

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

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Summary The aim of this study was to find predictors of neuropathy and reactions, determine the most sensitive methods for detecting peripheral neuropathy, study the pathogenesis of neuropathy and reactions and create a bank of specimen, backed up by detailed clinical documentation. A multi-centre cohort study of 303 multibacillary leprosy patients in Northern India was followed for 2 years. All newly registered MB patients requiring a full course of MDT, who were smear positive and/or had six or more skin lesions and/or had two or more nerve trunks involved, were eligible. A detailed history was taken and physical and neurological examinations were

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performed. Nerve function was assessed at each visit with nerve conduction testing, warm and cold detection thresholds, vibrometry, dynamometry, monofilaments and voluntary muscle testing. Because the latter two are widely used in leprosy clinics, they were used as 'gold standard' for sensory and motor impairment. Other outcome events were type 1 and 2 reactions and neuritis. All subjects had a skin biopsy at registration, repeated at the time of an outcome event, along with a nerve biopsy. These were examined using a variety of immunohistological techniques. Blood sampling for serological testing was done at every 4-weekly clinic visit. At diagnosis, 115 patients had an outcome event of recent onset. Many people had skin lesions overlying a major nerve trunk, which were shown to be significantly associated with an increased of sensory or motor impairment. The most important adjusted odds ratios for motor impairment were, facial 4.5 (1.3–16) and ulnar 3.5 (1.0–8.5); for sensory impairment they were, ulnar 2.9 (1.3–6.5), median 3.6 (1.1–12) and posterior tibial 4.0 (1.8–8.7). Nerve enlargement was found in 94% of patients, while only 24% and 3% had paraesthesia and nerve tenderness on palpation, respectively. These increased the risk of reactions only marginally. Seven subjects had abnormal tendon reflexes and seven abnormal joint position sense. In all but one case, these impairments were accompanied by abnormalities in two or more other nerve function tests and thus seemed to indicate more severe neuropathy. At diagnosis, 38% of a cohort of newly diagnosed MB leprosy patients had recent or new reactions or nerve damage at the time of intake into the study. The main risk factor for neuropathy found in this baseline analysis was the presence of skin lesions overlying nerve trunks. They increased the risk of sensory or motor impairment in the concerned nerve by 3–4 times. For some nerves, reactional signs in the lesions further increased this risk to 6–8 times the risk of those without such lesions. Patients with skin lesions overlying peripheral nerve trunks should be carefully monitored for development of sensory or motor impairment.

Introduction

Leprosy is feared because of the deformities and disability that it may cause.¹ Successful leprosy treatment should prevent or heal deformities and disabilities.² Most of these are secondary complications of impaired of nerve function, often caused by immunological reactions against *M. leprae* antigens.³ Unfortunately, people remain at risk of neuropathy resulting from such reactions during and even after successful anti-leprosy treatment.^{4–10}

In the past decade, several large cohort studies conducted in Ethiopia, Nepal, Bangladesh and Thailand have provided epidemiological data on prevalence and incidence of type 1 (reversal) and type 2 (ENL) reactions and sensory and motor impairment.^{5–12} These have revealed a number of factors that increase the risk of immune reactions and nerve function impairment (NFI). The main ones are extent of clinical disease,⁶ multibacillary (MB) classification,¹³ and neuropathy already present at the time of diagnosis.^{10,13} However, these three criteria apply to a fairly large proportion of cases detected in many programmes. Worldwide in 2003, 39% of new cases were MB.¹⁴ The proportion of patients with nerve function impairment (NFI) at diagnosis varies from 15% in Bangladesh¹⁵ to 55% in Ethiopia.¹⁰ Prospective testing of immunological and neurological markers may reveal additional factors that would enable more precise prediction of risk and thus more effective preventive measures.

Despite advances in the understanding of some of the mechanisms underlying immune reactions and neuropathy in leprosy, many questions related to the pathophysiology remain

unanswered. Much of the current knowledge of leprosy reactions has been gained from cross sectional studies, and there is little information on the longitudinal changes in immunological and histopathological parameters over time. Improved understanding of the precise mechanisms that trigger and modulate reactions may point to better methods of prevention and treatment. Early detection of NFI is likely to be the most effective method of prevention,^{7,13,16} indicating the importance of further studies to find out how early detection of neuropathy is best achieved.

It was therefore proposed to study all three of the above areas, prediction, detection and pathogenesis of reactions and NFI, in a large prospective study. The ILEP Nerve Function Impairment and Reaction or 'INFIR' Cohort Study described in this paper was set up for this purpose. The study is a multi-centre project involving two specialized leprosy referral hospitals and two immunology laboratories in India, designed to address the following three aims:

1. To find clinically relevant neurological and immunological predictors of NFI and reactions.
2. To determine which method or combination of methods of nerve function assessment will be most sensitive for the detection of sensory and motor impairment in leprosy.
3. To study the pathogenesis of peripheral neuropathy and reactions in leprosy with respect to time of development of immunological, pathological, neurophysiological, and clinical features.

This paper describes the main methods used in the study and the clinical profile of the intake cohort. The neurological, immunological and histological profiles of the cohort will be the subject of separate publications.

Materials and methods

DESIGN

This was a cohort study of newly registered MB patients. The patients were followed up monthly for 1 year and every 2nd month during the 2nd year.

LOCATION

Recruitment of subjects took place in The Leprosy Mission (TLM) hospitals in Naini and Faizabad, specialist leprosy referral centres in Uttar Pradesh, North India. The immunological and histopathological investigations were carried out at the LEPRO Blue Peter Research Centre (BPRC) in Hyderabad, Andhra Pradesh and at the TLM Stanley Browne Laboratories in Miraj, Maharashtra.

STUDY POPULATION

The study population comprised newly registered multibacillary leprosy patients requiring a full course of MDT.

STUDY SUBJECTS

Inclusion criteria

All newly diagnosed patients who were being registered for MDT and who were smear positive and/or had six or more skin lesions and/or had involvement of two or more nerve trunks were eligible for inclusion.

Exclusion criteria

The following categories of patients were excluded from the study, even if they met the inclusion criteria:

- Patients who did not give consent to be enrolled in the study.
- Any patients for whom MDT was contraindicated.
- Relapses, restarters, defaulters and any other re-treatment patients, unless last MDT dose was more than 5 years ago.
- Patients already on MDT, those transferred in from elsewhere and those transferred from PB to MB MDT, unless they were within 1 month from diagnosis and had taken only one dose of MDT.
- Patients under 12 or over 60 years of age.
- Patients already on steroid therapy for any reason.
- Patients with a history of alcohol abuse or diabetes.
- Patients with a history of or clinical signs of non-leprosy related peripheral neuropathies or poliomyelitis.
- Patients mentally unable to cooperate with sensory and motor testing procedures (cannot test).
- Patients living outside a predefined area around the study centre (within half a day travel).
- Patients who could not be expected to remain registered at the study centre for the time span of the study (e.g. people working in seasonal labour and other temporary residents).
- Patients with a serious additional infection or condition, such as tuberculosis.

SUBJECT SELECTION

Not all potentially eligible patients were asked to enter the study. Whenever several eligible new patients presented on a single day, those considered to be at highest risk of developing an outcome event were selected first. This meant that those with more extensive disease and positive skin smears were more likely to be selected than those with limited disease or a negative skin smear. Patients without an outcome event at registration were given preference over those with an event. Overall, however, it was only rarely necessary to choose between eligible patients. Those selected were invited to participate. They were informed about the procedure and could decline participation if they desired.

SAMPLING AND STUDY SIZE

The sample size calculations were based on odds ratio considerations for the 'predictor part' of the study. For a predictor present in 20% of the population, with an NFI frequency of 5% in the unexposed group, a study size of 240 would have been needed to detect a relative risk of 4.

To detect a difference of 20% between two predictive values (e.g. 60–40%), a sample size of 200 would have been sufficient. A study size of 300 was planned, including a contingency of 10–20% for loss to follow-up.

Patients who had a reaction or sensory or motor impairment at diagnosis were not excluded from the study. They were given steroid treatment, or other anti-inflammatory treatment as appropriate.

OUTCOME EVENTS

The following were counted as outcome events, neuritis, silent neuropathy (SN), type 1 or reversal reaction (T1R), erythema nodosum leprosum (ENL or T2R), sensory impairment (SI), motor impairment (MI). The latter two were only counted as outcome events if they were of ‘recent’ onset, defined as 6 months or less. For the present analysis, only events present at diagnosis were counted. The definitions are given in Appendix 1.

OUTCOME MEASURES

Predicting reactions and sensory and motor impairment

- The percentage of patients testing positive for a given measure or marker.
- The odds ratio of a given measure adjusted for other the effect of other measures that have a significant influence on the model.

Early detection of sensory or motor impairment

- The positive predictive value (PPV), sensitivity and specificity of each test compared with clinically significant NFI diagnosed with monofilaments (MF) or voluntary muscle test (VMT).

GENERAL EXAMINATION AT INTAKE

A standardized history using a checklist was taken from all patients admitted to the study. Patients were asked when they had first noticed signs or symptoms of the disease. They were given a full physical examination and a basic neurological examination (including reflexes, joint position sense and nerve palpation). Patients were assigned a leprosy classification according to the Ridley–Jopling system, but based on clinical criteria (appearance, extent and number of lesions, sensory impairment in the lesions and symmetry). The diagnosis of ‘pure neural’ leprosy was based on finding one or more definitely enlarged nerve trunks. To be eligible for the study, patients with pure neural leprosy had to have two or more enlarged nerves. The location and appearance of skin lesions were recorded, and whether they were overlying the course of a peripheral nerve trunk. Particular attention was given to signs and symptoms of T1R, T2R and peripheral neuropathy. Slit skin smears were made from both earlobes and from the edge of two active skin lesions. For serological investigations, 10 ml blood was taken. Basic blood (haemoglobin levels, ESR and blood cell counts) and urine analysis were performed at the local laboratory.

TREATMENT REGIMEN

All patients were put on WHO multidrug therapy for multibacillary patients (MB MDT), consisting of daily dapsone (100 mg) and clofazimine (50 mg) and monthly supervised rifampicine (600 mg) and clofazimine (300 mg). Patients whose average bacteriological index (BI) at diagnosis was <3 received 12 months of MB MDT; others were treated for 24 months.

NERVE FUNCTION ASSESSMENT

In view of the purpose of the study, namely to investigate in detail prediction, detection and pathogenesis of immunological reactions and neural impairment in leprosy, a number of tests of nerve function not routinely used in leprosy were incorporated in the protocol, along with standard tests. Motor and sensory impairment as outcome events were defined on the basis of an abnormal VMT or monofilament (MF) test result, because these are standard tests that are widely used. Nerve function assessment was done using the following methods.

History taking

A set of standardized questions was used that might detect current NFI or give warning signs for future outcome events. The questions are given in Appendix 2.

Motor nerve function

Voluntary muscle testing (VMT) using the 0–5 modified MRC scale (see Appendix 3).

Grip dynamometry, key pinch and pulp-to-pulp pinch testing. The dynamometer was made of a sphygmomanometer cuff inserted in a cylindrical cotton cover and inflated to a baseline pressure of 20 mmHg. Pulp-to-pulp and key-pinch strength was measured in a similar way using a neonatal sphygmomanometer cuff.¹⁷

Motor nerve conduction measurements (MNC). MNC parameters were measured on three nerves bilaterally (ulnar, median and lateral popliteal (peroneal)) using Neurocare 2000 EMG machines (BioTech Ltd., Mumbai). The Windows-driven software stores the compound muscle action potential traces in a database for future reference. The measured values for latency, amplitude and distance were stored automatically in a separate Access database. The Neurocare software calculated nerve conduction velocity (NCV) and area under the curve values. Skin temperatures were measured bilaterally at wrist and ankle with an electronic thermometer (Testo Quicktemp 925, with a surface probe no. 0602.0392). The NCV and distal latency values were corrected for temperature at the time of analysis using standard formulae.

Sensory nerve function

Touch sensation was tested using a standard set of coloured Semmes–Weinstein monofilaments (MF).¹⁸ The monofilaments used were 200 mg, 2 g, 4 g, 10 g and 300 g. Normal reference values were 200 mg for the hand and 2 g for the foot (excluding the heel).¹⁹ The test sites and scoring methods are given in Appendix 5.

Vibration perception thresholds (VPT). Vibration sensation was tested with a Vibrometer II (Somedic, Sweden). The instrument provides application force-controlled measurements of the VPTs in microns of skin displacement, using an algorithm of limits (slowly increasing vibration amplitude, until the person tested indicates that (s)he can feel the vibration. The test sites were the thenar and hypothenar eminences (soft tissue), for testing the median and ulnar nerve, respectively, the dorsal first webspace for the radial cutaneous nerve, the plantar pulp of the big toe (posterior tibial) and the mid-lateral border of the foot (sural). All tests were done bilaterally.

Thermal detection thresholds. Thermal thresholds were evaluated using an instrument called the Thermal Sensory Analyzer (TSA II), manufactured by MEDOC in Israel. The TSA is capable of measuring warm detection thresholds (WDT) and cold detection thresholds (CDT), as well as heat pain (HP) and cold pain (CP). In this study, only the former two were recorded. The WDT and CDT were measured relative to a baseline thermode temperature of 32°C. The algorithm used for determining the threshold was the ‘method of levels’.²⁰ The test sites were the same as for vibrometry, described above.

Sensory nerve conduction measurements (SNC). SNC parameters were measured bilaterally on four nerves (radial cutaneous, ulnar, median and sural) using the same equipment as described for MNC.

The neurological methods and results will be presented and discussed in subsequent papers. The thresholds for impairment were based on the normative studies done as part of this project and which are reported in separate publications (Nicholls *et al.*, in preparation; van Brakel *et al.*, in preparation). The diagnoses of sensory impairment and motor impairment were based on the results of the MF and the VMT, respectively.

ACTIVITIES OF DAILY LIVING (ADL)

A questionnaire-based ADL assessment was done, using the Green Pastures Activity Scale,²¹ to evaluate neurological disability at the time of diagnosis and when an outcome event was diagnosed.

BIOPSIES

A full-thickness biopsy was taken from the edge of an active skin lesion. In those experiencing an outcome event, a further skin biopsy was obtained from the same lesion. The biopsies were fixed according to standard protocols. Half of each biopsy was fixed in formalin; the other half was snap-frozen in liquid nitrogen. In patients with recent sensory or motor impairment (onset \leq 6 months), a nerve biopsy was taken from a cutaneous nerve on one affected limb. For the upper limb, the radial cutaneous nerve was used; for the lower limb, the sural nerve. Nerve biopsies were also fixed in liquid nitrogen. Biopsies were divided transversely and fixed in the same way as the skin biopsies. Reading of biopsies was done blinded to the clinical outcome diagnosis.

IMMUNOLOGICAL EVALUATION

Blood samples were taken during follow-up visits and at the time an outcome event was diagnosed. Samples were prepared according to standard procedures, detailed in the INFIR Field Procedures Manual. Serum was separated, aliquoted and stored in liquid nitrogen. As a

precaution, four blood drops from each sample were put on Whatman filter paper, dried and stored. Once a month, these were sent to the designated laboratories in liquid nitrogen transport containers. The immunological investigations were performed at the designated laboratories. Samples not needed for the immediate serological investigations are stored frozen at -70°C in a specimen bank. Once during the follow-up a separate blood sample was taken for future DNA analysis.

CLASSIFICATION AND CERTAINTY OF OUTCOME DIAGNOSIS

Initially, classification according to the Ridley–Jopling system and diagnosis of outcome events were based on clinical criteria initially. Treatment of reactions or nerve function impairment was therefore based on the physician’s diagnosis. After data collection of the first year of follow-up had finished, all outcome diagnoses were reviewed and checked for consistency with the criteria set in the protocol. Each outcome event was assigned a certainty grade 1–3 (1 = doubtful; 3 = definite). Subjects with a grade 2 or 3 outcome event were counted as ‘cases’; the remainder of the cohort were used as the control group. A few ‘missed events’ were also found and added as outcome in retrospect. Most frequently this had happened when NFI had developed slowly over a period of several months, but still within the stipulated maximum of 6 months. When the histological results became available, the classification data and outcome events were reviewed again and clinical and histological data were reconciled to give a final classification and outcome certainty level. With regard to the Ridley–Jopling classification, the histological findings took precedence over clinical classification, so a patient classified clinically as BT but with BL histology would have final BL classification. For multivariate analysis, the classification groups were collapsed to either T(uberculoid) or L(epromatous). ‘T’ includes skin smear negative BT and pure neuritic (PN) leprosy with BT histology. ‘L’ includes skin smear positive BT, BL, LL and PN with BL histology or >3 nerves enlarged.

For the final diagnosis of outcome events, the clinical diagnosis prevailed, but discrepancies were noted and a sub-group analysis exploring these will be done at a later stage. In cases with an outcome certainty level of ‘1’ (doubtful) in whom histology showed signs of T1R or T2R, the certainty level was changed to ‘2’. Subjects in whom a reaction was detected in the skin biopsy, but not clinically ($n = 44$), were *not* counted among cases with outcome events. Excluded from the control group were those with an event certainty status of ‘1’ and those who developed an incident reaction within 6 months of registration. Only events with a certainty level of 2 or 3 were included as outcome events in the analysis.

FOLLOW-UP

The patients were followed up monthly for the first year and every second month during the second year. Patients who did not report for their follow-up appointment were visited at home within 1 week of the due date. During these home visits the reason for missing the appointment was determined and the patient was counselled to return for treatment and investigations. At each follow-up, the patients had a physical examination and a full nerve function assessment as detailed above. A blood sample was also taken.

ANALYSIS OF RESULTS

Prevalence estimates are given as percentages with 95% confidence intervals for the point estimates. Delay in presentation was calculated as the interval between the time the patient had first notice a sign or symptom of the disease and the date of diagnosis. The significance of associations between outcome and predictor variables was tested using the Chi-squared or Fisher's exact test. Differences between means and differences between medians were tested with the *t*-test and the Kruskal–Wallis test, respectively. Univariate and multivariate analyses of associations between outcome and predictor variables were done with normal or stepwise logistic regression. Analyses were performed using Stata for Windows software, versions 7 and 8.

ETHICAL CONSIDERATIONS

No financial incentives were given to participants. However, travel expenses were refunded on occasion and, where relevant, lost earnings of daily labourers compensated. The study adhered to the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS/WHO, 1993). Permission for the study was obtained from the Indian Council of Medical Research and the Research Ethics Committee of the Central JALMA Institute for Leprosy in Agra gave ethical approval. This included permission for the skin and nerve biopsies. Written consent was obtained from individual study subjects before inclusion in the study, using a standard consent form.

Results

Three hundred and three subjects were enrolled in the study, of whom 83 were women (27%). The mean age was 32.8 years (range 12–60). Demographic and clinical details are presented in Table 1. Over 50% had grade 1 or 2 impairment and 36% were smear-positive. Twenty-one percent had an average BI of 3 or more.

The prevalence of reactions, sensory and motor impairment and neuritis is shown in Table 2. Altogether, 115 subjects had a reaction or NFI event at registration. Sixty-four (21%) had sensory or motor impairment of recent onset. Only six people (2.3%) had a T2R. Table 3 gives details of the reactional signs and symptoms. Involvement of other organs and nerve pain and tenderness disturbing sleep or activities were rare.

Many subjects had skin lesions overlying a major nerve trunk (Table 4). Table 5 shows the association between the presence of such a lesion, with and without a reaction, and neural impairment in that nerve. In univariate analysis, lesions overlying the facial and ulnar nerve had a statistically significant association with motor impairment, while lesions overlying the ulnar, median and posterior tibial nerve were associated with sensory impairment. However, when adjusting for the effects of age, sex, leprosy type, BI and old nerve damage, the association between lesions overlying the ulnar nerve and motor impairment was no longer significant at the 5% level. The odds ratios were generally higher for the presence of reactional skin lesions than for any skin lesions. The latter were more consistent and had smaller confidence intervals, probably because of the much larger number of people with non-reactional lesions. Even in multivariate analyses, associations between skin lesions

Table 1. Characteristics of the subjects in the INFIR Cohort Study (n=303) at the time of registration

Variable	Frequency	Percentage	
<i>Sex</i>			
Men	220	72.6	
Women	83	27.4	
<i>Age group</i>			
12–20	56	18.5	
21–30	97	32.0	
31–40	71	23.4	
41–50	61	20.1	
51–60	18	6.0	
<i>Classification</i>			
BT*	180	59.4	
BL*	81	26.7	
LL*	29	9.6	
PN*	13	4.3	
<i>WHO disability grade</i>			
0	150	49.5	
1	124	40.9	
2	29	9.6	
<i>Delay in presentation</i>			
Up to 6 months	91	30.0	
7–12	96	31.8	
13–24	52	17.2	
25–36	28	9.3	
37–60	19	6.3	
> 60 months	16	5.4	
<i>Average BI**</i>			
0	193	63.7	
Up to and including 1	20	6.6	
Up to and including 2	27	8.9	
Up to and including 3	21	6.9	
Up to and including 4	24	7.9	
Up to and including 5	16	5.3	
Up to and including 6	2	0.66	
<i>Visual acuity</i>			
	<i>n</i>	Right eye %	Left eye %
6/6	216	71.3	212 70.0
6/9–6/12	54	17.8	62 20.5
6/18–6/60	28	9.2	28 9.2
< 6/60	3	0.99	1 0.33
Missing	2	0.66	

* BT = borderline tuberculoid, BL = borderline lepromatous, LL = lepromatous and PN = pure neuritic (classification based on clinical and histological criteria).

** BI = bacteriological index of the skin smear (rounded; up if < 1, otherwise down to nearest integer).

overlying nerves and impairment of the facial, ulnar, median (sensory only) and posterior tibial nerves were statistically significant.

The prevalence of palpable nerve enlargement, tenderness, paraesthesia on palpation, absent joint position sense or abnormal tendon reflexes in each nerve is shown in Tables 6 and 7, respectively. Nerve enlargement was very common in this cohort (> 94% had one or more enlarged nerves). In contrast, paraesthesia and tenderness on palpation were much less common (24% and 3%, respectively). Very few subjects had abnormal reflexes or JPS, despite sensory or motor impairment in the same limb. However, detailed analysis of those

Table 2. Reactions observed at the time of registration among the subjects in the INFIR Cohort Study ($n = 303$)

Variable	Frequency	Percentage
<i>Type 1 reaction</i>		
All	60	19.8
Mild	23	7.6
Severe*	37	12.2
<i>Type 2 reaction</i>		
All	6	2.0
Mild	4	1.3
Severe*	2	0.7
<i>Nerve function impairment</i>		
Old	79	26.1
Recent**	64	21.1
Any	143	47.2
Sensory – old	96	31.7
Recent**	48	15.8
Any	127	41.9
Motor – old	26	8.6
Recent**	33	10.9
Any	59	19.5
Both sensory and motor (recent)	17	5.6
Other neuritis***	29	9.6

* A reaction was called 'severe' if one or more of the following signs or symptoms were present: sensory or motor impairment, ulcerating skin lesions, >10 reactional skin lesions, oedema that impaired function, 'visible' nerve tenderness on gentle palpation, despite distraction, paraesthesia or nerve pain disturbing sleep or impairing function or involvement of other organs, like eyes, joints, testis, etc.

** Onset 6 months ago or less.

*** See definition in Appendix 1.

patients whose JPS or reflexes were abnormal showed two things. Firstly, the abnormalities were mutually exclusive, i.e. those with abnormal reflexes had normal JPS and *vice versa*, and, secondly, abnormal reflexes or JPS were in all but one case accompanied by abnormalities in more than one other nerve function test (data not shown). Impaired JPS or reflexes were not associated with the presence reactions or NFI at diagnosis (Table 8).

Table 8 shows the results of univariate and multivariate analysis of the association between these neurological tests and the event status at registration. After adjusting for the effects of age, sex and leprosy type, none of the signs was independently associated with an increased risk of reaction or NFI. The presence of one or more tender nerves appeared to be associated with an increased risk of reaction or NFI (odds ratio 7.3), but this was not significant at the 5% level ($P = 0.084$).

Discussion

The current cohort consisted of 188 patients without reaction or NFI at intake and 115 with such an event. In the latter group, the progress of clinical, neurophysiological and immunological markers during reaction treatment were studied, as well as risk factors for reoccurrence of reaction or NFI during and after reaction treatment. In the former group, risk factors for occurrence of reactions and NFI and methods for early detection of sensory and

Table 3. Reactional signs and symptoms at the time of registration among the subjects in the INFIR Cohort Study ($n = 303$)

Variable	Frequency	Percentage
<i>Raised skin lesions</i>		
None	239	78.9
1–3	9	3.0
4–10	22	7.3
> 10	33	10.9
<i>Degree of inflammation</i>		
None	240	79.2
Erythema or nodules	35	11.6
Erythema and raised plaques or nodules	28	9.2
Ulceration	0	
<i>Reactional oedema</i>		
None	271	89.4
Minimal	19	6.3
Visible, but not affecting function	13	4.3
Affecting function	0	
<i>Fever due to reaction</i>		
< 37.5°C	299	98.7
37.6–38.9°C	4	1.3
≥ 39°C	0	
<i>Involvement of other organs*</i>		
None	301	99.3
Mild	1	0.33
Definite	1	0.33
<i>Nerve pain and/or paraesthesia</i>		
None	277	91.4
Intermittent; not limiting activity	23	7.6
Sleep disturbed and/or activity diminished	1	0.33
Incapacitating	2	0.66
<i>Nerve tenderness</i>		
None	275	90.8
Absent if attention distracted	23	7.6
Present if attention distracted	2	0.70
Withdraws limb forcibly	3	0.99

* For example, eyes, joints or testis.

Table 4. Prevalence of skin lesions overlying a major nerve trunk among subjects with reactional skin lesions ($n=62$) and in the whole cohort at the time of registration in the INFIR Cohort Study ($n = 303$)

Nerve	With reaction				Any skin lesions			
	Right		Left		Right		Left	
	n^*	%	n^*	%	n^*	%	n^*	%
Facial	28	45.2	23	37.1	75	24.8	70	23.1
Ulnar	30	48.4	27	41.9	126	41.6	120	39.6
Median	9	14.5	12	19.4	32	10.6	36	11.9
Peroneal	18	29.0	16	25.8	76	25.1	72	23.8
Posterior tibial	13	21.0	13	21.8	30	9.9	35	11.6

* n =number of subjects with a lesion overlying the nerve on that side.

Table 5. Association (odds ratios) between skin lesions overlying a nerve trunk and neural impairment in the same nerve in the INFIR Cohort Study ($n=512$ nerves*) at the time of registration

Nerve		Reactional skin lesions			Any skin lesions		
		Motor impairment	Sensory impairment	Sensory or motor impairment	Motor impairment	Sensory impairment	Sensory or motor impairment
Facial	Univariate	7.1** (2.2–23)			4.6 (1.4–15)		
	Multivariate***	6.8 (1.9–27)			4.5 (1.3–16)		
Ulnar	Univariate	3.1 (1.3–7.4)	1.4 (0.51–3.7)	1.7 (0.73–3.7)	3.3 (1.5–7.3)	2.3 (1.2–4.8)	2.6 (1.4–4.8)
	Multivariate	2.4 (0.88–6.5)	1.6 (0.50–5.1)	1.7 (0.66–4.2)	3.5 (1.0–8.5)	2.9 (1.3–6.5)	2.8 (1.5–5.6)
Median	Univariate	11 (0.97–127)	7.1 (2.2–24)	6.2 (1.9–20)	3.9 (0.34–44)	4.2 (1.5–12)	3.5 (1.3–9.6)
	Multivariate	28 (0.64–1238)	8.3 (1.9–39)	7.7 (1.6–36)	3.4 (0.21–56)	3.6 (1.1–12)	3.2 (1.0–10)
Peroneal	Univariate	No recent outcome events					
Posterior tibial	Univariate		2.6 (1.0–6.4)			3.0 (1.6–5.6)	
	Multivariate		4.3 (1.4–14)			4.0 (1.8–8.7)	

* Subjects who developed an incident reaction within the first 6 months from registration were excluded as controls ($n = 47$).

** OR = odds ratio; 95% CI = 95% confidence interval; the odds ratios blocked in *grey* are statistically significant at the 5% level.

*** Adjusted for age, sex, leprosy type, BI and longstanding neuropathy in the same nerve.

Table 6. Prevalence of palpable nerve enlargement, tenderness and paraesthesia in the INFIR Cohort Study ($n = 303$) at the time of registration

	Ulnar		Median		Radial		Peroneal		Post Tibial		Sural	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
<i>Enlarged</i>												
No	67	62	208	220	124	126	110	94	87	82	121	118
Possible	51	55	51	50	48	62	62	65	61	62	61	62
Definite	185	186	44	33	131	115	131	144	141	140	107	102
% definite	61.1	61.4	14.5	10.9	43.2	38.0	43.2	47.5	46.5	46.2	35.3	33.7
Missing									14	19	14	21
<i>Tender</i>												
No	284	288	293	294	296	295	293	290	268	261	280	271
Mild	16	11	9	8	5	6	9	11	22	23	12	12
Moderate	3	4	1	1	2	2	1	2	2	4		3
% moderate	0.99	1.3	0.33	0.33	0.66	0.66	0.33	0.66	0.66	1.3		0.99
Severe												
Missing									11	15	11	17
<i>Paraesthesia</i>												
No	274	272	287	288	294	288	275	276	266	258	276	271
Yes	29	31	16	15	9	15	28	27	26	30	16	15
% yes	9.6	10.2	5.3	5.0	3.0	5.0	9.2	8.9	8.6	9.9	5.3	5.0
Missing									11	15	11	17

Table 7. Prevalence of abnormal neurological test results in the INFIR Cohort Study ($n = 303$) at the time of registration

Test	Index finger		Little finger		Big toe		Right	Left	Right	Left
	Right	Left	Right	Left	Right	Left				
<i>Joint position sense</i>										
Absent	1	0	3	2	0	1				
% absent	0.33		0.99	0.66		0.33				
	Biceps		Triceps		Supinator		Knee jerk		Ankle	
<i>Reflexes*</i>										
Missing data								1		2
Normal	301	302	299	301	296	297	292	292	301	296
Brisk	2	1	1	1	2	1	11	9	1	1
Absent			3	1	5	5		1	1	4
% absent	0	0	0.99	0.33	1.65	1.65	0	0.33	0.33	1.32

* Only seven subjects had an absent tendon reflex on only one side.

motor impairment were investigated. These investigations will be reported elsewhere. A range of sero-immunological and immunohistochemical markers were studied as potential markers to predict reactions and NFI, as well as to increase our understanding of pathogenesis of such events. These results will be reported elsewhere. The cohort was selected with a bias towards patients expected to be at high risk of reactions and NFI. Therefore, no epidemiological conclusions can be drawn from the prevalence or incidence of these events in the cohort.

SKIN LESIONS OVERLYING A MAJOR NERVE TRUNK

Hogeweg *et al.* showed that skin lesions on the face, accompanied by signs of type 1 reaction (T1R), substantially increased the risk of motor impairment of the facial nerve.²² Many believe this to be true also for reactional skin lesions overlying other major nerve trunks, but

Table 8. Association between clinical and neurological parameters and outcome status at registration in the INFIR Cohort Study ($n=256^*$)

Sign	Reaction ($n=115$)	No reaction ($n=141$)	Univariate analysis	Multivariate analysis**
>4 nerves enlarged	64	54	2.0 (1.2–3.3)	1.7 (0.99–3.0)
Up to 4 nerves enlarged	51	87		
1 or more nerves tender	6	1	7.7 (0.91–65)	7.0 (0.75–66)
No tender nerves	109	140		
>1 nerve paraesthesia***	30	22	1.9 (1.0–3.5)	1.6 (0.83–3.2)
No or 1 nerve with paraesthesia	85	119		
Absent JPS	2	2	1.3 (0.17–9.0)	2.6 (0.31–21)
Normal JPS	111	139		
Abnormal reflexes	3	3	1.2 (0.24–6.2)	1.6 (0.28–8.5)
Normal reflexes	112	138		

* Subjects who developed an incident reaction within the first 6 months from registration were excluded as controls ($n = 47$).

** Adjusted for each of the variables in the table plus age, sex and leprosy type.

*** On palpation.

evidence for this has been lacking. The current study specifically noted the presence and location of skin lesions and thus provided the basis for a detailed risk analysis. Even after adjusting for the effects of major other risk factors such as age, sex, leprosy type and pre-existing nerve damage, the presence of skin lesions overlying most nerve trunks included in the study increased the risk of accompanying NFI by 3–4 times (Table 5). One caution is that the present cross-sectional analysis could not prove that the lesions actually preceded the reaction or NFI. The presence of a reaction increased the strength of the association for most nerves, although confidence intervals were much wider due to the much smaller number of subjects with reactional skin lesions. These findings indicate that the presence of skin lesions overlying a nerve trunk perhaps is more important than whether or not this lesion is in reaction. Patients with skin lesions overlying peripheral nerve trunks should be carefully monitored for development of sensory or motor impairment.

JOINT POSITION SENSE AND TENDON REFLEXES

These tests, though part of a routine neurological examination,²³ are often omitted in the examination of patients with leprosy, because proprioception and deep reflex pathways are not expected to be affected. However, Jennekens and Jennekens found abnormal position sense of one or more digits in 33% of the patients they examined.²⁴ van Brakel *et al.* found abnormal position sense in 2% of median and 10% of ulnar and posterior tibial nerves.²⁵ Ramadan *et al.* found 'diminished' reflexes in 18/40 of their patients and 'diminished joint and vibration sensation' in 13/40. However, the patient group in the latter study was older and had longer histories of leprosy than the present study group. In our study, although only ~2% of subjects had abnormal JPS or reflexes, this was accompanied by multiple other abnormal test results, perhaps indicating more advanced neuropathy. This confirms our earlier findings regarding JPS²⁵ and fits with the assessment that abnormal JPS indicates 'a severe impairment of the distal, thick sensory fibres'.²⁴ Prospective analysis from the INFIR Cohort Study will show whether these tests also have value for predicting neuropathy and prognostic value with regard to treatment outcomes.

NERVE ENLARGEMENT, TENDERNESS AND PARAESTHESIA

Nerve enlargement, tenderness and paraesthesia on palpation were associated with an increased risk of a reaction or a NFI event at diagnosis. However, after controlling for the effects of other variables, these associations failed to reach statistical significance. The reason in the case of tenderness may be that it occurred in only 0.7% of subjects (5% among those with an outcome event). This may be partly due to the fairly conservative criterion used for the diagnosis 'tenderness'. Only 'moderate' or 'severe' tenderness was counted in the analysis (see Appendix 1).

Conclusion

The main finding in this cross-sectional analysis is that skin lesions overlying major nerve trunks increase the risk of nerve damage in these nerves significantly, irrespective of whether these lesions show signs of a skin reaction. Absent joint position sense or tendon reflexes appear to indicate more advanced neuropathy. Nerve enlargement, tenderness and paraesthesia on palpation were associated with an increased risk of a reaction or a NFI event at diagnosis, but this association was not very strong. The nerve function of patients with these signs and symptoms should be monitored regularly.

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References

- ¹ Jopling WH. Leprosy stigma [editorial]. *Lepr Rev*, 1991; **62**: 1–12.
- ² Palande DD. Nerve involvement in leprosy – prevention and management of deformities: need for a paradigm-shift. *Lepr Rev*, 1994; **65**: 161–164.
- ³ Ridley DS, Job CK. The pathology of leprosy. In: Hastings RC (ed). *Leprosy* Churchill Livingstone, Edinburgh 1985.
- ⁴ Rose P, Waters MFR. Reversal reactions in leprosy and their management. *Lepr Rev*, 1991; **62**: 113–121.
- ⁵ Becc-Bleumink M, Berhe D. Occurrence of reactions, their diagnosis and management in leprosy patients treated with multidrug therapy; experience in the Leprosy Control Program of the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Ethiopia. *Int J Lepr*, 1992; **60**: 173–184.
- ⁶ van Brakel WH, Khawas IB. Nerve damage in leprosy: an epidemiological study of 396 patients in West Nepal – part 1: definitions, methods and frequencies. *Lepr Rev*, 1994; **65**: 204–221.

- ⁷ Richardus JH, Finlay KM, Croft RP, Smith WCS. Nerve function impairment in leprosy at diagnosis and at completion of MDT: a retrospective cohort study of 786 patients in Bangladesh. *Lepr Rev*, 1996; **67**: 297–305.
- ⁸ Schreuder PA. 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 [see comments]. *Int J Lepr*, 1998; **66**: 170–181.
- ⁹ Croft RP, Nicholls PG, Richardus JH, Smith WC. Incidence rates of acute nerve function impairment in leprosy: a prospective cohort analysis after 24 months (The Bangladesh Acute Nerve Damage Study) [In Process Citation]. *Lepr Rev*, 2000; **71**: 18–33.
- ¹⁰ Saunderson P, Gebre S, Desta K *et al*. The pattern of leprosy related neuropathy in the AMFES patients in Ethiopia: definitions, incidence, risk factors and outcome. *Lepr Rev*, 2000; **71**: 285–308.
- ¹¹ Scollard DM, Smith T, Bhoopat L *et al*. Epidemiologic characteristics of leprosy reactions. *Int J Lepr*, 1994; **62**: 559–567.
- ¹² de Rijk AJ, Gabre S, Byass P, Berhanu T. Field evaluation of WHO-MDT of fixed duration, at ALERT, Ethiopia: the AMFES project – II Reaction and neuritis during and after MDT in PB and MB leprosy patients. *Lepr Rev*, 1994; **65**: 320–332.
- ¹³ Croft RP, Nicholls PG, Steyerberg EW *et al*. A clinical prediction rule for nerve-function impairment in leprosy patients [In Process Citation]. *Lancet*, 2000; **355**: 1603–1606.
- ¹⁴ WHO. *World Health Organisation Leprosy Elimination Project. Status Report 2003*. World Health Organisation, Geneva, 2004.
- ¹⁵ Croft RP, Richardus JH, Nicholls PG, Smith WC. 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) [In Process Citation]. *Lepr Rev*, 1999; **70**: 140–159.
- ¹⁶ Meima A, Saunderson PR, Gebre S *et al*. Factors associated with impairments in new leprosy patients: the AMFES cohort [In Process Citation]. *Lepr Rev*, 1999; **70**: 189–203.
- ¹⁷ Soares D, Riedel A. A simple and inexpensive pinch meter to detect subclinical weakness among leprosy patients. *Lepr Rev*, 1997; **68**: 55–60.
- ¹⁸ Bell-Krotoski JA. ‘Pocket’ filaments and specifications for the Semmes–Weinstein Monofilaments. *J Hand Ther*, 1990; **Jan–Mar**: 26–31.
- ¹⁹ Anderson AM, van Brakel WH. Age specific normal thresholds for sensibility testing with monofilaments in a Nepali population. *Int J Lepr*, 1998; **66**: 69A.
- ²⁰ Yarnitsky D, Sprecher E. Thermal testing: normative data and repeatability for various test algorithms. *J Neurol Sci*, 1994; **125**: 39–45.
- ²¹ van Brakel WH, Anderson AM, Worpel FC *et al*. A scale to assess activities of daily living in persons affected by leprosy. *Lepr Rev*, 1999; **70**: 314–323.
- ²² Hogeweg M, Kiran KU, Suneetha S. The significance of facial patches and type I reaction for the development of facial nerve damage in leprosy. A retrospective study among 1226 paucibacillary leprosy patients. *Lepr Rev*, 1991; **62**: 143–149.
- ²³ Notermans NC, Wokke JHJ, Jennekens FGI. Clinical work-up of the patient with a polyneuropathy. In: de Jong JMBV (ed). *Handbook of Clinical Neurology* Elsevier, The Netherlands, 1991.
- ²⁴ Jennekens FGI, Jennekens-Schinkel A. Neurological examination of patients suffering from leprosy: is it worthwhile? *Lepr Rev*, 1992; **63**: 269–276.
- ²⁵ van Brakel WH, Shute J, Dixon JA, Arzet H. Evaluation of sensibility in leprosy – comparison of various clinical methods. *Lepr Rev*, 1994; **65**: 106–121.

Appendix 1: Outcome definitions and diagnostic cut-offs

Neuritis

A leprosy patient had neuritis if he/she had any of the following:

- Spontaneous nerve pain, paraesthesia or tenderness.
- New sensory or motor impairment of recent onset.
- Mixed signs neuritis.

A neuritis may be mild or severe (see below), acute (< 1 month duration), sub-acute (2–6 months) or long-standing (> 6 months). During the monthly study follow-ups, only acute neuritis was regarded as an outcome.

Silent neuropathy (SN)

A patient had silent neuropathy when he/she had sensory and/or motor impairment of recent onset (< 6 months duration) in an area innervated by one or more nerve without signs of a reaction (RR or ENL) or nerve pain and with or without tenderness.

Type 1 or reversal reaction (TIR)

A type 1 reaction was diagnosed when a patient had erythema and oedema of skin lesions. There may be accompanying neuritis and oedema of the hands, feet and face. The skin signs are obligatory; the nerve and general signs optional.

Erythema nodosum leprosum (ENL)

A patient had ENL if he/she had crops of tender subcutaneous skin lesions. There may be accompanying neuritis, iritis, arthritis, orchitis, dactylitis, lymphadenopathy, oedema and fever. The skin signs are obligatory; the nerve and general signs optional.

Severity of a reaction

A reaction was called 'severe' if one or more of the following signs or symptoms were present: sensory or motor impairment, ulcerating skin lesions, > 10 reactional skin lesions, oedema that impaired function, 'visible' nerve tenderness on gentle palpation, despite distraction, paraesthesia or nerve pain disturbing sleep or impairing function or involvement of other organs, such as eyes, joints, testis, etc.

Sensory impairment (SI)

A patient was diagnosed as having sensory impairment in any of the following situations: the monofilament threshold was increased from the normal threshold (200 mg for the hand and 2 g for the foot) by three or more levels (filaments) on any site, OR two levels on one site AND at least one level on another site, OR one level on three or more sites for one nerve. Only SI of *recent* onset (6 months or less) was counted as an outcome event.

Motor impairment (MI)

A patient was diagnosed as having motor impairment if the VMT score for any muscle was less than four on the 0–5 (modified) MRC scale. Only MI of *recent* onset (6 months or less) was counted as an outcome event.

New additional sensory or motor impairment

Where the baseline showed *partial or full longstanding* impairment for two or more consecutive assessments, then if the DIFFERENCE in 'levels' (between now and the

baseline) was 3 or more for monofilaments or 2 or more for VMT, then the patient had additional recent impairment and should be considered as having an outcome event.

Nerve enlargement

Scoring=none, possible or definite. Only nerves scoring 'definite' were included as 'enlarged' in the analysis.

Nerve tenderness

Scoring:

- Absent
- Mild – absent if patient's attention is distracted
- Moderate – present if patient's attention is distracted
- Severe – patient withdraws the arm forcibly

Only nerves scoring 'moderate' or 'severe' were counted for the analysis.

Paraesthesia

Nerves were marked positive for paraesthesia if the patient reported sensations of tingling, pricking or something equivalent while the nerve was gently palpated.

Joint position sense

Scoring:

- 0 or 1 correct responses in 3 trials
- 2 or 3 correct responses in 3 trials

Tendon reflexes

Scoring: absent, normal or brisk/exaggerated. Only single-sided absence of reflexes was counted as abnormal.

Appendix 2

Standard questions asked as part of the history taking:

- Did you notice any new loss of sensation in your hands or feet during the past month?
- Did you notice any new dryness of your hand palms or foot soles during the past month?
- Did you notice any new weakness in your hands or feet during the past month?
- Did you notice any new sensations of pin and needles or 'insects crawling' in your hands or feet during the past month?
- Did you notice any new pain sensations, such as burning or shooting pain in your hands or feet during the past month?

If a question was answered positively, the person was asked which limb was affected. This was recorded on the form. Paraesthesia and pain were graded on a 4-point scale:

- Absent
- Mild – only aware intermittently; does not limit activity
- Moderate – sleep disturbed and/or activities (including work) diminished
- Severe – incapacitating

Appendix 3: voluntary muscle testing

Movements tested per nerve

Tight eye closure – *facial nerve* (orbicularis oculi)

Thumb abduction – *median nerve* (abductor pollicis brevis)

Little finger abduction – *ulnar nerve* (abductor digiti minimi)

Wrist extension – *radial nerve* (extensor muscles)

Foot dorsiflexion – *lateral popliteal nerve* (tibialis anterior and peroneus longus and brevis)

If any particular muscle could not be tested (e.g. because of joint stiffness or previous surgery), a missing value (9) was recorded for the nerve score. Similarly, if test data are not available for any particular follow-up time, a missing value was recorded.

Grading criteria for the voluntary muscle test

Grade	Criteria
5	Full range of movement of the joint on which the muscle or muscle group is acting; normal resistance can be given (forced eye closure)
4	Full range of movement but less than normal resistance
3	Full range of movement but no resistance
2	Partial range of movement with no resistance (lidgap on tight eye closure)
1	Perceptible contraction of muscle(s) not resulting in joint (or eyelid) movement
0	Complete paralysis

Criteria for motor impairment was any muscle scoring <4.

Appendix 4: Semmes–Weinstein monofilaments

Recording

Coloured pens of appropriate colours (blue, purple (or black), red, orange, pink).

Test sites

On the ulnar side of the hand:

Hypothenar eminence

Fifth metacarpal head (MCP 5)

Volar surface of the distal phalanx of the little finger

On the median side of the hand:

Thenar eminence

Volar surface of the distal phalanx of the thumb

Volar surface of the distal phalanx of the index finger

For the radial cutaneous nerve:

Dorsal on the thumb, at the site of the motor point

On the foot:

Plantar surface of the distal phalanx of the big toe

First metatarsal head

Fifth metatarsal head

Plantar surface near lateral border of the foot

Lateral border of the foot (just distal from the head of the fifth metatarsal bone)

Scoring

Hand Colour	Approximate force	Score	Colour	Foot Approximate force	Score
Blue filament felt	200 mg	0	Purple filament felt	2 g	0
Purple filament felt	2 g	1	Red filament felt	4 g	1
Red filament felt	4 g	2	Orange filament felt	10 g	2
Orange filament felt	10 g	3	Pink filament felt	300 g	3
Pink filament felt	300 g	4	Pink not filament felt		4
Pink filament not felt		5			

The score for individual sites is summed for each nerve:

Ulnar: 3 sites

Median: 3 sites

Radial cutaneous: 1 site

Posterior tibial: 4 sites

Sural: 1 site

Criterion for sensory impairment

If a patient scores 3 or more for any nerve, the nerve had sensory impairment. The normal sensation level for all sites on the hand is 200 mg; The normal sensation level for all sites on the foot is 2 g.