. 23(5): p. 403-20.

17. Daniel, E. and P.S. Sundar Rao, *Evolution of vision reducing cataract in skin smear positive lepromatous patients: does it have an inflammatory basis?* Br J Ophthalmol, 2007. 91(8): p. 1011-3.

18. Fardet, L., et al., *Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion.* Br J Dermatol, 2007. 157(1): p. 142-8.

19. Morin, C. and L. Fardet, *Systemic glucocorticoid therapy: risk factors for reported adverse events and beliefs about the drug. A cross-sectional online survey of 820 patients*. Clin Rheumatol, 2015. 34(12): p. 2119-26.

20. Wood, A.J. and H.H. Zhou, *Ethnic differences in drug disposition and responsiveness*. Clin Pharmacokinet, 1991. 20(5): p. 350-73.

21. Swinburn, C.R., et al., *Evidence of prednisolone induced mood change ('steroid euphoria') in patients with chronic obstructive airways disease.* Br J Clin Pharmacol, 1988. 26(6): p. 709-13.

22. Naafs, B. and C.L. van Hees, *Leprosy type 1 reaction (formerly reversal reaction)*. Clin Dermatol, 2016. 34(1): p. 37-50.

23. Lambert, S.M., et al., *A Randomized Controlled Double Blind Trial of Ciclosporin versus Prednisolone in the Management of Leprosy Patients with New Type 1 Reaction, in Ethiopia*. PLoS Negl Trop Dis, 2016. 10(4): p. e0004502.

24. Liu, D., et al., *A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy.* Allergy Asthma Clin Immunol, 2013. 9(1): p. 30.

Chapter 8

Diet-related risk factors for leprosy: A case-control study

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PLoS Neglected Tropical Diseases 9(5): e0003766

Abstract

Introduction

Food shortage was associated with leprosy in two recent studies investigating the relation between socioeconomic factors and leprosy. Inadequate intake of nutrients due to food shortage may affect the immune system and influence the progression of infection to clinical leprosy. We aimed to identify possible differences in dietary intake between recently diagnosed leprosy patients and control subjects.

Methods

In a leprosy endemic area of Bangladesh, newly diagnosed leprosy patients and control subjects were interviewed about their socioeconomic situation, health and diet. Dietary intakes were recorded with a 24-hour recall, from which a Dietary Diversity Score (DDS) was calculated. Body Mass Index (BMI) was calculated and Household Food Insecurity Access Scale (HFIAS) was filled out for every participant. Using logistic regression, a univariate, block wise multivariate, and an integrated analysis were carried out.

Results

52 leprosy cases and 100 control subjects were included. Food shortage was more common, dietary diversity was lower and household food insecurity was higher in the patient group. Patients consumed significantly less items from the DDS food groups 'Meat and fish' and 'Other fruits and vegetables'. Lower food expenditure per capita, lower BMI, lower DDS and absence of household food stocks are the main factors associated with an increased risk of having leprosy.

Conclusion

Low income families have only little money to spend on food and consequently have a low intake of highly nutritious non-rice foods such as meat, fish, milk, eggs, fruits and vegetables. Development of clinical leprosy could be explained by deficiencies of the nutrients that these foods normally provide.

Introduction

Leprosy, an infectious disease caused by *Mycobacterium leprae*, affects skin and nerves and can lead to deformities of the hands, feet and face. The disease remains a public health problem in underdeveloped areas in the world, and is therefore known as a disease of the poor. Our understanding of risk factors for the transmission of *M. leprae* and the development of leprosy disease is not complete. One of the reasons is the long incubation period, which is on average 2-5 years. It is therefore difficult to investigate causal relationships between circumstances at the time of infection and the onset of clinical symptoms years later. The most important known determinant for contracting leprosy is being a household contact of a leprosy patient, which carries a five to eight times higher risk of contracting leprosy [1,2]. The specific risk factors that determine the risk for contacts include the Ridley-Jopling leprosy classification of the index patient, physical distance to the patient and age of the contact [3]. However, in endemic regions the majority of new leprosy patients are not close contacts of a known leprosy case [4,5]. Another possible risk factor is poverty, although not all poor countries have high leprosy prevalence rates. It is even the case that Brazil, an economically emerging country, has one of the highest new case detection rates in the world [6]. It remains unclear which aspects of poverty are associated with leprosy susceptibility and the progression to clinically detectable disease. Two recent case-control studies investigating socioeconomic factors in relation to leprosy found that food shortage was associated with leprosy. The setting of one of these studies was a poor, high leprosy endemic area in Brazil [7]. Among other factors, having experienced food shortage at any time in life was related to leprosy. The other study was set in two leprosy endemic districts in Bangladesh. In this study, food shortage in the past year was the only factor significantly associated with the clinical manifestation of leprosy [8].

The definition of food shortage used in the Bangladesh study was: 'the period in which a family had to reduce the number of meals a day, or reduce the intake of foods other than rice'. This is most likely to occur when rice prices are high, household food stocks depleted and income is low due to lack of labour opportunities. Multiple studies in Bangladesh documented that this situation typically arises in the period between September and the end of November, just before the major harvest Aman in December [9-12].

Food shortage worsens the often already inadequate intake of micro- and macronutrients. Nutritional deficiencies impair the immune system and thus the defence of the body against infections [13,14]. The risk of contracting subclinical *M. leprae* infection is not necessarily increased by food shortage, but it could facilitate the progression from infection to the clinical presentation of leprosy.

The findings of the two above-mentioned studies raise many questions regarding the exact mechanisms behind the relationship between food shortage and leprosy. Literature on this subject is scarce, and to further examine this relationship we designed a case-control study in rural Bangladesh during an expected period of food shortage. The aim was to assess possible differences in dietary intake between recently diagnosed leprosy patients and control subjects

without the disease that could lead to hypotheses on immunological mechanisms underlying the clinical development of leprosy.

Methods

Study population and sampling

This case-control study was conducted in October and November 2013 in northwest Bangladesh, in the mainly rural and agricultural Nilphamari and Rangpur districts. These are among the poorest regions in Bangladesh [15], and leprosy is still endemic in this area. In 2012, 512 new leprosy cases were found in these districts, which have a total population of four million (data from the Rural Health Program, Nilphamari).

Data of all patients diagnosed with leprosy in the first half of 2013 were gathered from the Rural Health Program database in Nilphamari, which is run by The Leprosy Mission International, Bangladesh (TLMIB). Our aim was to include an equal number of men and women, to have an even age distribution and take in only one person (case or control) per household. Furthermore, we only included patients aged between 18 and 50 years and with help of field staff we pre-selected patients with a low risk of stigma possibly caused by a home visit. Of the 180 patients, 92 were eligible for this study, 64 were outside the age bracket criterion and 24 were excluded to avoid the risk of stigma of a home visit. Controls were selected from a random cluster sample of the population, originally composed for the COLEP study [16]. Out of the 13 sub-districts in the area, 20 clusters were formed, each containing 1000 randomly selected people [17]. We selected three clusters that could be reached within approximately one hour by motorcycle from the TLMIB Leprosy Center in Nilphamari; two clusters in Nilphamari district, of which one rural and one suburban, and one rural cluster in Rangpur district. From each cluster, 34 controls were randomly selected using a computerized sampling method, with an even distribution of men and women. Controls were excluded if they or a household member had ever been diagnosed with leprosy and if they were under 18 or over 50 years of age. When a control subject was not available at the time of our first visit, we made two more attempts. If the control subject was still not available the third time, a neighbour of similar age was invited to participate.

Ethics

Ethical approval for the study protocol was given by the institutional review board of TLMI Bangladesh, Nilphamari. Informed written consent was obtained from all participants.

Data collection

Data on patients and controls were collected by means of a structured questionnaire, a 24-hour dietary recall and anthropometric measures. The questionnaires for cases and controls were

developed in English, translated separately by two translators to Bengali and each of them translated their colleague's version back to English. The translations were discussed and the questionnaire was optimized. The questionnaire was pre-tested on patients and controls and adjusted where necessary. The questions of the Household Food Insecurity Access Scale (HFIAS) were kindly provided in Bengali by the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). Data were collected during household visits by two staff members of the TLMIB Nilphamari Training Center, both were trained field workers fluent in Bengali and English.

The questionnaire focused on demographic, socioeconomic and health data of the subjects and their households. The questions dealt with the occupation of the income generator, household size (defined as the number of people eating in the house), average income and income variation, self-classification on a poverty scale (very poor to rich), land ownership, food expenditure, any health problems other than leprosy in the past year and the presence of a BCG scar. If a patient's income had changed since the diagnosis of leprosy, the pre-diagnosis income was used in the analyses. Income and food expenditure were then calculated per capita. Second, the HFIAS was administered [18]. This validated tool monitors problems with food access, dietary modifications and concerns about food insecurity over the past four weeks. Finally, subjects were asked questions about their history of food shortage, their household food stocks, and details of their coping mechanisms such as reducing the number or variety of meals. For the sake of comparability, these questions and the definition of food shortage were based on the study of Feenstra et al. [8]. Dietary intakes were assessed by a 24-hour recall, following the Food and Agriculture Organization (FAO) guidelines for measuring individual dietary diversity [19]. Subjects were asked to list the foods consumed during the previous day, starting from waking up in the morning. Details of all meals and snacks, consumed inside and outside the house during the full day, were recorded in chronological order. To be as accurate as possible, subjects were prompted about drinks, snacks and food consumed in and outside the house. Recipes of mixed meals were obtained to ascertain that all ingredients were recorded. The 24-hour recalls were carried out on weekdays, with exception of atypical holidays. Therefore, no interviews were held in the week after Eid al-Adha (Festival of Sacrifice). Also, two focus group discussions were held with women to gather information on commonly used ingredients and preparation methods.

Weight of subjects was measured using a portable scale and assessed to the nearest 0.5 kg. Subjects were asked to remove shoes. Height was measured with a measuring tape while the subject was standing barefooted with his/her back straight against a wall. Body mass index (BMI) was calculated as weight (kg)/ height^2 (m). Subjects were identified as underweight when their BMI was lower than 18.5 kg/m^2. From the 24-hour recalls, the Dietary Diversity Score (DDS) was calculated [19]. The DDS is a simple count of the food groups consumed by the subject, and is increasingly used to measure dietary quality [20,21]. Nine food groups were included in this study: 'Starchy staples', 'Dark green leafy vegetables', 'Other vitamin A rich fruits and vegetables', 'Other fruits and vegetables', 'Organ meat', 'Meat and fish', 'Eggs', 'Legumes, nuts and seeds' and 'Milk and milk products'. Vitamin A rich fruits and vegetables

were defined as containing a minimum of 60 RAE/100 g (RAE stands for retinol activity equivalents) [19]. The condiments garlic and chilies were not counted, because the amount consumed per person was likely to be very low. The possible score ranged from 0 to 9. A score ≥5 was considered as adequately diverse [22].

Analysis

Before statistical analysis, a framework was built using four blocks comprised of several related variables: Demographic factors (age, sex, religion, district, and household size); socioeconomic factors (income, food expenditure, poverty classification, occupation, and land ownership); health factors (diseased in the last year, BCG scar, and BMI); and diet-related factors (HFIAS, DDS, food shortage in the past year, food shortage at any time in life, and household food stocks).

Statistical analyses were performed using SPSS (version 22, SPSS Inc., Chicago, IL). All analyses were done using logistic regression, with case/control as dependent variable. Income and food expenditure were log transformed to normalize their distribution. To reduce the effect of matching, age and sex were continuously adjusted for in the univariate, multivariate per block and integrated analyses. Univariate analysis was carried out first, and the variables significantly ($p<0.10$) associated with leprosy were included in a multivariate backward stepwise logistic regression per block. The variables that remained statistically significant ($p \leq 0.05$) in these multivariate analyses were considered as the main result. Finally, for the integrated analysis, the significant variables of each block ($p<0.10$ in the Wald Chi Square test) were combined and again backward stepwise logistic regression was carried out, this time using a p-value of <0.05 as statistically significantly contributing to the model.

Table 1- Demographic, socioeconomic and health characteristics (% or mean (±SD))

		Cases (n=52)	Controls (n=100)
Sex	Male	29 (56%)	48 (48%)
Age	Mean (years)	35.0 ±9.5	33.3 ±10.4
	15-29	25.0 %	37.0 %
	30-44	32.7 %	27.0 %
	45-60	42.3 %	36.0 %
District	Nilphamari	27 (51.9%)	67 (67.0%)
	Rangpur	25 (48.1%)	33 (33.0%)
Household size	Mean	4.6 ±1.4	5.2 ±2.1
Income	Household mean (BDT)	5115 ±3621	8177 ±6398
	Per capita mean (BDT)	1180 ±886	1766 ±2011
Income variation	Mean (BDT)	3827 ±2852	5234 ±5719
Food expenditure	Household mean (BDT)	4545 ±2323	6540 ±3435
	Per capita mean (BDT)	1046 ±530	1340 ±841
Land owned	Landowner	8 (15.4%)	34 (34.0%)
	Mean size (m^2)	387 ±1214	3161 ±6820
BMI	Mean (kg/m^2)	20.3 ±3.1	21.6 ±3.0
	Underweight	25.0 %	14.0 %
	Normal weight	67.3 %	72.0 %
	Overweight	7.7 %	14.0 %
Leprosy	Paucibacillary	34 (65.4%)	-
	Multibacillary	18 (34.6%)	-
Disability grade	0	37 (71.2%)	-
	1	9 (17.3%)	-
	2	6 (11.5%)	-

BMI=Body Mass Index, categories: underweight <18.5, normal: 18.5-25, overweight: >25; BDT: Bangladesh Thaka, 100 BDT= ± $1.28

Results

Fifty-two leprosy cases and 100 control subjects were interviewed during home visits. The majority of the leprosy patients had paucibacillary (PB) leprosy (65%), was released from multidrug treatment (56%) and had no leprosy-related disabilities (71%). Seventy-one percent of the controls were acquainted with at least one person diagnosed with leprosy, in most cases a neighbour (59%). Other demographic and socioeconomic characteristics of the patient and control groups are shown in Table 1. In general, the socioeconomic variables for the patient group were less favourable.

Table 2 provides detailed data on food shortage, food security and coping mechanisms, from which it becomes evident that leprosy patients are once more in a disadvantaged position. The average DDS was significantly lower in the patient group, who consumed significantly less items from the groups 'Meat and fish' and 'Other fruits and vegetables' than the control group did (Figure 1). Control subjects consumed more items from the groups 'Milk and milk products' and 'Eggs' as well, but in this regard there was no significant difference with the patient group. Adequate dietary diversity (DDS ≥5) was reached by 25% of the control subjects and 17.3% of the patients. Food insecurity, measured by HFIAS, was more severe in the patient group, with a mean score of 10.2 and 6.4 for patient and control group, respectively (p=0.003). Figure 2 gives the proportions and the severity of food insecurity for each item of the scale for both groups. Food stocks were present in 74% of the controls' households, lasting on average for 32 days, and in 51% of the patients' households, sufficient for 15 days on average.

Food shortage, at any time in life as well as in the past year, was reported by many people in both groups, but significantly more often by patients (p= 0.03). Food shortage was most common in the months of September to November leading up to the major harvest in December, Aman. Income was reported to be the lowest of the year during these months by 79% of the subjects. Forty-one percent of interviewees who experienced food shortage in September-October last year reported food shortage as well in the period March-April, before the rice harvest in May/June, Boro. Food shortage in the past year lasted a mean of 101 days, during which one consumed less of certain food products or eliminated these from the diet, and often took fewer meals per day. Note that while control subjects mainly reduced their intake of most foods, patients had to eliminate foods more often. Intake of fish and meat was affected most frequently.

Regardless of being a case or control, people who had had to cope with food shortage in the past year had a DDS of 3.14 (±1.1) versus 4.59 (±1.3) in people without food shortage ($p<0.001$). Food insecurity was significantly higher among the people suffering from food shortage: their HFIAS score was on average 10.8, versus 0.6 for people not experiencing food shortage ($p<0.001$).

Table 2- Details on food shortage, coping mechanisms and household food stocks

		Cases (n=52)		Controls (n=100)	
DDS		3.2	± 1.1	3.8	± 1.4
Experienced food shortage at any time in life		96.2	%	84.0	%
Experienced food shortage in past year		80.8	%	64.0	%
Average duration of food shortage (days)		106	± 118	99	± 124
Experienced food shortage in					
March/April		5.7	%	5.4	%
September/October		54.3	%	57.1	%
Both periods		40.0	%	37.5	%
Coping mechanisms					
Reduced variety		16.7	%	29.7	%
Reduced number of meals		-		3.1	
Reduced both variety and number of meals		83.3	%	67.2	%
Changes in consumption of food items					
Fish	No change	9.5	%	1.6	%
	Reduced	38.1	%	64.5	%
	Eliminated	52.4	%	33.9	%
Meat	No change	7.1	%	-	
	Reduced	28.6	%	51.6	%
	Eliminated	64.3	%	48.4	%
Vegetables	No change	57.1	%	75.8	%
	Reduced	38.1	%	22.6	%
	Eliminated	4.8	%	1.6	%
Fruits	No change	50.0	%	53.2	%
	Reduced	19.0	%	25.8	%
	Eliminated	31.0	%	21.0	%
Lentils	No change	73.8	%	72.6	%
	Reduced	14.3	%	24.2	%
	Eliminated	11.9	%	3.2	%
Egg	No change	59.5	%	51.6	%
	Reduced	19.1	%	35.5	%
	Eliminated	21.4	%	12.9	%
Milk	No change	66.7	%	53.2	%
	Reduced	14.3	%	32.3	%
	Eliminated	19.0	%	14.5	%
HFIAS		10.2	± 7.4	6.4	± 7.0
Food secure		19.2	%	33.0	%
Mildly food insecure		3.8	%	9.0	%
Moderately food insecure		25.0	%	31.0	%
Severely food insecure		51.9	%	27.0	%
Household food stock present		51.9	%	74.0	%
Mean duration food stock (days)		14.9	±	32.0	± 56.1

DDS: Dietary Diversity Score, HFIAS: Household Food Insecurity Access Scale, categories following Coates et al. (2007)

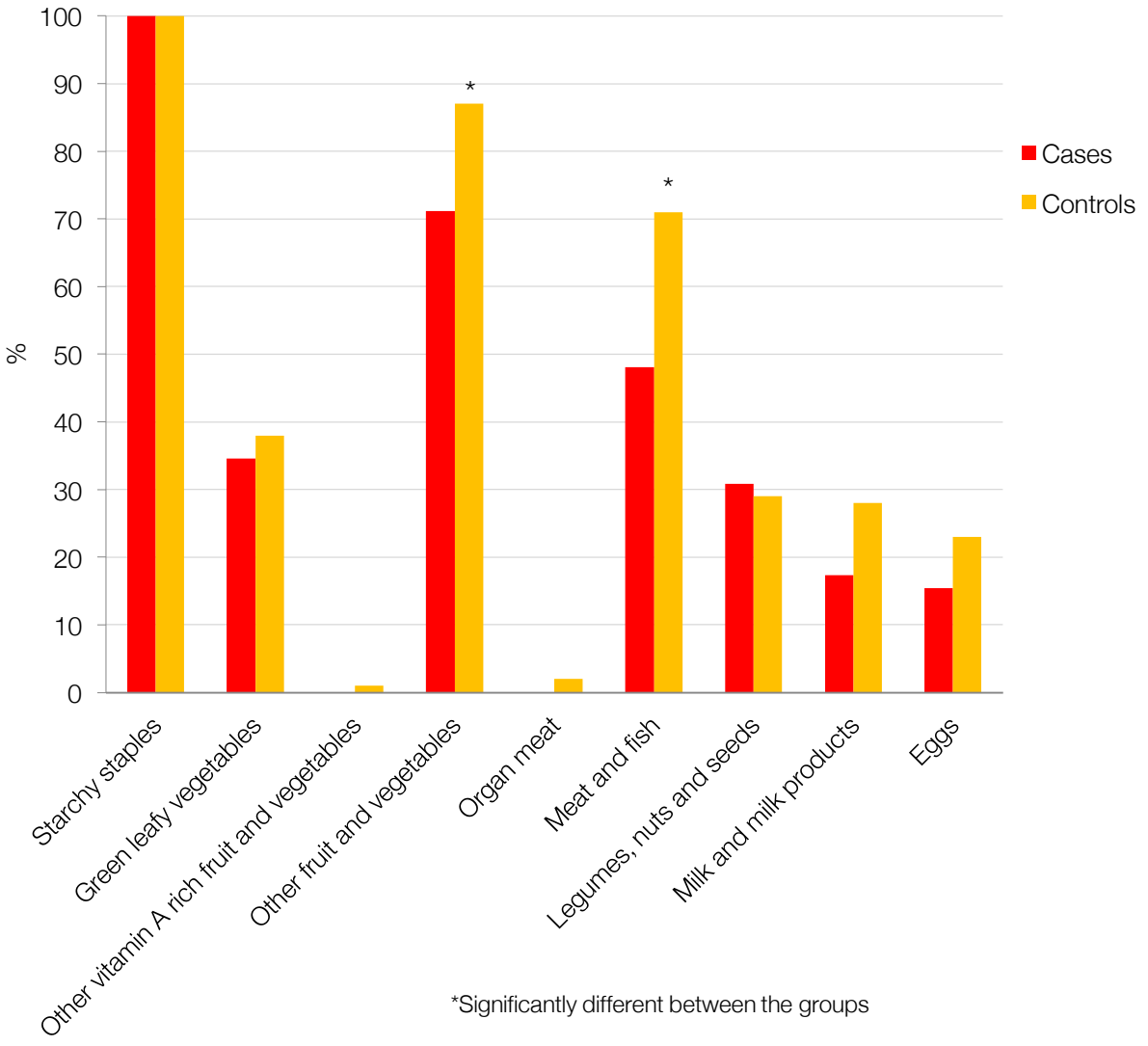

Figure 1- Proportion of leprosy patients and controls consuming items from the 9 food groups

The results of the univariate and multivariate analysis are summarized in Table 3, and are addressed below per block.

Block 1: Demographic factors

Both religion and household size were significantly related to leprosy in the demographic block ($p<0.10$). Hindus were two times more likely to be in the patient group. A larger household was associated with a lower risk on leprosy.

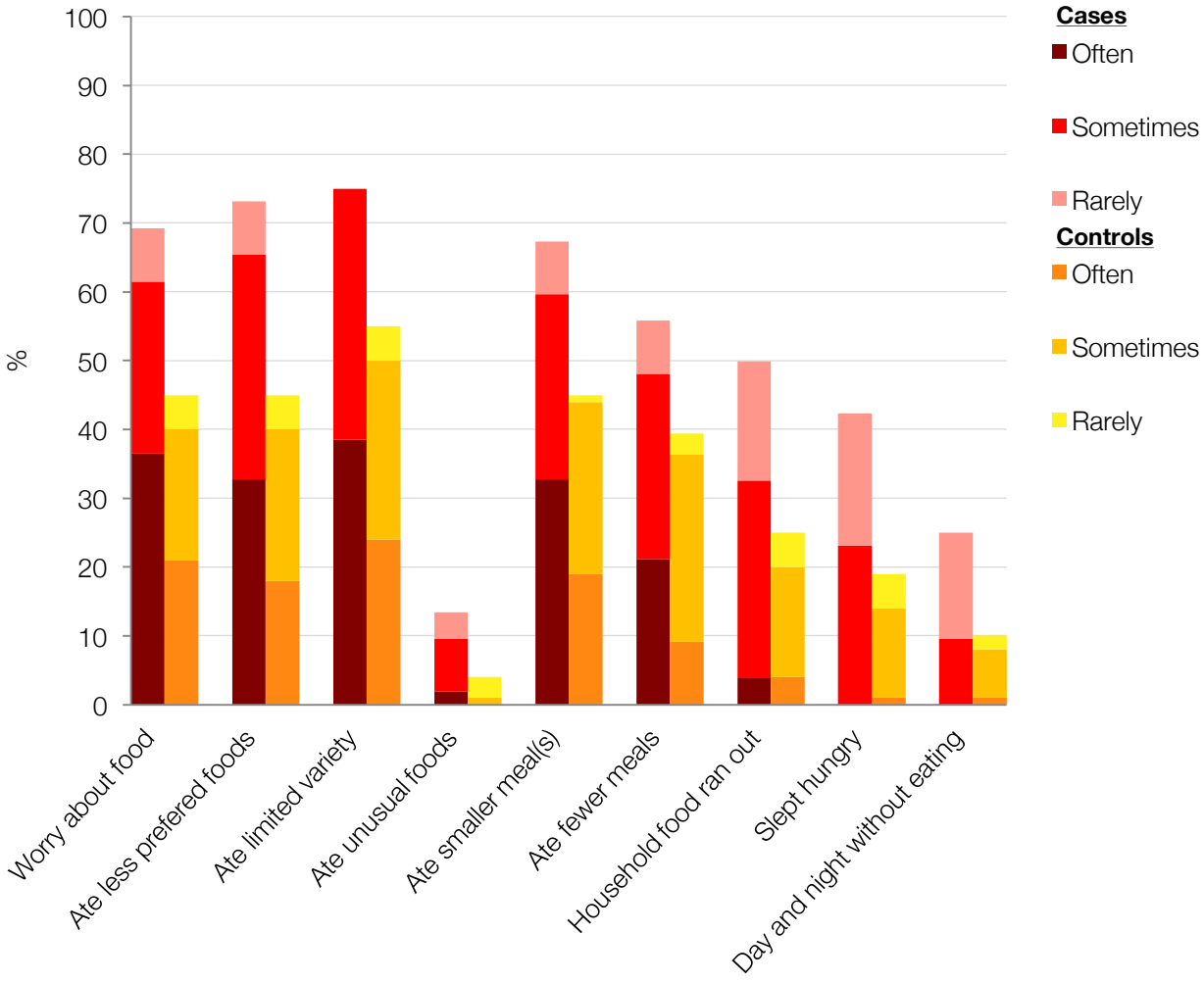

Figure 2- Frequency-of-occurrence for each Household Food Insecurity Access Scale item for leprosy patients and controls

Block 2: Socioeconomic factors

All variables in this block showed a significant association with leprosy in the univariate analysis. A higher income, land ownership, working as a farmer or a businessman and being part of a low/middle or middle income classified household lead to a decreased risk of leprosy. In the multivariate analysis of this socioeconomic block, step by step income per capita, land ownership, and self-classification were removed. The factors that remained significant were food expenditure per capita ($p<0.05$) and occupation ($p<0.10$).

Block 3: Health factors

BCG vaccination coverage was almost similar in both groups, and therefore did not show a relation to leprosy. BMI was the only significant factor in the uni- and multivariate analysis ($p<0.05$); a lower BMI increased the risk of leprosy.

Block 4: Diet-related factors

In the univariate analysis, higher HFIAS, food shortage experienced in the past year and at any time in life were significantly associated with an increased risk of leprosy, while higher DDS and household food stocks reduced the chance of having leprosy. In the multivariate analysis of this block, only DDS and household food stocks remained significant ($p<0.05$).

Integrated analysis

In addition to the analyses per block, we considered relationships between the blocks. Therefore, all significant factors from each of the four blocks were analysed together in a final integrated analysis, presented in Table 4. After stepwise elimination of the least significant factors, two factors remained significantly associated with leprosy: food expenditure (log) and household size ($p\leq0.05$).

Discussion

In this study, we set out to identify factors through which food shortage may have an effect on the development of leprosy, and in particular addressed the question whether recently diagnosed leprosy patients and controls had different dietary intakes. Our findings show that leprosy patients have a less favourable position with regard to socioeconomic, health and nutritional factors than a control population. Lower food expenditure per capita, lower BMI, lower dietary diversity score (DDS) and absence of household food stocks were the main factors associated with an increased risk of having leprosy.

Food shortage experienced in the past year was significantly associated with leprosy in the univariate analysis, but not in the multivariate analysis. In our study, the proportions of patients and control subjects experiencing food shortage in the past year were higher than in the study of Feenstra et al.: 80.8% vs. 47.8% for patients and 64.0% vs. 35.7% for the controls, respectively. This is remarkable because the studies were carried out in the same geographical area, used the same definition of food shortage, and no important changes were seen in income and food availability over the four years [23]. In the study of Feenstra et al., food shortage was only one item of an extensive socioeconomic questionnaire, however, while the focus of our questionnaire was entirely on diet and food insecurity. This could possibly explain the higher percentages observed in our study.

Dietary Diversity Score

Traditionally, a Bengali diet consists mainly of rice. Previous studies found that people from Bangladesh get between 74% and 86% of their energy intake from rice [12,22,24]. This suggests that consumption of nutritious non-rice foods is relatively low, which may explain the low DDSs found in our study. These studies demonstrated that the amount of rice a person consumes remains stable during the year, independent of rice price and season [12,25]. As a result, in the period September-November, when rice prices are high and income is low, expenditures on highly nutritious, generally more expensive, food products are likely to be lower. Consequently, dietary diversity scores are lower during these periods. To our knowledge, this is the first published study investigating the DDS in leprosy patients, and therefore there is no data to compare our results with. However, dietary diversity studies carried out among Bangladeshi women found a mean DDS of 3.6 ±1.1 and 3.4 ±1.1, which are very close to our findings of 3.2 ±1.1 and 3.8 ±1.4 for patient and controls, respectively [22,26].

For the DDS, the condiments chilies and garlic were not counted, in spite of the fact that they are used in almost all dishes according to the women participating in our focus group discussions. Still, the amounts consumed per person were likely to be very low, and therefore the contribution to the diet and dietary diversity should be negligible [22].

Food expenditure

Food expenditure was another important factor associated with leprosy. However, per capita food expenditure and per capita income were highly correlated (Spearman's correlation coefficient: 0.81, $p<0.001$) and additional analyses demonstrated that income and food expenditure are interchangeable in the block- and integrated analysis. The association with leprosy could therefore be just through poverty in general, which is a risk factor often associated with leprosy [27]. Two studies in Bangladesh have linked food expenditure and income to dietary diversity, a variable that was statistically significantly related to leprosy in our study [24,28]. In the study of Thorne-Lyman et al., household dietary diversity increased with increasing food expenditure, and primarily the intakes of animal source foods and fruits increased strongly with higher food expenditures. Our control population had significantly higher food expenditures, and accordingly had higher intakes of these food groups.

Household size

The second variable that remained statistically significant in the integrated analysis was household size. A larger household size gives a lower risk on leprosy. In the study of Feenstra et al., mean household size was larger in the control group as well, although this was not statistically significant. In theory, however, a larger household could increase the risk of leprosy, since it increases the chance of transmission. Nevertheless, in an Indonesia-based study an increased risk was found only for households larger than seven people [29], while in our study no more than 9 of 152 households counted more than seven people; two (3.8%) in the patient group and seven (7%) in the control group.

Table 3- Results of univariate logistic regression and multivariate logistic regression per block

Factors		Cases n=52	Controls n=100	Univariate† OR	(95% CI)	p-value	Multivariate OR	(95% CI)	p-value
Block 1: Demographic factors									
Age (years)		35.0 ± 9.5	33.3 ± 10.4	1.02	(0.99-1.05)	0.269			
Sex	Male	29 (56%)	48 (48%)						
	Female	23 (44%)	52 (52%)	0.69	(0.35-1.37)	0.294			
Religion	Muslim	40 (77%)	88 (88%)						
	Hindu	12 (23%)	12 (12%)	2.21	(0.90-5.38)	0.082#	2.23	(0.92-5.46)	0.079
Household size		4.6 ± 1.4	5.2 ± 2.1	0.83	(0.67-1.02)	0.075#	0.82	(0.66-1.02)	0.073
Block 2: Socioeconomic factors									
Income per capita (log)		2.96 ± 0.27	3.12 ± 0.30	0.10	(0.03-0.44)	0.002*			
Food expenditure per capita (log)		2.98 ± 0.17	3.08 ± 0.18	0.02	(0.00-0.22)	0.001*	0.03	(0-0.36)	0.006*
Self-classification						0.005*			
	Very poor	17 (33%)	14 (14%)	1.00					
	Poor	21 (40%)	29 (29%)	0.61	(0.24-1.50)				
	Low/middle	11 (21%)	35 (35%)	0.26	(0.10-0.69)				
	Middle	3 (6%)	22 (22%)	0.11	(0.03-0.47)				
	Rich	0 (0%)	0 (0%)	-					
Occupation						0.025*			0.058
	Laborer	26 (50%)	28 (28%)	1.00			1.00		
	Shopkeeper	10 (19%)	13 (13%)	0.84	(0.31-2.27)		1.28	(0.44-3.80)	
	Other	8 (15%)	25 (25%)	0.32	(0.12-0.86)		0.44	(0.16-1.22)	
	Farmer	5 (10%)	19 (19%)	0.28	(0.09-0.86)		0.24	(0.07-0.83)	
	Business	3 (6%)	15 (15%)	0.19	(0.05-0.76)		0.31	(0.07-1.34)	
Land						0.042*			
	Landless	41 (79%)	58 (58%)	1.00					
	Land leaser	3 (6%)	8 (8%)	0.49	(0.12-1.99)				
	Land owner	8 (15%)	34 (34%)	0.34	(0.14-0.81)				
Block 3: Health factors									
Disease other than leprosy	No	24 (46%)	49 (49%)						
	Yes	28 (54%)	51 (51%)	1.12	(0.57-2.22)	0.742			
BCG vaccination	No	26 (50%)	46 (46%)						
	Yes	26 (50%)	54 (54%)	0.89	(0.45-1.76)	0.743			
BMI (kg/m)		20.3 ± 3.1	21.6 ± 3.0	0.87	(0.77-0.98)	0.020*	0.87	(0.77-0.98)	0.020*
Block 4: Diet-related factors									
HFIAS (score 0-27)		10.2 ± 7.4	6.4 ± 0.19	1.08	(1.03-1.13)	0.003*			
DDS (score 0-9)		3.2 ± 1.1	3.8 ± 1.4	0.67	(0.50-0.89)	0.007*	0.71	(0.52-0.96)	0.024*
Recent food shortage	No	10 (19%)	36 (36%)						
	Yes	42 (81%)	64 (64%)	2.42	(1.07-5.47)	0.034*			
Ever food shortage	No	2 (4%)	16 (16%)						
	Yes	50 (96%)	84 (84%)	4.30	(0.93-19.77)	0.061#			
Household food stocks	No	25 (48%)	26 (26%)						
	Yes	27 (52%)	74 (74%)	0.38	(0.19-0.78)	0.008*	0.45	(0.22-0.95)	0.036*

†Adjusted for age and sex; BCG: Bacillus Calmette-Guérin; BMI: Body Mass Index; HFIAS: Household Food Insecurity Access Scale; DDS: Dietary Diversity Score; * statistically significant at 0.05 level; # statistically significant at 0.10 level

Table 4- Results of the integrated logistic regression analyses containing the significant variables of the multivariate analysis per block

Factors		Before backward elimination			After backward elimination		
		OR	(95% CI)	p-value	OR†	(95% CI)	p-value
Age		1.00	(0.97-1.05)	0.797	1.02	(0.98-1.05)	0.424
Sex	Male	1.00			1.00		
	Female	0.45	(0.20-1.00)	0.050	0.52	(0.25-1.10)	0.086
Religion	Muslim	1.00					
	Hindu	1.41	(0.52-3.88)	0.502			
Household size		0.76	(0.55-1.04)	0.084	0.68	(0.52-0.89)	0.005*
Food expenditure		0.02	(0.00-0.45)	0.014*	0.005	(0.00-0.08)	<0.001*
Occupation				0.294			
	Labourer	1.00					
	Shopkeeper	2.08	(0.62-6.98)				
	Farmer	0.47	(0.12-1.89)				
	Business	0.66	(0.13-3.25)				
	Other	0.59	(0.20-1.72)				
BMI		0.90	(0.78-1.04)	0.163			
DDS		0.83	(0.58-1.18)	0.299			
Household food stocks	No	1.00					
	Yes	0.66	(0.29-1.50)	0.320			

†Calculated OR's are adjusted for all other variables in the column, * statistically significant at 0.05 level; BMI: Body Mass Index; DDS: Dietary Diversity Score

Effect of nutrition on leprosy

The exact role of malnutrition on susceptibility to leprosy and the development to a clinical stage remains unclear [30,31]. A recent review on micronutrients and the immune response in leprosy emphasizes this knowledge gap, as only few studies in this field have been carried out and most of the evidence is derived from other diseases, mainly tuberculosis. Apart from this, previous studies are based either on blood analyses, or focussed on diets of (cured) leprosy patients after they developed clinical leprosy. In both cases, it is difficult to determine if leprosy is a cause or a consequence of nutritional deficiencies [32-36]. *Mycobacterium leprae* is an intracellular micro-organism, thus a cell-mediated immune response is important in the defence of the human host [37]. Protein-energy malnutrition, as well as inadequate intake of vitamins and/or minerals are linked to a reduced cell-mediated immunity [38]. With lower intakes of several food groups during food shortage periods, deficiencies may have put leprosy patients in our study at risk for a reduced cell-mediated immunity.

Another interesting theory that could possibly apply to leprosy is that of immune reconstitution, a well-known phenomenon in HIV after anti-retroviral therapy [39,40]. When the immune response is restored after a period of suppression, the immune system will start to respond to

infections present in the body. We hypothesize that, when food intake is improved after a long period of food shortage and nutrient deficiencies, the body may start to respond to *M. leprae*, causing the development of clinical disease.

This study has some limitations. First, data were collected referring to the period after the diagnosis of leprosy, which makes it hard to determine a causal relationship. However, the interviews were held shortly after diagnosis (maximum of nine months) and we specifically asked for changes in the patients' diet and income after diagnosis, to be able to correct for this difference. Second, a cross-sectional design was employed because we aimed to collect data during a food shortage period. We assumed that the persons experiencing food shortage this year have also experienced this in the previous years and that their diets did not change over time. Ideally, a longitudinal study, collecting detailed data on diet and health, and taking blood samples to determine nutrient absorption, should be carried out to compare long-term data of the persons who developed leprosy with data of persons who did not. However, this will be a lengthy and expensive process. A third limitation is that most of the data were self-reported through questionnaires, introducing recall and response biases. Especially for very poor people with an unstable income their average income is difficult to estimate. By asking the same questions to cases and controls we tried to limit the effect on our results. In addition to the 24-hour recall, biomarkers for micro- and macronutrients in blood, urine and/or faeces can be analysed to assess dietary intake more objectively [41]. However, we decided not to use this method because collection and analyses of biomarkers is laborious and costly, especially because a high number of nutrients need to be analysed since the key nutrients are unknown.

Fourth, the DDS might have been over or underestimated as it was based on one 24h-recall, which may not be representative of the usual diet. We tried to get the most reliable information possible by avoiding a recall for atypical days such as religious holidays. Furthermore, in developing countries diets tend to have a low day-to-day variability, thus one 24h-recall may be enough to get a good idea of the usual diet. In addition, when comparing population groups, a single dietary recall should give an accurate estimation of the intake of a whole group [42]. The DDS informs about the last 24 hours, while we extrapolated this to the diet before leprosy was diagnosed. Only few patients indicated that their food intake was different from that of last year, before diagnosis, however. Data of these patients were kept in the analyses, because there was little difference in the numbers of people who consumed more (4 patients) and who consumed less (5 patients) than in the period before they were diagnosed.

In conclusion, this study adds to the current knowledge on food shortage, nutrition and leprosy. We found that DDS and household food stocks are the most important diet-related factors associated with leprosy in Bangladesh. Furthermore, BMI and food expenditure per capita have a strong association with leprosy in our study. People living in poverty have less money available to spend on food. This results in a low consumption of animal source foods, fruits and vegetables. Deficiencies of the nutrients that these types of foods provide could result in an impaired immune response, which may be an explanation for the development of clinical leprosy. It is evident that little research has been carried out on the association between leprosy

and nutrition, and that the immunological pathway leading to the clinical development of leprosy and the influence of nutrition should be studied further. Our results can be a starting point to elucidate the relation between nutrition and leprosy.

References

1. Fine PE, Sterne JA, Ponnighaus JM, Bliss L, Saui J, et al. (1997) *Household and dwelling contact as risk factors for leprosy in northern Malawi*. Am J Epidemiol 146: 91-102.
2. van Beers SM, Hatta M, Klatser PR (1999) *Patient contact is the major determinant in incident leprosy: implications for future control*. Int J Lepr Other Mycobact Dis 67: 119-128.
3. Moet FJ, Pahan D, Schuring RP, Oskam L, Richardus JH (2006) *Physical distance, genetic relationship, age, and leprosy classification are independent risk factors for leprosy in contacts of patients with leprosy*. J Infect Dis 193: 346-353. Epub 2005 Dec 2028.
4. Deps PD, Guedes BV, Bucker Filho J, Andreatta MK, Marcari RS, et al. (2006) *Characteristics of known leprosy contact in a high endemic area in Brazil*. Lepr Rev 77: 34-40.
5. Richardus JH, Meima A, van Marrewijk CJ, Croft RP, Smith TC (2005) *Close contacts with leprosy in newly diagnosed leprosy patients in a high and low endemic area: comparison between Bangladesh and Thailand*. Int J Lepr Other Mycobact Dis 73: 249-257.
6. World Health Organization *Epidemiological Record* 88: 365-380.
7. Kerr-Pontes LR, Barreto ML, Evangelista CM, Rodrigues LC, Heukelbach J, et al. (2006) Socioeconomic, environmental, and behavioural risk factors for leprosy in North-east Brazil: results of a case-control study. Int J Epidemiol 35: 994-1000. Epub 2006 Apr 1027.
8. Feenstra SG, Nahar Q, Pahan D, Oskam L, Richardus JH (2011) *Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study*. PLoS neglected tropical diseases 5: e1029.
9. Abdullah M, Wheeler EF (1985) *Seasonal variations, and the intra-household distribution of food in a Banlgadeshi village*. Am J Clin Nutr 41: 1305-1313.
10. Hassan N, Huda SN, Ahmed A (1985) *Seasonal patterns of food intake in rural Bangladesh*. Ecology of Food and Nutrition 17: 175-186.
11. Hillbruner C, Egan R (2008) *Seasonality, household food security, and nutritional status in Dinajpur, Bangladesh*. Food and Nutrition Bulletin 29: 221-231.
12. Tetens I, Hels O, Khan NI, Thilsted SH, Hassan N (2003) *Rice-based diets in rural Bangladesh: how do different age and sex groups adapt to seasonal changes in energy intake?* Am J Clin Nutr 78: 406-413.
13. Chandra RK (1997) *Nutrition and the immune system: an introduction*. Am J Clin Nutr 66: 460S-463S.
14. Wintergerst ES, Maggini S, Hornig DH (2007) *Contribution of selected vitamins and trace elements to immune function*. Ann Nutr Metab 51: 301-323. Epub 2007 Aug 2028.
15. Bangladesh Bureau of Statistics (2010) *Report of the household income and expenditure survey* 2010.
16. Moet FJ, Pahan D, Oskam L, Richardus JH (2008) *Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster*

randomised controlled trial. BMJ 336: 761-764. doi: 710.1136/bmj.39500.885752.BE. Epub 882008 Mar 885710.

17. Moet FJ, Schuring RP, Pahan D, Oskam L, Richardus JH (2008) T*he prevalence of previously undiagnosed leprosy in the general population of northwest Bangladesh*. PLoS neglected tropical diseases 2.
18. Coates J, Swindale A, Bilinsky P (2007) *Household Food Insecurity Access Scale (HFIAS) for Measurement of Food Access: Indicator Guide* v. 3. Washington DC: Food and Nutrition Technical Assistance Project.
19. Kennedy G, Ballard T, Dop M (2013) *Guidelines for measuringhousehold and individual dietary diversity.* Rome: FAO.
20. Arimond M, Wiesmann D, Becquey E, Carriquiry A, Daniels MC, et al. (2010) *Simple food group diversity indicators predict micronutrient adequacy of women's diets in 5 diverse, resource-poor settings* 1-7. Journal of Nutrition 140: 2059S-2069S.
21. Ruel MT (2002) *Is dietary diversity an indicator of food security or dietary quality? A review of measurement issues and research needs*, FCND Discussion Paper No.104. Washington DC: International Food Policy Research Institute
22. Arimond M, Torheim LE, Wiesmann D, Joseph M, Carriquiry A (2009) *Dietary Diversity as a Measure of the Micronutrient Adequacy of Women's Diets: Results from Rural Bangladesh Site*. Washington DC: Food and Nutrition Technical Assistance II Project, FHI 360.
23. World Food Programme (2013) *Bangladesh Food Security Monitoring Quarterly Bulletin.*
24. Thorne-Lyman AL, Valpiani N, Sun K, Semba RD, Klotz CL, et al. (2010) *Household dietary diversity and food expenditures are closely linked in rural Bangladesh, increasing the risk of malnutrition due to the financial crisis*. Journal of Nutrition 140: 182S-188S.
25. Torlesse H, Kiess L, Bloem MW (2003) *Association of household rice expenditure with child nutritional status indicates a role for macroeconomic food policy in combating malnutrition*. J Nutr 133: 1320-1325.
26. Arsenault JE, Yakes EA, Islam MM, Hossain MB, Ahmed T, et al. (2013) *Very low adequacy of micronutrient intakes by young children and women in rural bangladesh is primarily explained by low food intake and limited diversity.* Journal of Nutrition 143: 197-203.
27. Lockwood DNJ (2004) Commentary: Leprosy and poverty. International Journal of Epidemiology 33: 269-270.
28. Rashid DA, Smith L, Rahman T (2006) *Determinants of dietary quality: evidence from Bangladesh*. American Economics Association Annual Meeting. Long Beach, California.
29. Bakker MI, Hatta M, Kwenang A, Van Mosseveld P, Faber WR, et al. (2006) *Risk factors for developing leprosy--a population-based cohort study in Indonesia*. Lepr Rev 77: 48-61.
30. Keusch GT (2003) *The history of nutrition: malnutrition, infection and immunity*. J Nutr 133: 336S-340S.
31. Scrimshaw NS, SanGiovanni JP (1997) *Synergism of nutrition, infection, and immunity: an overview*. Am J Clin Nutr 66: 464S-477S.

32. Diffey B, Vaz M, Soares MJ, Jacob AJ, Piers LS (2000) *The effect of leprosy-induced deformity on the nutritional status of index cases and their household members in rural South India: a socio-economic perspective*. Eur J Clin Nutr 54: 643-649.

33. Khadapani T, Mishra BK (2010) *Health problems and nutritional status of selected leprosy victims of Burla town, Orissa, India*. Current Research Journal of Social Sciences 2: 350-357.

34. Montenegro RMN, Zandonade E, Molina MCB, Diniz LM (2012) *Reactional state and nutritional profile among leprosy patients in the primary health care system, greater Vitoria, Espirito Santo State, Brazil*. Cadernos de Saude Publica 28: 31-38.

35. Oh SY, Paik HY, Ju D (1998) *Dietary habits, food intake and functional outcomes in those with a history of Hansen's disease in Korea.* International Journal of Leprosy 66: 34-42.

36. Rao PSS, John AS (2012) *Nutritional status of leprosy patients in India.* Indian Journal of Leprosy 84: 17-22.

37. Harboe M (1985) T*he immunology of leprosy*. In: Hasting RC, editor. Leprosy. New York: Longman Group Ltd. pp. 53-87.

38. Ferguson A, Griffin GE (2000) N*utrition and the immune system*. In: Garrow JS, James WPT, Ralph A, editors. Human Nutrition and Dietetics. London: Elsevier Science Ltd. pp. 747-764.

39. Hirsch HH, Kaufmann G, Sendi P, Battegay M (2004) *Immune reconstitution in HIV-infected patients.* Clinical Infectious Diseases 38: 1159-1166.

40. Lawn SD, Bekker LG, Miller RF (2005) *Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals*. Lancet Infectious Diseases 5: 361-373.

41. Jenab M, Slimani N, Bictash M, Ferrari P, Bingham SA (2009) *Biomarkers in nutritional epidemiology: Applications, needs and new horizons*. Human Genetics 125: 507-525.

42. Gibson RS, Ferguson EL (2008) *An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries*. Washington, DC: IFPRI and CIAT.

Chapter 9

General discussion

In this thesis, methods to improve the detection and treatment of leprosy neuropathy were studied in order to prevent irreversible nerve function impairment and disabilities. Also, nutritional risk factors that could be related to the development of clinical leprosy were assessed. In this section, I will answer the research questions posed in the introduction by discussing the key findings of the previous chapters. Also, some practical implications and recommendations for future studies will be given.

Research Question 1

How can the detection of nerve function impairment in leprosy at field level be improved?

Answer:

Detection of NFI can be improved by including the radial cutaneous and in particular the sural nerve in routine sensory testing. These two nerves are often affected and are affected in an early stage. This can lead to a higher sensitivity for earlier detection of NFI in general. Furthermore, NFI can be detected even earlier in the field when using portable warm and cold sensation tests such as the Thermal Sensibility Tester and the NeuroQuick.

Leprosy patients are at risk of developing nerve function impairment, even after they have been treated with multidrug therapy [1-3]. Since sensory and motor nerve function can lead to severe disabilities and deformities of hands, feet and eyes, it is very important that NFI is detected at an early stage so that it can be treated with prednisolone and nerve function can be restored. In the TENLEP study, we followed the recommendation of the INFIR study to include the sural and radial cutaneous nerves in the assessments in our TENLEP trials. The sensory function of the sural and radial cutaneous was assessed with monofilaments on three test-sites for the first time. We established the normal monofilament threshold values for the sural and radial cutaneous nerves (*Chapter 3*), so that these tests can be applied routinely in the field. In *Chapter 4* we determined that the monofilament assessment of the sural and radial cutaneous nerves can be performed reliably when executed by trained staff.

The sural and radial cutaneous nerves are rarely examined in clinical practice, because impairment of these nerves is not likely to lead to ulceration or burns due to the position of the area they innervate - the lateral border of the foot for the sural nerve, and the dorsal side of the hand for the radial cutaneous nerve. In the INFIR study, however, the sural nerve was the second most affected nerve when assessed with MFT [4]. Furthermore, the sural and radial cutaneous nerves were affected most frequently when assessed for subclinical neuropathy by nerve conduction studies [4]. These two nerves may not be so relevant from a practical point of view, but they can function as sensitive sentinels for NFI in general. Including the sural and radial cutaneous in the clinical assessment routinely would likely lead to earlier detection of NFI and early treatment can prevent further damage to more significant peripheral nerves.

The significance of assessing the sural and radial cutaneous was originally demonstrated in INFIR study, and subsequently confirmed in the TENLEP study. Adding the sural and radial cutaneous nerves to the Composite Nerve Score increased its reliability (*Chapter 6*).

Other methods that may improve early detection of NFI were discussed in *Chapter 5*. In patients with subclinical neuropathy, new, portable devices were compared with the standard reference tests Warm Detection Threshold and Nerve Conduction Studies. The Thermal Sensibility Tester (TST) and the NeuroQuick showed high sensitivity and specificity for the detection of subclinical neuropathy. The TST assesses warm sensation and was originally designed to test sensation in leprosy skin lesions. One of the ends becomes warm when switched on, with a temperature between 45°C and 60°C depending on environmental temperature. The NeuroQuick tests cold sensation using an air flow produced by the integrated fan. The adjustable fan speed can be increased until the patient perceives the air flow.

Both the NeuroQuick and the TST examine small fibre function. The INFIR study found, however, that there was variation between patients in which modality and type of nerve fibre - large or small - was first affected [5]. This variation should be covered when testing for early neuropathy and therefore we recommend adding a second test method that assesses large fibre function. Unfortunately, the two devices in our study testing large fibres, the NC-stat DPNCheck and the Vibratip, are not very suitable for early detection of leprosy neuropathy. So far nerve conduction studies are the only reliable option for detection of early large fibre neuropathy.

Monofilaments as well as the portable TST and the NeuroQuick are very useful to detect neuropathy under field conditions. They are relatively cheap compared to TSA and the monofilament test is reliable. Reliability for the TST and the NeuroQuick needs to be assessed further. It is important to underline that for these methods some training is required and therefore it is important to regularly carry out a quality control such as reliability testing.

To improve early detection of NFI we recommend including the radial cutaneous and in particular the sural nerve in routine sensory testing, especially in referral centres. Also, the use of early, subclinical detection methods such as Thermal Sensibility Tester and/or the NeuroQuick is advised. Good training protocols should be developed to support a reliable execution of the testing. Suggestions for further research are:

- For the Thermal Sensibility Tester and the NeuroQuick study, reliability tests should be done. Test-retest and inter-rater reliability should be studied in preferable different leprosy populations.
- The sensitivity and specificity of the Thermal Sensibility Tester and the NeuroQuick test should be determined for testing on the hands.
- The search for devices should be continued, to find alternative portable, battery operated tests that accurately and reliably detect subclinical large fibre neuropathy.

Research Question 2

How can irreversible nerve function impairment best be prevented in leprosy?

Answer:

A prednisolone course of 20 weeks was sufficient to improve or restore nerve function in the majority of leprosy patients with recent nerve function impairment (NFI). Longer prednisolone treatment could be a solution for the 15% of patients who needed additional prednisolone due to reactions or new NFI.

While prednisolone is commonly used to treat recent nerve function impairment (NFI) in leprosy patients, the optimal treatment duration has not yet been established [6]. As early treatment is stimulated in leprosy neuropathy, the detection and treatment of subclinical neuropathy would be a logical step. However, the first results of studies into prophylactic treatment or treatment of subclinical neuropathy are not very promising [7,8]. The TENLEP study on clinical neuropathy was designed to evaluate whether a 32-week prednisolone course is more effective than a 20-week course in restoring and improving nerve function (*Chapter 6*). We found that there was no difference in the percentage of patients with restored and improved nerve function between the two courses. Both treatment groups showed improvement rates of 78%, with 24-30% of these patients recovering completely. Unfortunately, in other patients the immune response against (dead) *M. leprae* bacilli seems too persistent to be permanently suppressed, even by a 32-week prednisolone course [9]. In both groups, around 15% of patients needed additional prednisolone due to reaction or new NFI.

Our study provides insight in the characteristics of this group of patients that needed additional treatment with prednisolone, as they were different than the group that did not need extra prednisolone. At baseline, the former group contained significantly more MB patients - mainly BL and LL-, had higher average bacterial index (BI), had a higher EHF score, suffered more often from both type 1 and type 2 reactions, and more often showed signs of neuritis and reactions, i.e. inflamed and raised skin lesions, peripheral oedema, nerve pain and tenderness. This information can be an important addition to the Clinical Prediction Rule, developed by Croft et al. to determine which patients have a high risk of developing NFI [10]. Three risk levels - low, medium and high - were defined in their study, where MB patients with NFI at baseline have the highest risk of developing NFI within 2 years of diagnosis. Our result can strengthen the Clinical Prediction Rule, by defining the group that need extra surveillance when on treatment for NFI. People at risk for ‘resistance’ of prednisolone treatment are quite similar to the people at risk for new NFI, i.e. MB patients with previous reaction or nerve function impairment problems.

Future studies should focus on this group of patients who do not seem to benefit from prednisolone treatment of the duration assessed in the TENLEP study. First, it is important to unravel the immunological and pathophysiological mechanisms behind NFI and to study the actual effect of prednisolone on NFI. Second, an even longer prednisolone course could be beneficial for this group. Naafs et al. found that some leprosy patients may need up to 20

months of treatment [11]. However, the TENLEP study showed that longer prednisolone leads to more Serious Adverse Events (*Chapter 7*). An option would be to test longer, low-dose steroid courses, as is practiced for the treatment of autoimmune diseases, such as rheumatoid arthritis (4.5-10 mg/day for 2 years) [12] or autoimmune hepatitis (5-12 mg/day for 3 years) [13]. Third, an effort should be made to develop alternative drugs. Currently, prednisolone is the drug of choice for the treatment of NFI, but other anti-inflammatory drugs are available as well, mainly as second line treatment for reactions [14]. Clofazimine, thalidomide, cyclosporine and azathioprine have been studied for reactions, but so far their effect is not as durable as prednisolone.

As non-drug option, surgical treatment is available for patients with severe nerve inflammation, i.e. nerve decompression. This might be an alternative method to restore NFI in some patients. Through an incision of the thickened nerve sheath, pressure on the nerve due to oedema is relieved [15]. However, so far there is not enough evidence of the effectiveness of surgical nerve decompression [16].

In conclusion, for a large group of patients, a 20-week course of prednisolone is sufficient to improve or restore nerve function. It is important to have a better understanding on which patient characteristics determine the outcome of prednisolone treatment. In addition, the pathophysiology of NFI needs to be unravelled. So far, an alternative for prednisolone is lacking and there is no sufficient evidence that surgical treatment is beneficial. Recommendations for further research are:

- Validate the characteristics of the group of patients not responding to a 20-week or 32-week course of prednisolone.
- Assess longer, low-dose prednisolone courses in the group not responding to a 20 or 32-week course of prednisolone for the treatment of NFI.
- Continue the search for alternative drugs for the treatment of NFI.
- Carry out a – preferably multicentre - trial to establish the effect of surgical nerve decompression.

Research Question 3

Which nutritional factors possibly underlie the development of clinical leprosy?

Answer:

Leprosy patients are more likely to have a low dietary diversity. Reduced intake of meat, fish, fruits and vegetables leads to protein-energy malnutrition and/or inadequate intake of vitamins and minerals. These nutrient deficiencies could reduce the body's immune response towards *M. leprae*, and therefore give way to the development of clinical leprosy.

The risk factors for contracting leprosy and the further development into the clinical stage of the disease are largely unknown. Recently, two studies linked food shortage to the clinical manifestation of leprosy [17,18]. We investigated this association further by studying whether during a period of food shortage the dietary intake differed between recently diagnosed leprosy patients and control subjects (*Chapter 8*). Food shortage was defined as 'the period in which a family had to reduce the number of meals a day, or reduce the intake of foods other than rice'. Our study took place in a leprosy endemic region in Bangladesh, where food shortage is mainly experienced in the period between September and the end of November, just before the major rice harvest in December. Our findings show that leprosy patients have a less favourable position with regard to socioeconomic, health and nutritional factors than a control population from the same area. Leprosy patients had a lower dietary diversity and were more often and more severe food insecure. They consumed less meat, fish, fruits and vegetables, and to a lesser extent eggs, milk and milk products, than the control group. Furthermore, food stocks were lower and more often absent in households of leprosy patients. Most likely, this is explained by the lower food expenditure in patients' households, a factor highly correlated with income. Using a retrospective study makes it difficult to study the causal relation between food shortage and leprosy, as the association could work both ways - food shortage could cause leprosy, or leprosy could cause food shortage. However, by interviewing patients shortly after diagnosis and asking for changes compared to last year we were reasonably certain that our outcomes were a good representation of the situation in the previous year.

The exact role of malnutrition on leprosy susceptibility and the further development to a clinical stage remains unclear [19,20]. The risk of contracting subclinical *M. leprae* infection is not necessarily increased by food shortage, but malnourishment can cause progress of a latent infection into a clinical, active disease [21]. The cell-mediated immunity plays an important role in the response against *M. leprae* [22], and this immune function can be deteriorated by protein-energy malnutrition as well as micronutrient deficiencies [23]. Considering our finding that leprosy patients had significant lower intake of protein sources, such as meat and fish, and micronutrient sources as fruit and vegetables, a reduced cell-mediated immunity seems a likely intermediary step towards the development of clinical leprosy. There is hardly any evidence for this theory though, because of the complex interactions between the different deficiencies,

environmental factors such as hygiene, safe water, housing, access to health services, and the previous described two-way causal relationship. It is therefore important to better understand the mechanisms and interactions of the mentioned factors on the development of clinical leprosy, and to find ways to influence these factors through e.g. vaccination, improved nutrition, and general socioeconomic support. Such support, in particular targeted at high risk groups for leprosy, would be an important addition to leprosy control programs.

Looking at the evidence so far, it is crucial that future research is focused on preventing clinical leprosy, especially because once nerves are clinically impaired, it is difficult to achieve complete recovery of nerve function (*Chapter 6*). Interrupting the transmission of *M. leprae* is the most promising intervention. Other possible sources of infection should be investigated and eliminated, for example reservoirs outside the human body such as soil, water, and armadillo's (in the America's) [24]. Also early diagnosis and treatment of leprosy is key to prevent further transmission of leprosy. Therefore, sensitive diagnostic tests need to be developed that can detect subclinical leprosy [25]. Additionally, prophylactic treatment of leprosy contacts can interrupt transmission. Studies targeting leprosy contacts show good results up to two years after chemoprophylaxis with single dose rifampicin [26].

In conclusion, the nutritional status of poor people living in leprosy endemic regions needs to be improved in order to reduce the chance that clinical leprosy develops after subclinical infection. This should be done by increasing their dietary diversity and food security. As long as transmission of *M. leprae* is ongoing, efforts should be made to prevent the development of clinical leprosy, and therefore we need to understand the mechanisms better. Recommendations for further research are:

- Study the immunological pathway and mechanisms leading to the clinical development of leprosy and the factors that can influence this
- Investigate if and how improving nutrition status of poor people living in leprosy endemic regions reduces leprosy prevalence and transmission
- Improve methods to detect subclinical leprosy

<u>Challenges in studying leprosy</u>

Leprosy prevalence has fallen significantly in the previous decades, mainly because of the introduction of multidrug therapy [27] and improved case finding [28]. However, the new case detection rate is still substantial - around 250.000 per year-, and has barely decreased over the last decade. The diminished decline can be very well explained by the knowledge gaps that still exist, even though leprosy is one of the oldest diseases known. The first uncertain factor is the method of *M. leprae* transmission, which is of course very important in relation to the number of new cases. Most likely, people get infected through nasal droplets via the respiratory tract [29]. In some cases, it might also be possible that *M. leprae* enters the body through abraded skin [27,30]. A second major factor is that no specific leprosy vaccine is available that can prevent

clinical leprosy, although much research has been done on this subject. Furthermore, there is not yet a reliable test available for early diagnosis and detection of subclinical disease. Establishing which person with subclinical infection would continue to progress to clinical disease and providing subsequent treatment would reduce the time a person is infectious and can spread the disease to others. Third, we do not completely understand the immunological pathways involved in leprosy infection and the reasons why some infected people develop clinical leprosy and others do not. It is most likely related to genetic differences [31,32], social distance, nutritional status, health status, and hygiene. Fourth, the immune mechanisms leading to NFI are largely unknown, as pointed out before in this thesis. And methods to identify high risk patients for NFI are lacking as well. Last, no ideal treatment regimen has been established to treat nerve function impairment and reactions.

The reason why so little is known about leprosy is because the disease is difficult to study. To explain, I will list some biological characteristics of *M. leprae* and mention epidemiological and social reasons. Because of the slow multiplication of *M. leprae*, the incubation time of leprosy is usually long. In extreme cases, symptoms appear after 20 years, but in most patients the incubation time is between 2 and 5 years [33]. This makes it nearly impossible to determine when and under which circumstances the disease was contracted, hence the knowledge gap on transmission of leprosy. A second characteristic that interferes with studying the pathology of leprosy is that *M. leprae* cannot be cultured in vitro. Furthermore, no suitable animal model is available for vaccine testing and to study pathogenesis. Experiments are performed on nude mice and armadillo's, but mice do not live long enough to follow the effects of the slowly growing *M. leprae* [34], and armadillo's develop mainly lepromatous infections.

Also from an epidemiologically point of view it is hard to study leprosy, as the incidence is relatively low. To study the pathology and transmission of the disease in humans, prospective studies are preferred, as the long incubation time makes retrospective studies less reliable. Since leprosy is such a rare disease many people need to be followed for a long time to get good results. This is time consuming and costly. In addition, because of the low prevalence it is very challenging to produce possible new vaccines cost-effectively. Last, but not least, a large social problem influences the success to study leprosy: stigma. Fear of stigma may lead to refusal to participate in scientific studies. And more importantly, stigma leads to delayed diagnosis and thus to a longer period of infectiousness and more severe and irreversible neuropathy.

All the above challenges in studying leprosy and in developing effective preventive and curative measures for leprosy and related disability are a main cause of the steady new case detection rate of leprosy and of the steady numbers of new cases with grade 2 disability in many endemic countries. Fortunately, progress is being made, and large multicentre studies such as TENLEP help increase our insight into the many difficult issues in leprosy that need to be addressed in order to achieve elimination of the disease and its related disabilities.

Recommendations

From the results and experience of the studies described in this thesis, I developed a set of recommendations to improve detection and treatment of neuropathy in the field:

- The sural and radial cutaneous nerves should be included in standard routine monofilament assessment
- Use more sensitive methods to detect neuropathy in an earlier stage. The portable Thermal Sensitivity Tester and NeuroQuick are promising devices for this goal
- Implement nutrition support for poor people during food shortage periods in leprosy endemic regions to increase dietary diversity

Furthermore, the results reported in this thesis lead to new questions and identified the need for more research and new studies. The topics that need to be studied further are:

- The sensitivity and specificity of the Thermal Sensibility Tester and the NeuroQuick test should be determined for testing on the hands
- The reliability of the Thermal Sensibility Tester and the NeuroQuick should be examined. Test-retest and inter-rater reliability should be studied in preferable different leprosy populations
- The search for devices should be continued, to find alternative portable, battery-operated tests that accurately and reliably detect subclinical large fibre neuropathy
- The characteristics of the group of patients not responding to a 20-week or 32-week course of prednisolone need to be determined in more detail
- Assess longer, low-dose prednisolone courses in the group of patients that is not responding to a 20- or 32-week course of prednisolone for the treatment of NFI.
- Set up studies on nutrition in new leprosy patients and test serum levels of different micronutrients in blood around first symptoms
- Investigate if and how improving nutrition status of poor people living in leprosy endemic regions reduces leprosy prevalence and transmission
- Study the immunological pathway and mechanisms leading to the clinical development of leprosy and the factors that can influence this
- Continue the search for alternative drugs for the treatment of NFI
- Carry out a multi-centre trial to establish the effect of surgical nerve decompression
- Focus on finding methods to prevent leprosy (early detection of leprosy (infection), prophylaxis, identify all ways of transmission (soil/water))

References

1. Croft, R.P., et al., *Incidence rates of acute nerve function impairment in leprosy: A prospective cohort analysis after 24 months (The Bangladesh Acute Nerve Damage Study)*. Leprosy Review, 2000. 71(1): p. 18-33.
2. Richardus, J.H., et al., *Nerve function impairment in leprosy at diagnosis and at completion of MDT: A retrospective cohort study of 786 patients in Bangladesh*. Leprosy Review, 1996. 67(4): p. 297-305.
3. Saunderson, P., et al., *The pattern of leprosy-related neuropathy in the AMFES patients in Ethiopia: Definitions, incidence, risk factors and outcome*. Leprosy Review, 2000. 71(3): p. 285-308.
4. van Brakel, W.H., et al., *Early diagnosis of neuropathy in leprosy--comparing diagnostic tests in a large prospective study (the INFIR cohort study)*. PLoS Negl Trop Dis, 2008. 2(4): p. e212.
5. McKnight, C.J., *Clinical testing and prognostic markers for the development of leprosy neuropathy,* in Dept. of Infectious and Tropical Diseases. 2010, London School of Hygiene & Tropical Medicine.
6. van Veen, N.H., et al., *Corticosteroids for treating nerve damage in leprosy. A Cochrane review*. Lepr Rev, 2008. 79(4): p. 361-71.
7. Smith, W.C.S., et al., *Steroid prophylaxis for prevention of nerve function impairment in leprosy: Randomised placebo controlled trial (TRIPOD 1)*. British Medical Journal, 2004. 328(7454): p. 1459-1462.
8. Brandsma, J.W., E.P. Post, and I.M. Wagenaar, *Prednisolone treatment of subclinical neuropathy in leprosy patients* (unpublished, in preparation). 2016.
9. Shetty, V.P., et al., *Persistence of Mycobacterium leprae in the peripheral nerve as compared to the skin of multidrug-treated leprosy patients*. Leprosy Review, 1992. 63(4): p. 329-336.
10. Croft, R.P., et al., A *clinical prediction rule for nerve function impairment in leprosy patients-revisited after 5 years of follow-up*. Lepr Rev, 2003. 74(1): p. 35-41.
11. Naafs, B., J.M. Pearson, and H.W. Wheate, R*eversal reaction: the prevention of permanent nerve damage. Comparison of short and long-term steroid treatment*. Int J Lepr Other Mycobact Dis, 1979. 47(1): p. 7-12.
12. Mota, L.M., et al., *Guidelines for the drug treatment of rheumatoid arthritis*. Revista brasileira de reumatologia, 2013. 53(2): p. 158-183.
13. Kanzler, S., et al., *Duration of immunosuppressive therapy in autoimmune hepatitis*. J Hepatol, 2001. 34(2): p. 354-5.

14. Lockwood, D.N. and P.R. Saunderson, N*erve damage in leprosy: A continuing challenge to scientists, clinicians and service providers*. International Health, 2012. 4(2): p. 77-85.

15. Husain, S., *Decompression of peripheral nerve trunks in leprosy prevents the development and progression of deformities?* Indian Journal of Leprosy, 2014. 85(4): p. 163-169.

16. van Veen, N.H., et al., *Decompressive surgery for treating nerve damage in leprosy. A Cochrane review*. Lepr Rev, 2009. 80(1): p. 3-12.

17. Feenstra, S.G., et al., *Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study*. PLoS neglected tropical diseases, 2011. 5(5): p. e1029.

18. Kerr-Pontes, L.R., et al., *Socioeconomic, environmental, and behavioural risk factors for leprosy in North-east Brazil: results of a case-control study*. Int J Epidemiol., 2006. 35(4): p. 994-1000. Epub 2006 Apr 27.

19. Keusch, G.T., *The history of nutrition: malnutrition, infection and immunity*. J Nutr., 2003. 133(1): p. 336S-340S.

20. Scrimshaw, N.S. and J.P. SanGiovanni, *Synergism of nutrition, infection, and immunity: an overview*. Am J Clin Nutr., 1997. 66(2): p. 464S-477S.

21. Chandra, R.K., 1990 McCollum Award Lecture. *Nutrition and immunity: Lessons from the past and new insights into the future*. American Journal of Clinical Nutrition, 1991. 53(5): p. 1087-1101.

22. Harboe, M., *The immunology of leprosy,* in Leprosy, R.C. Hasting, Editor. 1985, Longman Group Ltd: New York. p. 53-87.

23. Ferguson, A. and G.E. Griffin, *Nutrition and the immune system*, in Human Nutrition and Dietetics, J.S. Garrow, W.P.T. James, and A. Ralph, Editors. 2000, Elsevier Science Ltd.: London. p. 747-764.

24. Bratschi, M.W., et al., *Current knowledge on Mycobacterium leprae transmission: a systematic literature review*. Lepr Rev, 2015. 86(2): p. 142-55.

25. Roset Bahmanyar, E., et al., *Leprosy Diagnostic Test Development As a Prerequisite Towards Elimination: Requirements from the User's Perspective*. PLoS Neglected Tropical Diseases, 2016. 10(2).

26. Moet, F.J., et al., *Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomised controlled trial*. BMJ, 2008. 336(7647): p. 761-4.

27. World Health Organization. *Disease Watch Focus Leprosy.* 2003 [cited 2016 22 June]; Available from: http://www.who.int/tdr/publications/disease_watch/leprosy/en/.

28. Schreuder, P.A., S. Noto, and J.H. Richardus, *Epidemiologic trends of leprosy for the 21st century.* Clin Dermatol, 2016. 34(1): p. 24-31.

29. Hatta, M., et al., *Distribution and persistence of Mycobacterium leprae nasal carriage among a population in which leprosy is endemic in Indonesia*. Trans R Soc Trop Med Hyg, 1995. 89(4): p. 381-5.

30. Job, C.K., S.K. Chehl, and R.C. Hastings, *New findings on the mode of entry of Mycobacterium leprae in nude mice*. Int J Lepr Other Mycobact Dis, 1990. 58(4): p. 726-9.

31. Moet, F.J., et al., *Physical distance, genetic relationship, age, and leprosy classification are independent risk factors for leprosy in contacts of patients with leprosy*. J Infect Dis, 2006. 193(3): p. 346-53.

32. Scollard, D.M., et al., *The continuing challenges of leprosy*. Clin Microbiol Rev, 2006. 19(2): p. 338-81.

33. Noordeen, S.K., *The epidemiology of leprosy, in Leprosy*, C. Hastings, Editor. 1985, Churchill Livingstone: Edinburgh.

34. Faizal, M., *Animal Models in Leprosy*. South American Journal of Medicine 2013. 1(1).

Summary

In this thesis, methods were studied that could improve the detection and treatment of leprosy neuropathy, in order to prevent irreversible nerve function impairment and disabilities. Also, nutritional risk factors that could be related to the development of clinical leprosy were assessed

Leprosy is an infectious disease caused by the bacteria *Mycobacterium leprae*. The disease is often seen as a disease of the poor, especially since nowadays leprosy is mainly prevalent in low resource countries. 89% of the newly detected patients are found in eight countries: Bangladesh, Brazil, Democratic Republic of Congo, Ethiopia, India, Indonesia, Nepal and Nigeria, with India alone contributing as much as 60%. According to the WHO, leprosy is officially eliminated worldwide, meaning that there is less than 1 patient per 10 000 people. Unfortunately, for certain regions in the mentioned countries this is not the case yet, and leprosy is still being spread. The disease is transmittable, but not everyone who comes into contact with the bacillus will get leprosy. Whether the disease develops depends on multiple variables; it is assumed that genes, general health status, nutrition, hygiene and social distance to a patient play a role.

Leprosy affects the skin and the nerves, but the results of the nerve damage makes that the disease is considered harmful. Neuropathy may lead to nerve function impairments, for sensory as well as motor nerves. When sensory function is impaired, leprosy patients often do not feel when they burn themselves or have a wound, and in addition, they do not take good care of the wounds because they do not feel the pain. As a result of impaired motor function, muscles in the hand and feet can weaken. Both motor and sensory nerve function impairments can result in disabilities. Therefore, it is very important to detect nerve function impairment in an early stage so it can be treated. In general, it is assumed that impaired nerve function of less than 6 months will improve when treated with prednisolone. The optimal duration of prednisolone treatment, however, is unknown.

We have set up a study to investigate whether a longer than usual treatment with prednisolone has a more profound effect on the improvement of nerve function impairment. This study is known as the TENLEP study (Treatment of Early Neuropathy in LEProsy). Results of this and other studies are described in this thesis. The aim of this thesis was to answer the following research questions:

1. How can the detection of nerve function impairment in leprosy at field level be improved?
2. How can irreversible nerve function impairment best be prevented in leprosy?
3. Which nutritional factors possibly underlie the development of clinical leprosy?

Chapter 1 gives an introduction in the disease leprosy and extensive information on neuropathy. The four studies in the second part focus on methodology. In **chapter 2,** I describe the details of the protocol of the two randomized controlled TENLEP trials that we have carried out in six leprosy centres in four Asian countries. In the Clinical trial we assessed whether a

longer treatment with prednisolone, 32 weeks compared to 20 weeks, shows better results in the improvement of recent nerve function impairment. Additionally, in a parallel, placebo controlled trial, we studied whether prednisolone can prevent nerve function impairment in patients with subclinical neuropathy. This trial is named the Subclinical trial.

In the following three chapters I attempt to answer the first research question. In **chapter 3**, a study is described in which we determined the normal values for the monofilaments test of the radial cutaneous and sural nerves, by testing people without leprosy in India and Nepal. Earlier research has shown that these two nerves are the first affected nerves in leprosy neuropathy. Patients rarely have wounds in the innervation areas of the radial cutaneous and sural nerves however, therefore these nerves are seldom included in routine sensory testing. This is a missed opportunity, because the additional information can lead to earlier detection and treatment of nerve function impairment. To be able to interpret the outcomes of monofilament tests in leprosy patients, it is important to know what the threshold of normal sensitivity is in people without leprosy. We established that the purple 2-g monofilament is the normal threshold for de radial cutaneous nerve and the red 4-g filament is normal threshold for the sural nerve.

For the monofilament test (MFT) and voluntary muscle test (VMT), we have done reliability studies in the six leprosy centres. These studies are described in **chapter 4**. We can conclude that monofilament tests of the radial cutaneous nerve and sural nerve can be done reliably by trained staff. **Chapter 5** presents a study into alternative methods to detect nerve function impairment in an earlier stage. In this study we carry out diagnostic accuracy tests for five different 'gadgets' in 209 leprosy patients who have subclinical neuropathy. These five tests have been selected on their usefulness in the field: they are portable, battery operated, affordable and easy to carry out without much training. The five gadgets all examine the sensory nerves, but test different modalities: the Neuropad® tests sweat function, the Vibratip™ examines sensitivity for vibration, the NC-Stat®DPNCheck™ tests the nerve conduction, the NeuroQuick examines cold sensation and the Thermal Sensibility Tester (TST) tests warm sensation. The tests were performed on the sural nerve and/or the posterior tibial nerve. Additional assessments with two gold standard tests, the Warm Detection Threshold with the TSA II and Nerve Conduction with electromyography machines, allowed us to calculate the sensitivity and specificity of each gadget. The NeuroQuick and the TST showed the best results and we recommend to verify the reliability of these two tests. Using this type of gadgets would be a very good way to improve the early detection of nerve function impairment in leprosy patients in the field.

The third part contains three chapters that present the results of various studies. **Chapter 6** describes the most important results of the Clinical TENLEP trial, and discusses the second research question. In the Clinical trial, we compared in 868 leprosy patients a prednisolone treatment of 32 weeks and 20 weeks. By testing the nerve function with MFT and VMT monthly during the 32 weeks of the treatment period and at 52 and 78 weeks, we gained good insight into the effect of the two treatments. Our study shows that the two treatment durations lead to a similar result: in 78% of the patients nerve function was improved or restored. Also the

secondary outcomes, such as the Participation scale, the Activity limitation scale and Reaction Severity scale, did not differ between the two treatments. **Chapter 7** presents the Serious and Minor Adverse Events (SAE and MAE) of prednisolone treatment for both TENLEP trials. Examples of SAEs are: high blood pressure, diabetes, peptic ulcer and tuberculosis. For 20 weeks as well as 32 weeks of prednisolone treatment, the percentage of patients developing an SAE was low, 0.9% and 2.7% respectively. The patients who followed the longer treatment duration had significantly more SAEs though. Taking into account the results of **chapter 6**, i.e. the longer treatment was not more beneficial, the 32-week treatment is not worth the increased SAE risk.

The study in **chapter 8** answers the third research question. In an earlier study in Bangladesh, a recent period of food shortage was associated with the development of clinical leprosy. We aimed to further explore this relationship in a case-control study in Bangladesh, in which we compared the diet and food security of 52 recently diagnosed leprosy patients with a healthy control population of 100 people from the same region. By interviewing controls and patients, data was collected on dietary intake in the last 24 hours, household food security, income, and food expenditure. Our study showed that food shortage was more common in households of leprosy patients, and that the dietary diversity and food security were lower. Patients reported a lower food expenditure, had a lower body mass index and had less household food stocks. Because patients could spend less money on food, they had a lower intake of highly nutritious non-rice foods such as meat, fish, milk, eggs, fruits and vegetables. It could be that because of the low intake of micronutrients, the immune system, which might give opportunity for clinical leprosy to develop.

In the last part of this thesis the conclusions and recommendations are addressed. In **chapter 9** the findings of the different studies are discussed and the research questions are answered. Even though leprosy is one of the oldest diseases known, many aspects of the disease are not completely understood. It is complicated to study *M. leprae*, because the bacteria cannot be cultured *in vitro* and the incubation time of leprosy is long (between 2 and 5 years on average). For these reasons, it is not completely clear how the disease is transmitted, how and when the immune system responds to the bacilli and how neuropathy and reactions develop. In addition, no specific leprosy vaccine is available that can prevent clinical leprosy. These challenges in studying leprosy and in developing effective preventive and curative measures for leprosy are a main cause of the steady new case detection rate of leprosy and of the steady numbers of new cases with grade 2 disability in many endemic countries. Fortunately, progress is being made, and large multicentre studies such as TENLEP help increase our insight into the many difficult issues in leprosy that need to be addressed in order to achieve elimination of the disease and its related disabilities.

From the results and experience of the studies described in this thesis, I developed a set of recommendations to improve detection and treatment of neuropathy in the field:

- The sural and radial cutaneous nerves should be included in standard routine monofilament assessment

- Use more sensitive methods to detect neuropathy in an earlier stage. The portable Thermal Sensitivity Tester and NeuroQuick are promising devices for this goal
- Implement nutrition support for poor people during food shortage periods in leprosy endemic regions to increase dietary diversity

Samenvatting

In dit proefschrift beschrijf ik verschillende methodes om neuropathie in leprapatiënten vroeg op te sporen en te kunnen behandelen, zodat permanente, onomkeerbare verslechterde werking van de zenuwen voorkomen kan worden. Ook heb ik verschillende risicofactoren op het gebied van voeding bestudeerd in relatie tot de ontwikkeling van klinische lepra.

Lepra is een infectieziekte die wordt veroorzaakt door de bacterie *Mycobacterium leprae*. Het wordt vaak gezien als een ziekte van de armen, zeker nu lepra vooral voorkomt in lage-inkomenslanden. 89% van de nieuw gediagnosticeerde leprapatiënten komen uit acht landen: Bangladesh, Brazilië, Democratische Republiek Congo, Ethiopië, India, Indonesië, Nepal en Nigeria, waarvan India veruit de grootste bijdrager is met ongeveer 60%. Officieel is lepra wereldniveau geëlimineerd, wat wil zeggen dat er minder dan 1 patiënt per 10.000 inwoners voorkomt volgens de Wereldgezondheidsorganisatie (WHO). Helaas geldt dit nog niet voor bepaalde regio's in bovengenoemde landen en er vindt daar dus nog steeds verspreiding plaats. De ziekte is besmettelijk, maar niet iedereen die besmet is krijgt het. Bij het wel of niet krijgen van lepra spelen genen, algemene gezondheidsstatus, voeding, hygiëne en sociale afstand tot een patiënt een rol.

De leprabacterie tast de huid en zenuwen aan, maar de gevolgen van de zenuwbeschadiging maakt dat lepra als ernstige ziekte beschouwd wordt. Zenuwbeschadiging kan leiden tot verslechterde functie van de zenuwen, zowel sensorisch als motorisch. Door verslechterde sensorische functie voelen leprapatiënten vaak niet dat ze zich verbranden of een wond hebben, en verzorgen de wonden minder goed omdat ze geen pijn voelen. Door verslechterde motorische functie kunnen de spieren in de handen en voeten soms verzwakken. Door beide soorten beschadiging kan de patiënt gehandicapt raken. Het is dus van belang om eventuele zenuwbeschadiging snel op te merken en te behandelen. In het algemeen wordt aangenomen dat als een verslechterde zenuwfunctie binnen 6 maanden behandeld wordt met prednisolon de functie verbeterd kan worden. De optimale duur van een prednisolonbehandeling voor verslechterde zenuwfunctie is echter onbekend.

We hebben een onderzoek opgezet om antwoord te vinden op de vraag of een langer dan gebruikelijke behandeling met prednisolon een grotere verbetering van de zenuwfunctie tot gevolg heeft. Dit onderzoek heeft de naam TENLEP gekregen (Treatment of Early Neuropathy in LEProsy).

De resultaten van dit en andere onderzoeken zijn beschreven in dit proefschrift. Het doel van dit proefschrift is om de volgende vragen te beantwoorden:

1. Hoe kan de opsporing van verslechterde zenuwfunctie in lepra in 'het veld' verbeterd worden?
2. Hoe kan onomkeerbare verslechterde zenuwfunctie het beste voorkomen worden?
3. Welke aan voeding gerelateerde factoren hebben invloed op het ontwikkelen van klinische lepra?

Hoofdstuk 1 geeft een inleiding in de ziekte lepra en uitgebreide informatie over neuropathie. De vier studies in het tweede deel focussen op methodologie. In **hoofdstuk 2** beschrijf ik de details van het protocol van de twee gerandomiseerde, gecontroleerde TENLEP-studies die we hebben uitgevoerd in zes lepracentra in vier Aziatische landen. In de Klinische studie vergelijken we of een langere behandelduur met prednisolon, 32 vergeleken met 20 weken, betere resultaten laat zien in de verbetering van verslechterde zenuwfunctie. Daarnaast bestuderen we in een parallelle, placebo-gecontroleerde studie, genaamd de Subklinische studie of 20 weken prednisolon verslechterende zenuwfunctie kan voorkomen in leprapatiënten met subklinische zenuwbeschadiging.

De volgende drie hoofdstukken geven antwoord op de eerste onderzoeksvraag. In **hoofdstuk 3** beschrijven we een onderzoek waarbij we van twee zenuwen de gevoeligheid bepalen met een monofilament test bij mensen zonder lepra in India en Nepal. Uit eerder onderzoek is gebleken dat de nervus radialis en de nervus suralis de eerst aangedane zenuwen zijn bij lepra neuropathie. Maar doordat verwondingen bijna nooit voorkomen in de innervatiezone van deze zenuwen worden de nervus radialis en de nervus suralis vaak overgeslagen bij de routinetesten. Dit is een gemiste kans, omdat deze zenuwen juist extra informatie geven die de vroege opsporing van verslechterde zenuwfunctie kunnen bevorderen. Om de bevindingen van de monofilament test bij patiënten te kunnen interpreteren hebben we daarom in deze studie vastgesteld wat de grens van de normale waarde voor de monofilament test is. Het paarse 2-grams filament is de normaalwaarde voor de nervus radialis en het rode 4-grams filament is normaal voor de nervus suralis.

Voor de monofilament- en spiertesten hebben we betrouwbaarheidsstudies uitgevoerd in de zes lepracentra; deze staan beschreven in **hoofdstuk 4**. We kunnen daaruit concluderen dat monofilament testen van de nervus radialis en de nervus suralis betrouwbaar uitgevoerd kunnen worden door getrainde staf. **Hoofdstuk 5** beschrijft een studie naar alternatieve methodes om verslechterde zenuwfunctie vroeg op te kunnen sporen. In de studie doen we diagnostische nauwkeurigheidstesten met vijf verschillende ‘gadgets’ bij 209 leprapatiënten met subklinische neuropathie. Deze testen zijn geselecteerd omdat ze draagbaar, batterij-gevoed, betaalbaar en makkelijk uitvoerbaar zijn en daarmee geschikt zijn voor gebruik tijdens veldwerk. De vijf verschillende gadgets testen de sensorische zenuwen via verschillende modaliteiten: de Neuropad® test zweetfunctie, de Vibratip™ test vibratiegevoeligheid, de NC-Stat®DPNCheck™ test de zenuwgeleiding, de NeuroQuick test de gevoeligheid voor kou en de Thermal Sensibility Tester (TST) test de gevoeligheid voor warmte. Door de gadgets te vergelijken met twee gouden standaarden, de warmtedetectiegrens met de TSA II en zenuwgeleiding met een elektromyografie apparatuur, konden we de sensitiviteit en specificiteit van de gadgets berekenen voor de nervus suralis en nervus tibialis posterior. De NeuroQuick en de TST lieten veruit de beste resultaten zien en we adviseren dat voor deze twee testen de betrouwbaarheid wordt geëvalueerd. Dit soort gadgets zou een erg goede manier zijn om de vroege opsporing van verslechterde zenuwfunctie door lepra in het veld te verbeteren.

Het derde deel omvat drie hoofdstukken waarin de resultaten van verschillende studies worden gepresenteerd. **Hoofdstuk 6** beschrijft de belangrijkste resultaten van de Klinische TENLEP-studie en gaat daarbij in op de tweede onderzoeksvraag. In de Klinische studie vergeleken we in 868 patiënten of een prednisolonbehandeling van 32 weken beter is dan van 20 weken in de verbetering en volledige genezing van verslechterde zenuwfunctie. Door de zenuwfunctie van de patiënten maandelijks te testen met monofilament- en spiertesten tijdens de 32 weken van de behandelfase en vervolgens op 52 en 78 weken, kregen we een goed beeld van het effect van de twee behandelingen. Uit onze studie blijkt dat beide behandelingen hetzelfde resultaat geven, namelijk dat in 78% van de patiënten de verslechterde zenuwfunctie verbeterde of volledig genas. Dit betekent dat het geven van een langere behandeling geen extra verbetering oplevert. Ook de secundaire uitkomsten, zoals de schalen die participatie, activiteit-beperkingen, en ernst van reacties meten, verschilden niet tussen de twee behandelduren. **Hoofdstuk 7** beschrijft de ernstige en minder ernstige ongewenste voorvallen, ofwel bijwerkingen, van het gebruik van prednisolon binnen de TENLEP-studies. Onder ernstige ongewenste voorvallen vallen onder andere hoge bloeddruk, diabetes, maagzweer of tuberculose. Bij beide behandelduren, 20 en 32 weken, is het percentage patiënten dat ernstige ongewenste voorvallen krijgt erg laag, respectievelijk 0,9% en 2,7%. De patiënten die de langere behandeling van 32 weken volgden kregen significant meer ernstige ongewenste voorvallen. Rekening houdend met het resultaat beschreven in **hoofdstuk 6**, namelijk dat de behandeling van 32 weken geen betere uitkomsten geeft dan de behandeling van 20 weken, is de lange behandeling het verhoogde risico op ernstige ongewenste voorvallen niet waard.

Het onderzoek in **hoofdstuk 8** geeft antwoord op de derde onderzoeksvraag. In een eerder onderzoek in Bangladesh werd een recente periode van voedseltekort geassocieerd met het ontstaan van ziekteverschijnselen van lepra. In ons onderzoek zijn we hier dieper op ingegaan, en hebben een case-control studie in Bangladesh uitgevoerd, waarin het voedingspatroon en de voedselzekerheid van 52 recent gediagnosticeerde leprapatiënten wordt vergeleken met die van een gezonde controlepopulatie uit dezelfde regio, bestaande uit 100 mensen. Door middel van interviews hebben we data verzameld over de voedselinname van patiënten en controles in de voorafgaande 24 uur en over de voedselzekerheid, de inkomsten, en uitgaven aan voedsel van het huishouden. Het bleek dat in huishoudens van leprapatiënten voedseltekorten vaker voorkwamen, dat de diversiteit van het voedingspatroon lager was en dat er een hogere voedselonzekerheid was. Daarbij gaven patiënten minder geld uit aan voeding, hadden ze een lagere body mass index (BMI) en hadden minder voedselvoorraden in huis. Doordat er minder geld aan voeding uitgegeven kon worden was het duidelijk dat voedingsmiddelen met hoge nutritionele waarden, zoals vlees, vis, fruit, groenten, eieren en melk, minder geconsumeerd werden Het is mogelijk dat door lagere inname van micronutriënten het immuunsysteem niet optimaal werkt en daardoor klinische lepra kan ontstaan.

In het laatste deel komen de conclusies en aanbevelingen aan bod. In **hoofdstuk 9** worden de bevindingen bediscussieerd en de onderzoeksvragen beantwoord. Ondanks dat lepra één van de oudst bekende ziektes is, zijn er nog veel aspecten van deze ziekte onbekend. Het is ingewikkeld om de bacterie te bestuderen, mede doordat deze niet buiten het lichaam groeit (in

vitro) en omdat de bacterie een erg lange incubatietijd heeft (tussen de 2 en 5 jaar gemiddeld). Hierdoor begrijpt men nog niet goed hoe de ziekte zich verspreidt, hoe en wanneer het immuunsysteem precies op de bacillen reageert en hoe zenuwbeschadiging en reacties ontstaan. Ook is er tot op heden geen effectief vaccin ontwikkeld om lepra te voorkomen. Mede door deze nog bestaande uitdagingen is lepra nog niet overal geëlimineerd. Gelukkig wordt er in studies steeds vooruitgang geboekt en helpen grootschalige studies als de TENLEP-studie om onze kennis verder uit te breiden.

Naar aanleiding van de bevindingen in dit proefschrift heb ik de volgende praktische aanbevelingen gedaan om de opsporing en behandeling van verslechterde zenuwfunctie in het veld te verbeteren:

- De nervus radialis en de nervus suralis zouden geïncludeerd moeten worden in de standaard routine van de monofilament test
- Maak gebruik van sensitievere methodes om neuropathie in een vroeg stadium op te kunnen sporen. De draagbare Thermal Sensitivity Tester en de NeuroQuick zijn veelbelovend voor dit doel
- Zorg voor ondersteuning van mensen met een laag inkomen in lepra-endemische regio's, zodat zij over nutriënt-dichte voedingsmiddelen kunnen beschikken tijdens periodes van voedseltekort en zij zo de diversiteit van hun dieet kunnen vergroten

Dankwoord

Heerlijk dat het boekje eindelijk af is. Maar dit betekent helaas ook het einde van een ontzettend leuke en bijzondere periode. Ik heb het voorrecht gehad om op een mooi project te mogen werken, waar ik heel veel geleerd heb, veel heb gereisd, en een heleboel inspirerende mensen heb leren kennen.
Dit proefschrift was er nooit gekomen zonder hulp van een groot aantal mensen. Die wil ik hier graag bedanken!

Allereerst mijn promotor en copromotor natuurlijk. Jan Hendrik, ik wil u bedanken voor de goede en uitgebreide begeleiding, ondanks uw eigen reizen en andere projecten wist u altijd tijd voor mij vrij te maken. Het was erg fijn dat u mij het vertrouwen gaf om zelf studies op te zetten en uit te voeren, maar er tegelijkertijd altijd te zijn voor advies. Al die keren dat ik weer met een frisse blik en nieuwe moed uit onze overleggen kwam zijn heel belangrijk geweest. Wim, ik heb erg geluk gehad met jou als begeleider. Je heb zo veel kennis en praktijkervaring, en daar heb ik de afgelopen jaren graag gebruik van gemaakt. Van onze allereerste reis samen, toen je me leerde leprasymptomen te herkennen en allerlei tests uit te voeren, tot het schrijven van papers. Erik, ook al ben je officieel niet mijn copromotor, je mag natuurlijk niet in dit rijtje ontbreken. Je bent in het TENLEP-project de allerbelangrijkste factor geweest. We hebben over zo veel onderwerpen scherp gediscussieerd, en ik waardeer jouw creatieve manier van denken, je sterke mening en dat je altijd (en het liefst meteen) met oplossingen wil komen. And of course Peter, you played a vital role in the TENLEP project as part of our ‘core’ team. Thank you for your time and effort handling the enormous amounts of TENLEP data. I really enjoyed the trip to Guildford, many thanks for your hospitality.

Graag wil ik ook hierbij de leescommissie bedanken voor het beoordelen van mijn proefschrift en alle overige leden van de promotiecommissie voor hun tijd en inspanning.

This thesis would have never been accomplished without all the leprosy patients and their families who participated in our studies. The same can be said for the work of the wonderful teams in India, Nepal, Bangladesh and Indonesia. Working together with all of you was a pleasure, and I want to thank each and every one of you for going out of your ways to make me feel at home when I was visiting.
Ook wil ik graag de Master studenten bedanken die een deel van de onderzoeken hebben gedaan. Lisanne, wat fijn dat we zo’n leuk onderzoek hebben opgezet, en dat je zo zelfstandig de data hebt verzameld en uitgewerkt. Nicolette, ook al waren het uitdagende omstandigheden, je hebt je er wel doorheen geslagen, en ik heb veel aan jouw voorwerk in Mumbai gehad.

Zonder mijn collega’s was het doen van promotieonderzoek een veel saaiere onderneming geweest. Cherry, you were my roommate from the beginning. I really appreciated our talks and discussions about the Netherlands, China, the Dutch language and foods. Even though I wasn’t often at MGZ in the last years, I will miss the nice talks in our ‘girls’ room, thanks for those years

Nana, Raquel and Moniek! Fenna en Astrid, bedankt voor de gezelligheid, ook naast het werk. Suzette, wat hebben we een goede tijd gehad op MGZ. Bedankt voor alle thee- en koffiebreaks, en altijd je luisterend oor. Ik zal onze vakantie samen nooit vergeten!

Grote dank gaat uit naar mijn familie, vrienden en teamgenoten die mij de zo belangrijke ontspanning naast het werk gaven. Alle meiden van mijn teams bij Erasmus Volley, RVC Libanon en ZUVO ontzettend bedankt voor alle belangstelling voor mijn werk, maar ook dat ik lekker even helemaal nergens aan hoefde te denken en kon sporten en kletsen.
Frank, Jeroen, Wim, Eeske en Marleen: ook al is het niet zo vaak, het blijft bijzonder dat als we elkaar zien het gewoon 'net als vroeger' is. Ik hoop dat we deze traditie in stand kunnen houden!

Melina, wat is het fijn om een vriendin te hebben zoals jij. Zo enthousiast, open en meelevend, en ik ben dankbaar dat je me bij zal staan als paranimf. Ik zou onze skype avondjes nooit willen missen en hoop dat we ooit weer eens in hetzelfde land zullen wonen!

Lieve mam en pap, door jullie steun, zorg en liefde ben ik zo ver gekomen. Ik heb mijn eigen weg kunnen gaan, met het veilige gevoel dat jullie er zijn om me op te vangen als het niet zou lukken. Het is erg bijzonder om zulke slimme ouders te hebben die zelfs over onderzoek buiten hun veld nog advies kunnen geven. Bedankt voor alle wijze raad, en dat ik altijd bij jullie terecht kan. Aukje, geweldige zus, ik bewonder je ambitie en doorzettingsvermogen ontzettend. Het is zo fijn dat je altijd voor me klaar staat, wanneer ik mijn hart moet luchten of ik weer eens advies nodig heb, ook al heb je het zelf zo druk. Dennis, jij ook bedankt voor je interesse in mijn werk en reizen, en natuurlijk voor de gezellige borrelavondjes! Jelle, mijn grote kleine broer, wat ben ik trots op je! Ik vind het zo leuk dat je uiteindelijk ook de 'gezondheidskant' hebt gekozen, en ik weet zeker dat jij je weg wel vindt. Ik ben erg blij dat je als paranimf naast me staat en me een beetje kalm kan houden.

Lieve Cyril, you are the one who had to live with me every day, going through the ups and downs of writing this thesis. Thank you for being there for me and for your patience (living in the cold and rainy Netherlands for years so I could finish my PhD). You stimulated me to work a bit harder, but also made sure I could relax: taking me out for sports, cooking delicious dinners and travelling to so many incredible places with me. I love our life together and I am looking forward to the new things and places that we will discover! Je t'aime!

Curriculum Vitae

Inge Margriet Wagenaar was born in Wageningen, the Netherlands, on 29 August 1985. After graduating from her secondary education (VWO) in 2003, she started her studies at Wageningen University, where she obtained her Bachelor's degree in Human Nutrition and Health. She continued her studies in Wageningen, and she graduated in 2009 from a two year Master's in Nutritional Epidemiology and Public Health. During this master, she did an internship in Nepal for the UN World Food Program, and she completed an internship at Heinz Innovation Centre in Nijmegen.

In 2010, she started her PhD at the department of Public Health at the Erasmus University of Rotterdam, under supervision of Jan Hendrik Richardus. The TENLEP project was carried out in cooperation with the Royal Tropical Institute in Amsterdam During her PhD she completed a Master's degree in Public Health at the Netherlands Institute of Public Health (Erasmus MC). As of May 2016 she works as an epidemiologist on the surveillance of antimicrobial resistance in eastern Europe and central Asia at the National Institute of Public Health and the Environment (RIVM) in Bilthoven, the Netherlands.

List of publications

Wagenaar I, Brandsma JW, Post E, Prakoeswa CRS, Tamang K, Hagge D, Bowers R, Husain S, Shetty V, Nicholls P, Richardus JH. *Effectiveness of 32 versus 20 weeks of prednisolone in leprosy patients with recent nerve function impairment: a randomized controlled trial* (submitted).

Wagenaar I, Post E, Brandsma JW, Ziegler D, Rahman M, Alam K, Richardus JH. *Early detection of neuropathy in leprosy: A comparison of five tests for field settings* (submitted).

Post E, **Wagenaar I,** Brandsma JW, Prakoeswa CRS, Tamang K, Hagge D, Bowers R, Husain S, Shetty V, Nicholls P, Richardus JH. *Prednisolone Adverse Events in the Treatment of Leprosy Neuropathy* (submitted).

de Vlas SJ, Stolk WA, le Rutte EA, Hontelez JA, Bakker R, Blok DJ, Cai R, Houweling TA, Kulik MC, Lenk EJ, Luyendijk M, Matthijsse SM, Redekop WK, **Wagenaar I,** Jacobson J, Nagelkerke NJ, Richardus JH. *Concerted Efforts to Control or Eliminate Neglected Tropical Diseases: How Much Health Will Be Gained?* PLoS Negl Trop Dis. 2016 Feb 18;10(2):e0004386.

Wagenaar I, van Muiden L, Alam K, Bowers R, Hossain MA, Kispotta K, Richardus JH. *Diet-related risk factors for leprosy: a case-control study.* PLoS Negl Trop Dis. 2015 May 12;9(5):e0003766.

Wagenaar I, Brandsma W, Post E, Richardus JH. *Normal threshold values for a monofilament sensory test in sural and radial cutaneous nerves in Indian and Nepali volunteers*. Lepr Rev. 2014 Dec;85(4):275-87.

Brandsma JW, **Wagenaar I**, Post E, Nicholls P, Richardus JH. *Reliability of clinical nerve function assessment in peripheral neuropathies*. Lepr Rev. 2014 Mar;85(1):29-35.

Wagenaar I, Brandsma W, Post E, van Brakel W, Lockwood D, Nicholls P, Saunderson P, Smith C, Wilder-Smith E, Richardus JH. *Two randomized controlled clinical trials to study the effectiveness of prednisolone treatment in preventing and restoring clinical nerve function loss in leprosy: the TENLEP study protocols.* BMC Neurol. 2012 Dec 18;12:159

PhD portfolio

	Year	Workload or ECTS
1. PhD training		
Research skills		
- Master of Public Health, Netherlands Institute for Health Sciences (NIHES) Rotterdam	2013	30 ECTS
Courses and workshops		
- Online training Good Clinical Practice, Royal Holloway, University of London	2011	30 hours
- Systemic reviews of measurement properties , Kenniscentrum meetinstrumenten	2011	8 hours
- Developing a Cochrane Systematic Review of Interventions, Dutch Cochrane Centre	2013	20 hours
- Biomedical English writing, Erasmus MC	2015	3 ECTS
Conferences and presentations		
- 18th International Leprosy Congress, Brussels, Belgium ▪ Oral presentation 'The Treatment of Early Neuropathy in Leprosy trials' ▪ Poster presentation 'Normal threshold values of a monofilament test in radial cutaneous and sural nerves'	2013	2 ECTS
- Congres Nederlandse Vereniging Tropen Geneeskunde, Amsterdam ▪ Oral presentation: 'Diet-related risk factors for leprosy'	2015	1 ECTS
- 19th International Leprosy Congress, Beijing, China ▪ Oral presentation 'Effectiveness of 32 versus 20 weeks of prednisolone in leprosy patients with recent nerve function impairment' ▪ Oral presentation 'Diet-related risk factors for leprosy' ▪ Poster presentation ' Early detection of neuropathy in leprosy: A comparison of five tests for field settings'	2016	2 ECTS
Other		
- Leprosy Research Initiative: proposal review "Leprosy, parasites and nutrition: New paradigms to fight an old disease"	2015	15 hours
- Progress report annual meeting, Netherlands Leprosy Relief, Amsterdam	2015	8 hours
2. Teaching		
- Revising essays for STOLA tropical medicine course, Stola foundation	2011-2015	250 hours
- Supervising Master student VUMC	2011	15 hours
- Supervising Master student Wageningen University	2013	100 hours
- Supervising Master student VUMC	2015	50 hours
- Supervising Community Project Medical Students	2014	20 hours