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Reference values for nerve function assessments among a study population in northern India – II: Thermal sensation thresholds

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Abstract

Objective: This paper presents normal reference values for thermal sensation for a study population with no known neurological condition in northern India. It was part of the INFIR Cohort Study, a prospective study of people newly diagnosed with multibacillary leprosy, the objective being to identify early changes in nerve function predictive of new onset impairment and reactions. *Methods:* Data on warm and cold sensation in five bilateral nerves was collected from 326 healthy subjects stratified by sex and by age and drawn from the same general population as the subsequent leprosy-affected cohort. Reference values were computed from log-transformed data after the exclusion of outliers. *Results:* Normal reference values are presented in the form of 95th percentiles for warm and 5th percentile for cold sensation within eight age and sex groups and by centre. The prevalence of impairment at diagnosis among the leprosy-affected cohort is described and illustrated The high prevalence of lost warm sensation in the leprosy-affected cohort suggests that this is an important early indicator for nerve involvement in leprosy.

INTRODUCTION

Monitoring nerve function provides essential information in assessing the needs of individuals at risk of peripheral neuropathy.[1] Rigorous clinical evaluation involves assessing the ability to detect sensation of vibration, warm and cold temperature and other stimuli, collectively known as Quantitative Sensation Testing (QST). While these methods have been widely applied for use in diabetic patients, only limited use has been made in the context of leprosy.[2]

Thermal Sensation Assessment (TSA) has been demonstrated to be sensitive to abnormalities in the small fibres of thin myelinated and unmyelinated nerves, by cold and warm temperature threshold testing respectively.[3,4] Deterioration in temperature thresholds reflects changes in nerve status and is an indicator for small fibre damage. In combination with other assessments it supports the diagnosis of the presence and extent of neuropathy. Compared to other technical tests such as nerve conduction, TSA is simple to perform and gives readily interpretable results on the functional status of the peripheral nerves.[5] It has potential applications in a number of conditions, for example, a northern India study on detecting small fibre neuropathy.[6] A cross-sectional study in Brazil found extreme warm and cold sensation thresholds in the skin lesions of 112 individuals newly diagnosed with leprosy.[7] In the case of leprosy, loss of sensation may occur prior to and during treatment with multi-drug therapy with an increased risk of secondary impairments. Identifying the early indicators of nerve involvement is therefore a primary concern.

Accurate determination of the limits of normal function requires the collection and analysis of data from healthy, non-neuropathic subjects drawn from a study population within a defined geographical area.[11] The purpose of the present study was to compute normal reference values within appropriate age and sex groups in a study population in Uttar Pradesh State, northern India for which no reference data has previously been published. The resulting reference values were then applied to classify nerve function in a prospective cohort study of newly diagnosed

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multibacillary leprosy, the INFIR Cohort Study, the objective being to identify early changes in nerve function predictive of new onset impairment and reactions. See the first paper of the present series[12] for more details.

METHODS

Equipment

The equipment used in each field centre was a Thermal Sensation Analyser (TSA) II manufactured by Medoc, Israel and supported and maintained by agents in India. The TSA II is a computer-linked system that heats or cools a thermode. Starting from a baseline temperature of 32˚C it allows determination of thermal sensation thresholds by a stepwise warming or cooling of the thermode placed in turn on each of a series of test sites. Subjects indicate when they perceive a change in temperature. The equipment records the appropriate temperature as the limit of warm or cold sensation alongside demographic data in a standard spreadsheet format. Several different algorithms are available for determining thresholds, including the 'forced-choice, two-alternative method', the 'method of limits' and the 'method of levels'.[5] Since the method of levels has good test repeatability and little inter-session bias compared to the other methods.[13] this was the preferred method for the present study.

Subject selection and sample size

As with the study of vibration thresholds we planned to recruit subjects from among the healthy relatives accompanying individuals attending general and dermatology outpatient clinics at the two participating centres, applying inclusion and exclusion criteria as follows:

Inclusion criteria: Individuals were selected to obtain an equal number of male and female subjects and equal numbers of subjects in each of four age bands up to 60 years, the maximum age for recruitment to the subsequent cohort study.

Exclusion criteria: Since the diagnosis of leprosy can only be made on clinical grounds all subjects were screened by an experienced leprologist leading to the exclusion of anyone exhibiting clinical signs or symptoms of leprosy. Subjects with any known neurological disorder, previous contact with leprosy or a history of diabetes were also excluded. Individuals aged above 60 years or those less than 10 years were excluded.

To ensure adequate precision we planned to assess 40 subjects within each of the four age groups for men and for women. The overall target for the number of normal subjects was therefore 320, equal numbers to be recruited in each centre.

Protocol for testing and data recording

The test sites and bilateral nerves assessed in the study were the same as those to be assessed in the Cohort Study. These were the cutaneous areas innervated by five of the nerves commonly affected during leprosy neuropathy. For the ulnar nerve, the palmar hypothenar area, for the median, the palmar thenar area, for the radial cutaneous, the dorso-radial web space between the thumb and the first digit, for the sural, the dorso-lateral area of the foot and for the posterior tibial nerve, the plantar surface of the distal phalanx of the big toe. At each site cold and warm sensation thresholds were assessed, giving a total of 20 data points for each subject. The test procedure is outlined in Table 1. Further details on the study design are available in an earlier publication.[8]

Producing reliable data requires good levels of concentration on the part of subjects. Subjects were informed of the testing process and given practice in responding to the thermal stimulus. Verbal encouragement and instructions were given throughout the procedure. A small number of subjects were unable to detect warming at temperatures approaching 50ºC or cooling at temperatures approaching 10ºC, the range of measurable temperatures. All these assessments were excluded from the calculation of reference values.

The testing was undertaken by a team of physiotherapists also responsible for vibrometry and nerve conduction testing. After initial training the teams in each centre, four physiotherapists and a physio-technician in Naini and four physiotherapists in Faizabad, completed inter-rater reliability testing. Within each centre each pairing was asked to complete up to 20 assessments of volunteer subjects. For this purpose only we recruited subjects with a variety of neurological conditions that would ensure testing across a range of warm and cold sensation thresholds. The results were analysed and demonstrated a good level of reliability.

Identification of outliers and computation of reference values

A total of 326 subjects were assessed and the

Table 1: Instructions for thermal sensation testing

Procedure for thermal sensation testing

At the start of the day:

1. Switch on the TSA unit after the computer has completed the boot process.
2. Check the water level in the TSA unit, add distilled water if necessary.
3. Start the TSA software. The computer communicates with the unit in 'real-time' and so expects continuous feed-back from the unit.
4. Wait for 30 seconds for the system to initialise. The thermode and water temperature are reported in real-time in the top right-hand corner of the screen.
5. Ensure that "Device" is set to thermal

To perform a test:

1. The subject should be seated in front of the examiner in a relaxed position. Explain the procedure to the subject, including the type of sensation (s)he will be experiencing. Let him/her feel the thermode surface.
2. Click once on the large 'Test' button or press Alt-t.
3. Click 'Open departments or directories'. Select the folder for data storage (Faizabad or Naini).
4. For new subjects click Add and enter demographic data. Click OK. Select existing subjects from list.
5. The system will automatically select the algorithm that uses the "Levels" method.
6. Either, click Level 5 for warm sensation testing or Level 6 for cold sensation testing.
7. Proceed through the testing first warm and then cold sensation at each site on 5 bilateral nerves using the following procedure:
   - 7.1 Select a test site from the list and click Continue
   - 7.2 Strap the thermode to the selected site making sure that it stays in place until testing on that site is complete. Hold the response button in your left hand.
   - 7.3 For the method of levels: Tell the subject that a warm (or cold) stimulus will be given, followed by an audible sound. Tell him/her that you will be asking him/her whether (s)he felt the stimulus or not, *after* the sound. A 'don't know' response will be interpreted as a 'No'. Run one trial series to familiarise the person with the procedure. This may only be necessary the first one or two times the person comes for testing.
   - 7.4 Alert the subject that you are about to begin with testing. Press the space bar to start the test. After a count-down of 4 seconds, the first stimulus will appear. A tone-sound can be heard when the maximum has been reached. There is a brief pause while the temperature returns to baseline.
   - 7.5 When using the method of levels, a prompt appears: "Use Y/N keys or Patient Response Unit". At this point, ask the subject: "Did you feel this, yes or no?" Indicate the response using the corresponding key on the keyboard or the corresponding mouse button. As soon as you have done this, the count-down for the next stimulus starts.
   - 7.6 Repeat 9.4 and 9.5 until a blue bar appears indicating that the threshold has been determined. (See Figure 1.)
8. Click 'Save' to save the results of the warm sensation test at the current site.
9. Repeat the process for cold sensation at the same site (Return to point 6, above.)
10. Repeat for each of the five bilateral nerves.
11. Finally file a printout of the results.
12. At the end of the working day make a back-up of the saved data.

data from the resulting spreadsheets merged into a single spreadsheet. Since the data collection procedure was fully automated data checking focussed on the accuracy of demographic data and the availability of data from all subjects assessed.

As with the analysis relating to reference values for vibration perception[12], we identified outliers using regression analysis of log-transformed warm and cold sensation data on age. In view of differences between centres as well as between sexes these analyses were repeated within four groups defined by centre and sex. Any assessments for which the standardised residual exceeded 2.58

were identified as outliers and excluded from all further analysis. Where a subject was found with 3 or more outliers all assessments were excluded. The same process was repeated to exclude further suspect outliers. We then used analysis of variance to assess differences between sexes, between age groups, between left and right sides and between centres.

In the absence of differences between left and right side assessments we planned to pool the remaining assessments, effectively doubling the sample size and then used regression analysis of log transformed data on age within each sex and centre grouping to compute reference values. These were reverse-transformed to the original units of temperature (˚C). Presented here are reference values based on 95th percentiles for reduced warm and 5th percentile for reduced cold sensation in each of the five sites tested. Details of 97.5th /2.5th and 99th /1st percentiles are available from the authors.

All the analysis was carried out using STATA.

Application of reference values

The reference values were then applied to classify nerve function among the 303 cases newly diagnosed with multibacillary leprosy and recruited to the prospective INFIR Cohort Study, the objective being to identify early changes in nerve function predictive of new onset impairment and reactions. Other papers in the present series[12,14] describe reference values for vibration perception thresholds and for parameters of sensory and motor nerve conduction. The first of these provides more information on the cohort study and references to other published work. The reference values reported here are based on a re-analysis of the normative data using methods that show greater sensitivity to differences relating to sex, age and centre.

Ethical Approval

Permission for all aspects of the INFIR study was obtained from the Indian Council of Medical Research. The Research Ethics Committee of the Central JALMA Institute for Leprosy in Agra gave ethical approval. Written consent was obtained from cases before enrolment in the Cohort Study and verbal consent from subjects participating in the normative study.

RESULTS

A total of 326 subjects were enrolled during the study period ending in July 2001. The first round of outlier identification resulted in the exclusion of 10 ulnar nerve assessments (5 warm and 5 cold), 8 median nerve assessments (4, 4), 9 radial cutaneous nerve assessments (4, 5), 2 posterior tibial nerve assessments (0, 2) and 8 sural nerve assessments (1, 7). Three of the 23 subjects involved had three or more outliers and were eliminated from all further analyses. Other outliers were reset as missing values. In the second round, a further 16 subjects were identified with one or more outliers. In total, 2.2% of all assessments were identified as outliers and excluded from any further analysis.

Demographic data on subjects remaining in the analysis is presented in Table 2. The mean age for men and women in Faizabad were 39.2 years (standard deviation 13.4) and 39.9 years (13.7) respectively and in Naini 39.5 years (13.2) and 38.8 years (12.3).

Summary statistics for warm and cold sensation are summarised by centre, in Tables 3A and 3B. The variability in numbers is caused by eliminated or missed assessments.

It is apparent from Table 3 that for each site mean values for warm sensation thresholds for Faizabad were marginally higher than those for Naini while mean cold sensation thresholds were marginally lower. For each test site and modality we used analysis of variance to assess the statistical significance of effects relating to age, sex, side and centre. The oldest age group had the highest value for warm sensation and the lowest value for cold sensation for each site ($p<0.001$). We found statistically significant differences between sexes for palmar hypothenar warm and cold sensation and for palmar thenar warm sensation ($p<0.05$ for each). The importance of age-related trends and differences between sexes is evident from Figure 1. There were statistically significant differences between left and right side only for the foot dorso-lateral test site ($p<0.001$ for both warm and cold sensation). No interaction effects reached statistical significance. Including centre in each of the analysis did not affect any of the findings above. However, between centre differences reached statistical significance in both warm and cold assessments at all five sites ($p<0.001$).

We decided that the small differences in left and right side sural nerve functions, though statistically significant, were too small to be of clinical significance and therefore proceeded with calculation of reference values based on pooled left and right side assessments. The resulting

Table 2: Age and sex distribution plus row and column percentages for subjects assessed in two centres

Faizabad
Age groups (years)

Sex	<=30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	Total
Female	20 25.3, 52.6	19 24.1, 45.2	20 25.3, 52.6	20 25.3, 51.3	79 50.3
Male	18 23.0, 47.4	23 29.5, 54.8	18 23.0, 47.4	19 24.4, 48.7	78 49.7
Total	38 24.2	42 26.8	38 24.2	39 24.8	157

Naini
Age groups (years)

Sex	<=30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	Total
Female	22 27.2, 52.4	20 24.7, 48.8	23 28.4, 51.1	16 19.8, 44.4	81 49.4
Male	20 24.1, 47.6	21 25.3, 51.2	22 26.5, 48.9	20 24.1, 55.6	84 50.6
Total	42 25.6	41 25.0	45 27.4	36 22.0	164

reference values based on the appropriate 95th or 5th percentile are presented in Table 4, broken down by sex and age group and by centre and for combined centres.

The strength of association with age, sex and centre is evident in the trends in reference values for both warm and cold sensation thresholds in Table 4. This is reinforced in Figure 1 which presents scatter plots for hand and foot assessments overwritten with the appropriate 95th or 5th percentiles based on data from combined centres plus a shaded area illustrating the distance between centre-specific reference values.

The scatter plots for plantar surface of the distal phalanx draw attention to the lack of sensitivity at the big toe. With increasing age the site becomes increasingly insensitive, some subjects being unable to detect warming to 50˚C, the upper limit of the TSA equipment. A similar trend was found in cold sensation where the ability to detect cooling approach the lower 10ºC limit. The scatter plots also draw attention to the differences between men and women and the lower levels of variability in the hand palmar hypothenar, compared to the great toe.

We applied these reference values to assess the nerve status of the 303 cases newly diagnosed with MB leprosy and recruited to the INFIR cohort study at the same two field centres. Centre, age, sex and nerve-specific rates for impaired function beyond these reference values at time of diagnosis are presented in Table 5. More details of the cohort have already been published.[8,9]

The scatter plots presented in Figure 2 illustrate the extent of impaired function found in the ulnar nerves of the leprosy cohort. We note that the rate of impairment in warm sensation exceeds that in cold sensation, suggesting that, in leprosy, warm sensation is affected earlier than cold. The same was true of the median, radial cutaneous and sural nerves. (Details available from authors).

DISCUSSION

Thermal sensation assessment provides a unique functional measurement in the monitoring of peripheral nerve function. This is the first major study to produce reference values for warm and cold sensation for a study population drawn from Uttar Pradesh state in northern India. The strength

Figure 1. Scatter plot of ulnar and posterior tibial warm and cold sensation (degrees centrigrade) for normal subjects by age and by centre with 95th or 5th percentiles based on combined centres plus shaded area highlighting the difference between centre-specific percentiles.

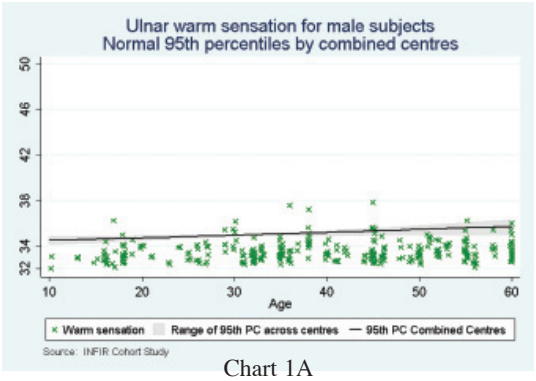

Chart 1A

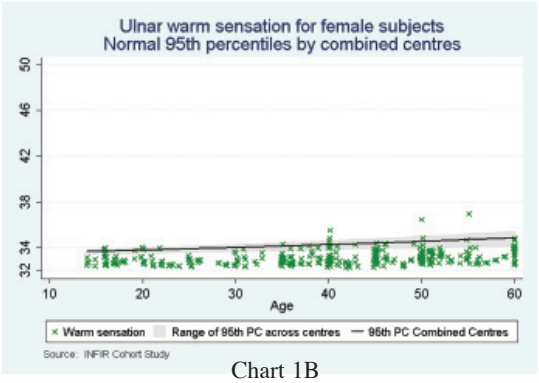

Chart 1B

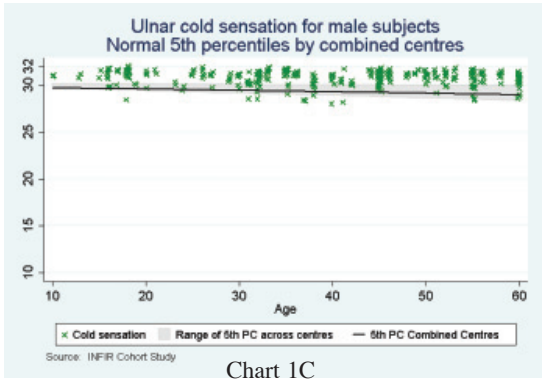

Chart 1C

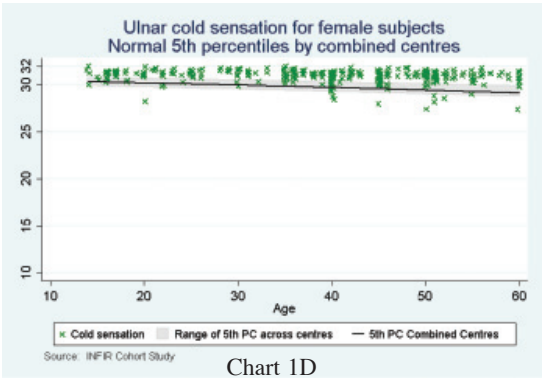

Chart 1D

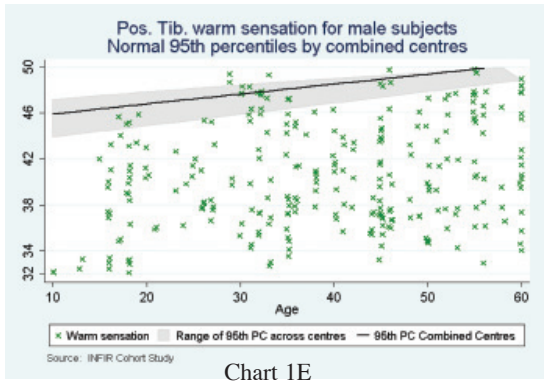

Chart 1E

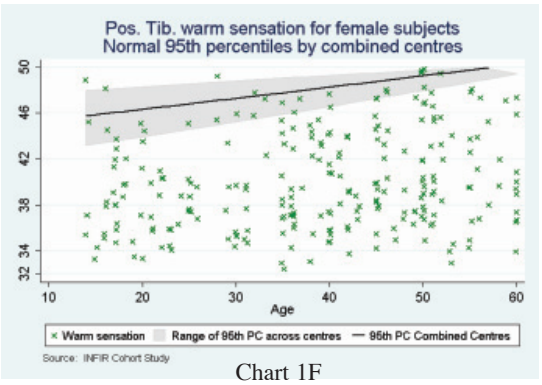

Chart 1F

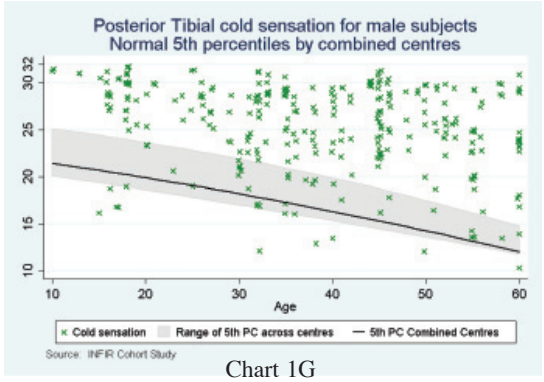

Chart 1G

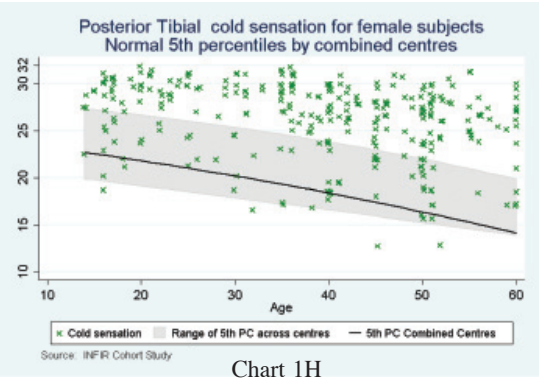

Chart 1H

Table 3A: N, mean, standard deviation and median warm sensation thresholds by nerve, centre, sex, age group and side

Age Group	Side	Ulnar	Median	Radial Cutaneous	Posterior Tibial	Sural
Warm sensation thresholds for male subjects by age group in Faizabad						
≤30 yrs	**Right**	18, 33.4 (0.8), 33.2	18, 32.8 (0.3), 32.7	18, 32.8 (0.4), 32.8	16, 39.5 (4.8), 39.2	18, 33.7 (0.9), 33.6
	Left	18, 33.7 (1.2), 33.4	18, 32.9 (0.4), 32.9	18, 32.9 (0.5), 32.7	16, 40.5 (4.9), 39.8	18, 33.9 (1.2), 33.6
31-40 yrs	**Right**	23, 33.3 (0.6), 33.2	22, 32.9 (0.5), 32.7	23, 33.3 (0.8), 33.2	20, 44.0 (3.4), 44.3	23, 34.9 (1.8), 34.2
	Left	23, 33.6 (0.7), 33.4	23, 33.3 (0.8), 33.1	23, 33.2 (0.7), 33.1	15, 43.3 (4.7), 45.3	22, 35.3 (2.5), 34.9
41-50 yrs	**Right**	18, 33.7 (1.0), 33.6	16, 33.1 (0.5), 33.2	18, 33.7 (1.0), 33.2	12, 41.0 (4.7), 41.7	17, 35.6 (2.7), 35.4
	Left	16, 34.3 (1.3), 33.8	18, 33.5 (0.8), 33.2	18. 34.0 (0.9), 33.9	11, 41.8 (4.6), 41.6	17, 36.3 (4.0), 34.7
51-60 yrs	**Right**	19, 34.0 (0.8), 34.0	19, 33.5 (0.8), 33.2	19, 33.9 (1.3), 33.4	15, 43.8 (4.3), 45.4	18, 35.9 (2.4), 35.0
	Left	18, 34.1 (0.8), 33.9	19, 33.4 (0.5), 33.2	17, 34.0 (1.0), 33.8	15, 42.9 (5.0), 41.6	17, 37.0 (3.7), 35.9
Warm sensation thresholds for female subjects by age group in Faizabad						
≤30 yrs	**Right**	20, 32.9 (0.4), 32.7	20, 32.7 (0.4), 32.5	20, 32.7 (0.4), 32.5	14, 41.1 (4.7), 40.3	20, 33.8 (1.2), 33.7
	Left	20, 33.1 (0.5), 32.9	19, 32.7 (0.4), 32.7	20, 32.9 (0.5), 32.7	17, 40.9 (4.0), 40.3	20, 34.6 (1.8), 33.9
31-40 yrs	**Right**	19, 33.2 (0.8), 32.9	19, 33.0 (0.4), 32.9	18, 33.2 (0.6), 33.2	14, 40.0 (4.2), 39.6	19, 35.9 (3.6), 34.4
	Left	19, 33.3 (0.7) 33.2	18, 33.0 (0.4), 32.9	19, 34.3 (2.7), 33.2	13, 42.2 (5.0), 44.4	19, 36.8 (4.3), 35.1
41-50 yrs	**Right**	20, 33.2 (0.5), 33.1	20, 33.0 (0.3), 33.0	20, 33.3 (0.8), 33.0	13, 40.7 (5.3), 38.6	19, 35.8 (3.0), 34.6
	Left	20, 33.5 (0.9), 33.2	20, 33.1 (0.5), 33.1	20, 33.4 (1.3), 33.1	12, 41.8 (5.5), 40.0	20, 37.2 (4.5), 36.1
51-60 yrs	**Right**	20, 33.4 (0.6), 33.6	19, 33.1 (0.6), 32.9	20, 33.6 (1.0), 33.6	9, 44.2 (3.1), 45.4	20, 36.8 (3.7), 35.3
	Left	20, 33.7 (1.0), 33.4	20, 33.3 (0.7), 33.1	20, 33.5 (1.5), 33.2	15, 41.8 (4.5), 39.9	20, 37.5 (4.3), 36.5
Warm sensation thresholds for male subjects by age group in Naini						
≤30 yrs	**Right**	20, 33.3 (0.6), 33.2	20, 33.0 (0.6), 32.9	20, 32.8 (0.6), 32.5	18, 39.0 (4.3), 39.4	20, 34.6 (2.0), 34.0
	Left	20, 33.5 (1.0), 33.2	20, 33.1 (0.7), 33.2	20, 33.0 (0.5), 32.9	20, 39.2 (4.4), 39.5	20, 34.4 (1.9), 33.9
31-40 yrs	**Right**	21, 33.4 (0.7), 33.2	21, 33.0 (0.4), 33.1	21, 33.1 (0.5), 32.9	19, 38.4 (4.0), 37.4	21, 34.6 (1.8), 33.9
	Left	21, 33.8 (1.4), 33.4	21, 33.2 (0.7), 32.9	20, 33.0 (0.6), 32.9	16, 37.9 (2.2), 37.7	21, 34.3 (1.6), 33.9
41-50 yrs	**Right**	22, 32.9 (0.3), 32.9	22, 32.8 (0.4), 32.8	22, 32.9 (0.5), 32.7	22, 38.7 (3.8), 37.5	22, 34.7 (3.0), 33.6
	Left	22, 33.1 (0.5), 33.1	22, 33.0 (0.6), 32.7	22, 33.2 (0.8), 33.1	22, 39.7 (3.7), 39.8	22, 34.6 (3.1), 33.6
51-60 yrs	**Right**	20, 33.5 (0.7), 33.2	20, 33.2 (0.7), 33.0	20, 33.4 (0.7), 33.4	16, 41.2 (4.5), 40.4	20, 36.2 (2.6), 35.7
	Left	20, 33.6 (0.9), 33.6	20, 33.4 (1.0), 33.1	20, 33.7 (1.2), 33.5	16, 41.5 (5.1), 41.9	20, 35.6 (2.4), 35.3
Warm sensation thresholds for female subjects by age group in Naini						
≤30 yrs	**Right**	22, 32.8 (0.4), 32.7	22, 32.6 (0.3), 32.5	22, 32.6 (0.3), 32.5	20, 37.5 (3.0), 37.5	21, 33.6 (0.8), 33.4
	Left	22, 32.8 (0.4), 32.7	22, 32.6 (0.2), 32.6	22, 32.6 (0.3), 32.5	20, 37.2 (2.9), 36.4	22, 33.7 (1.2), 33.4
31-40 yrs	**Right**	20, 32.8 (0.4), 32.7	20, 32.7 (0.4), 32.5	20, 32.6 (0.3), 32.5	18, 39.1 (4.0), 37.7	19, 33.8 (1.0), 33.6
	Left	20, 33.0 (0.5), 33.0	20, 32.7 (0.4), 32.5	20, 32.7 (0.5), 32.5	18, 39.2 (4.1), 37.5	19, 34.3 (1.9), 33.6
41-50 yrs	**Right**	22, 32.9 (0.5), 32.9	23, 32.9 (0.5), 32.7	23, 32.9 (0.5), 32.7	21, 41.1 (4.2), 39.2	23, 34.3 (1.4), 33.9
	Left	22, 33.0 (0.7), 32.8	23, 33.0 (0.8), 32.7	22, 32.9 (0.6), 32.7	22, 40.5 (3.6), 39.5	23, 34.9 (2.9), 33.8
51-60 yrs	**Right**	16, 33.1 (0.4), 33.0	16, 32.9 (0.3), 33.0	16, 32.9 (0.5), 32.8	13, 39.4 (4.7), 38.1	16, 36.1 (2.8), 35.9
	Left	16, 33.1 (0.4), 33.2	16, 32.8 (0.4), 32.8	16, 33.1 (0.5), 33.1	12, 39.2 (4.0), 37.8	15, 34.9 (1.4), 34.4

Table 3B: N, mean, standard deviation and median cold sensation thresholds by nerve, centre, sex, age group and side

Age Group	Side	Ulnar	Median	Radial Cutaneous	Posterior Tibial	Sural
Cold sensation thresholds for male subjects by age group in Faizabad						
≤30 yrs	**Right**	17, 31.0 (0.6), 31.1	18, 30.8 (0.7), 31.1	18, 31.1 (0.4), 31.1	18, 26.3 (3.9), 27.1	18, 29.1 (1.5), 28.9
	Left	18, 30.7 (0.8), 31.0	18, 31.3 (0.4), 31.3	18, 30.8 (0.8), 31.1	18, 24.2 (4.3), 25.1	18, 28.9 (1.5), 28.9
31-40 yrs	**Right**	23, 30.5 (0.8), 30.8	23, 30.9 (0.5), 30.8	22, 30.8 (0.7), 31.0	22, 23.8 (4.3), 24.2	23, 28.7 (2.4), 29.6
	Left	23, 30.2 (1.0), 30.2	23, 30.9 (0.7), 31.1	23, 30.6 (0.9), 30.8	20, 21.7 (4.8), 22.2	23, 28.0 (2.1), 28.5
41-50 yrs	**Right**	18, 30.6 (0.6), 30.4	18, 30.9 (0.5), 31.0	18, 30.9 (0.7), 31.1	13, 24.3 (4.1), 24.1	18, 28.8 (1.7), 28.7
	Left	18, 30.4 (0.8), 30.4	18, 30.9 (0.5), 31.1	18, 30.6 (1.3), 31.2	14, 23.8 (2.0), 23.6	18, 28.1 (1.7), 28.5
51-60 yrs	**Right**	19, 30.3 (0.8), 30.4	18, 30.9 (0.4), 30.9	18, 30.8 (0.7), 31.0	16, 21.2 (5.2), 23.7	19, 27.7 (3.3), 28.9
	Left	19, 30.2 (0.9), 30.2	19, 30.7 (0.8), 31.0	16, 29.9 (1.9), 30.8	13, 21.4 (4.6), 22.6	17, 27.2 (2.2), 27.0
Cold sensation thresholds for female subjects by age group in Faizabad						
≤30 yrs	**Right**	20, 31.0 (0.6), 31.0	20, 30.8 (1.1), 31.1	19, 31.2 (0.5), 31.5	20, 26.0 (3.3), 26.5	20, 29.7 (1.5), 29.8
	Left	20, 30.9 (0.8), 31.1	19, 31.2 (0.3), 31.3	20, 30.9 (0.8), 31.1	20, 25.3 (3.5), 25.3	20, 28.2 (2.2), 28.9
31-40 yrs	**Right**	19, 30.8 (0.8), 31.1	19, 30.9 (0.8), 31.1	18, 30.9 (0.8), 31.1	18, 24.6 (4.6), 26.6	19, 28.8 (2.3), 30.0
	Left	19, 30.6 (0.7), 30.8	19, 30.9 (0.7), 31.1	18, 30.7 (1.2), 30.9	19, 23.3 (4.0), 23.8	18, 28.5 (2.1), 28.9
41-50 yrs	**Right**	20, 30.9 (0.4), 30.8	20, 30.8 (1.0), 31.1	20, 31.0 (0.7), 31.2	17, 22.9 (4.8), 23.4	19, 28.7 (1.9), 29.2
	Left	20, 30.6 (1.1), 31.1	20, 31.1 (0.4), 31.2	20, 30.6 (0.8), 30.8	15, 22.4 (3.0), 22.0	19, 27.2 (1.9), 27.0
51-60 yrs	**Right**	18, 30.5 (0.9), 30.8	20, 30.3 (1.5), 31.1	20, 30.8 (0.6), 30.8	17, 22.0 (4.6), 24.3	19, 28.9 (1.9), 29.9
	Left	20, 30.5 (1.0), 30.8	20, 30.9 (0.5), 31.0	20, 30.8 (0.9), 31.0	16, 24.0 (3.5), 24.9	19, 27.8 (2.3), 28.7
Cold sensation thresholds for male subjects by age group in Naini						
≤30 yrs	**Right**	20, 31.1 (0.6), 31.2	19, 31.2 (0.5), 31.3	19, 31.4 (0.4), 31.5	19, 27.7 (4.1), 29.9	20, 30.7 (0.9), 30.9
	Left	20, 31.0 (0.7), 31.2	20, 31.4 (0.4), 31.5	19, 31.3 (0.5), 31.5	19, 29.2 (2.1), 29.6	20, 30.8 (0.9), 31.1
31-40 yrs	**Right**	21, 31.1 (0.6), 31.3	21, 31.3 (0.4), 31.3	21, 31.4 (0.3), 31.5	21, 26.9 (3.5), 28.0	21, 30.4 (1.0), 30.7
	Left	21, 31.0 (0.7), 31.1	21, 31.1 (0.5), 31.3	21, 31.3 (0.5), 31.5	19, 27.5 (3.6), 28.7	21, 30.5 (1.0), 30.7
41-50 yrs	**Right**	21, 31.3 (0.4), 31.3	22, 31.3 (0.4), 31.5	22, 31.3 (0.4), 31.3	20, 26.9 (4.6), 28.5	22, 30.4 (0.6), 30.5
	Left	22, 31.2 (0.4), 31.3	22, 31.3 (0.3), 31.3	22, 31.4 (0.3), 31.5	19, 28.0 (3.3), 29.0	22, 30.3 (1.2), 30.8
51-60 yrs	**Right**	20, 31.0 (0.6), 31.1	20, 31.1 (0.5), 31.3	19, 31.0 (0.7), 31.3	17, 26.0 (4.3), 27.4	20, 30.3 (1.0), 30.4
	Left	20, 31.1 (0.5), 31.2	20, 31.1 (0.4), 31.3	20, 30.8 (0.9), 31.2	17, 24.9 (4.5), 25.7	20, 30.2 (1.1), 30.3
Cold sensation thresholds for female subjects by age group in Naini						
≤30 yrs	**Right**	22, 31.4 (0.3), 31.5	21, 31.5 (0.2), 31.5	22, 31.5 (0.3), 31.5	22, 29.4 (2.3), 29.9	22, 30.9 (0.9), 31.2
	Left	22, 31.4 (0.2), 31.4	22, 31.4 (0.3), 31.5	22, 31.5 (0.2), 31.5	20, 29.9 (1.1), 29.8	22, 30.9 (0.8), 31.1
31-40 yrs	**Right**	20, 31.3 (0.4), 31.3	20, 31.4 (0.4), 31.3	20, 31.2 (0.4), 31.3	18, 29.2 (1.8), 29.5	19, 30.7 (1.0), 30.9
	Left	20, 31.1 (0.6), 31.3	19, 31.4 (0.4), 31.5	20, 31.4 (0.3), 31.5	18, 29.1 (1.6), 29.3	18, 30.7 (0.8), 31.1
41-50 yrs	**Right**	23, 31.2 (0.6), 31.3	23, 31.2 (0.4), 31.3	23, 31.2 (0.5), 31.3	23, 27.1 (2.8), 27.7	20, 30.6 (0.9), 30.7
	Left	23, 31.1 (0.5), 31.2	21, 31.3 (0.3), 31.5	23, 31.4 (0.3), 31.5	23, 26.8 (2.8), 27.4	22, 30.4 (1.2), 30.9
51-60 yrs	**Right**	16, 31.0 (0.4), 31.1	16, 31.2 (0.4), 31.3	16, 31.2 (0.4), 31.3	15, 27.7 (2.2), 28.0	15, 30.2 (0.8), 30.3
	Left	16, 31.0 (0.5), 31.2	15, 31.4 (0.2), 31.5	16, 31.2 (0.6), 31.5	15, 27.1 (4.0), 28.0	15, 30.3 (0.7), 30.2

Table 4: Normal reference values for warm and cold sensation for five nerves based on the 95th or 5th percentiles, presented as mid-points of four 10 year age bands and calculated by sex, by centre and by combined centres

Faizabad	Ulnar				Median				Radial Cutaneous				Posterior Tibial				Sural			
	Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
25 years	34.7	34.1	29.7	29.7	33.7	33.4	30.1	29.6	34.1	34.2	29.5	29.9	48.2	48.5	17.8	18.5	35.5	36.6	26.3	27.3
35 years	35.1	34.4	29.4	29.4	34.0	33.7	29.9	29.5	34.6	34.7	29.2	29.6	48.7	49.0	16.2	17.3	36.5	38.0	25.5	26.7
45 years	35.6	34.8	29.2	29.2	34.4	34.0	29.7	29.3	35.3	35.4	28.8	29.3	49.3	49.5	14.5	15.9	37.8	39.9	24.6	26.0
55 years	36.0	35.2	28.9	28.9	35.2	34.3	29.3	29.1	36.1	36.3	28.4	29.0	49.8	49.9	12.7	14.6	39.3	42.4	23.7	25.3

Naini	Ulnar				Median				Radial Cutaneous				Posterior Tibial				Sural			
	Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
25 years	34.9	33.6	30.1	30.6	34.2	33.3	30.6	30.8	34.2	33.2	30.5	30.9	45.4	44.6	22.8	26.1	36.9	35.1	29.5	30.0
35 years	34.9	33.8	30.0	30.4	34.3	33.5	30.5	30.8	34.4	33.4	30.4	30.7	46.4	45.9	20.9	24.6	37.6	35.9	29.2	29.7
45 years	35.0	34.0	30.0	30.2	34.4	33.7	30.4	30.7	34.7	33.8	30.2	30.5	47.4	47.3	18.7	23.0	38.0	37.0	29.0	29.4
55 years	35.0	34.2	29.9	30.0	34.6	33.9	30.3	30.7	35.0	34.1	30.0	30.2	48.4	48.7	16.2	21.0	39.3	38.3	28.7	29.1

Combined Centres	Ulnar				Median				Radial Cutaneous				Posterior Tibial				Sural			
	Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
25 years	34.9	33.9	29.5	30.1	34.0	33.4	30.3	30.1	34.2	33.7	29.9	30.4	47.2	46.8	19.1	21.0	36.2	35.9	27.6	28.5
35 years	35.1	34.1	29.4	29.9	34.2	33.6	30.2	30.0	34.6	34.2	29.7	30.1	48.1	47.8	17.3	19.3	37.1	37.1	27.2	28.0
45 years	35.3	34.4	29.2	29.6	34.4	33.9	30.0	29.9	35.0	34.7	29.5	29.8	49.0	48.8	15.3	17.4	38.1	38.6	26.6	27.5
55 years	35.6	34.7	29.1	29.3	34.7	34.1	29.9	29.7	35.6	35.4	29.2	29.5	49.8	49.8	13.2	15.3	39.4	40.6	26.0	26.9

Table 5: Impairment rates (percentages) for warm and cold sensation in five nerves among 303 newly diagnosed cases of multibacillary leprosy, presented by sex, by centre and for combined centres.

Ulnar				Median				Rad. Cut.				Pos. Tib.				Sural			
Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold		Warm		Cold	
M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Faizabad																			
25.8	20.5	14.6	9.2	29.8	36.4	14.8	13.6	35.0	34.1	20.4	38.6	58.2	50.6	40.8	32.2	45.2	39.8	30.6	36.4
Naini																			
17.7	26.3	10.3	13.2	18.5	34.2	12.5	19.7	40.9	50.7	34.8	36.0	53.7	44.7	42.1	34.2	39.3	37.3	40.2	38.7
Combined Centres																			
21.7	23.8	10.5	10.4	22.8	34.8	12.6	11.6	37.0	41.7	25.6	36.8	50.6	44.8	37.5	30.7	42.7	38.7	32.7	33.7

of the study is its large sample size, allowing calculation of normal reference values stratified by age, by sex and by centre for five bilateral nerve sites. Our inclusion and exclusion criteria mean that the resulting reference values may be applied in similar areas of India. They may also be applied in studying the impact of other conditions in which warm and cold sensation are affected, specifically in diabetes.

The first paper in the present series[12] discussed the impact of uncertainty about age on vibration perception thresholds. A tendency to underestimate age may be partly responsible for the extreme values identified for warm and cold sensation thresholds in the present data set. However, the data for the lateral dorsum of the foot exhibit the most extreme values and greatest variability (Table 3A and 3B and Figure 1) and raise concerns about the sensitivity of testing when thresholds are close to the maximum measurable temperatures. Inability to detect such high temperatures also gives rise to concerns about the potential for damage to skin through testing. However, in none of the subjects or cases tested did we see any such damage.

Given the vulnerability of the sole of the foot to injury, maintaining sensation in the foot is a primary concern in preventing secondary injury in leprosy. A study by Withington *et al*[9] found the onset of disabilities in leprosy to be related to socio-economic factors and manual occupations. Such factors may well contribute to the variability in thresholds reported here.

While normal thresholds for the posterior tibial nerve are close to the limits of what could be tested, those for the sural nerve were less extreme. Testing sural nerve function may therefore be preferred. However, the pattern of onset in nerve function impairment in leprosy is not well understood. Further analysis is therefore required to determine sensitivity and specificity between the two nerves.

Working in Israel Yarnitsky and Sprecher[10] used the same Medoc Thermal Sensation Analyser to assess warm and cold sensation at the thenar eminence of the hand in a group of 106 normal subjects. Using the method of levels they computed reference values using the 97.5th percentiles for warm sensation at the thenar eminence ranging from 32.5˚C in the age group 20 - 39 years and 32.6˚C in the age group 60 to 79 years. The equivalent for cold sensation was 31.3˚C to 31.2˚C. In the present study we found both warm and cold sensation thresholds based on the less demanding 95th percentile to be more extreme- 34.0-.7 and 30.3-29.7 respectively. These findings confirm the importance of determining population-specific reference values.

Researchers in UK[14] compared thermal sensation thresholds of healthy subjects in centres in Sweden, Belgium and UK in order to determine the causes of variability within their data. They found differences relating to occupation, age-related effects and a skin temperature effect but no significant difference between left and right side assessments. Compared to workers of other vocations, manual labourers were less able to detect temperature decrease and displayed a wider range between detectable warm and cold sensation. We did not record data on occupation

Figure 2. Scatter plot of ulnar and posterior tibial warm and cold sensation (degrees centigrade) for leprosy cohort cases by age and by centre with superimposed normal 95th or 5th percentiles based on combined centres plus shaded area highlighting the difference between centre-specific percentiles.

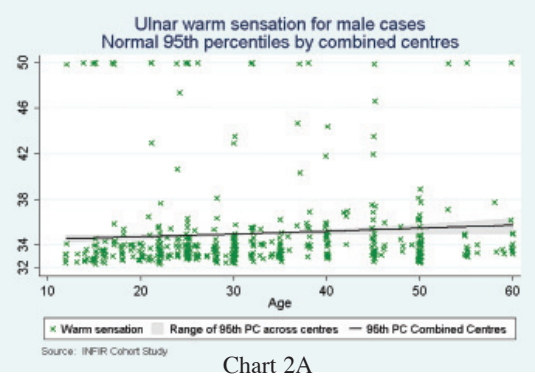

Chart 2A

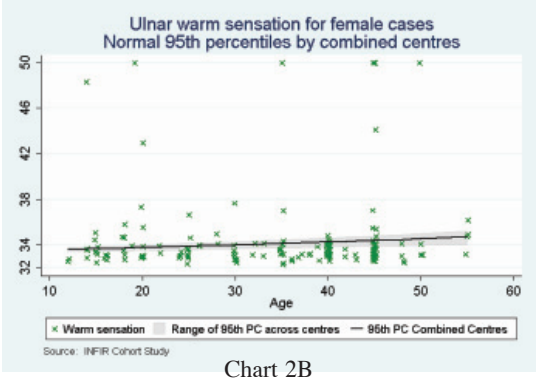

Chart 2B

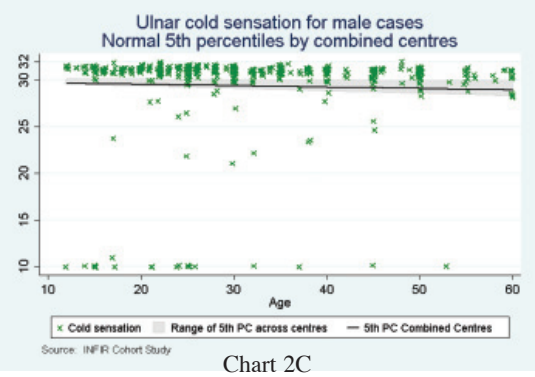

Chart 2C

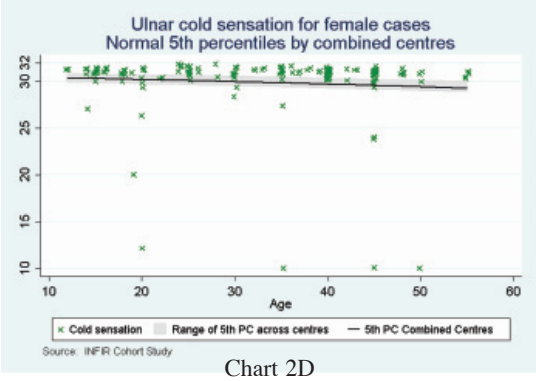

Chart 2D

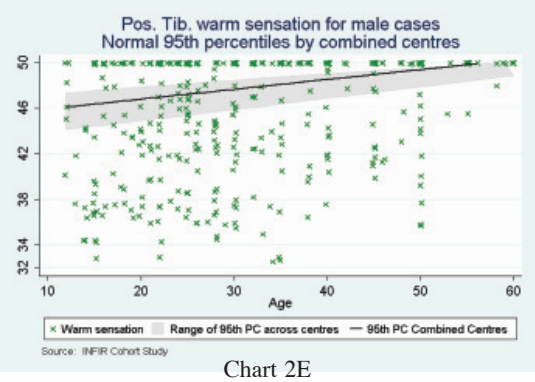

Chart 2E

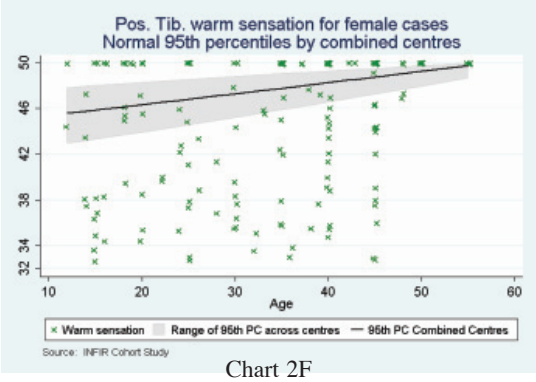

Chart 2F

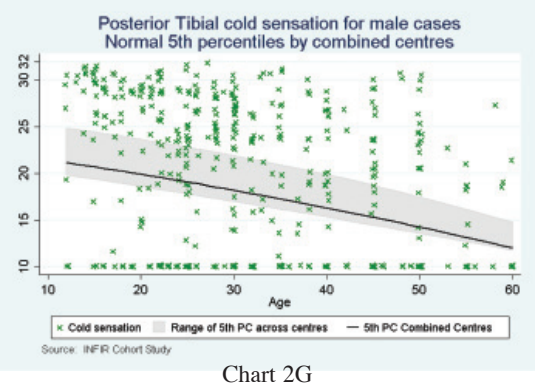

Chart 2G

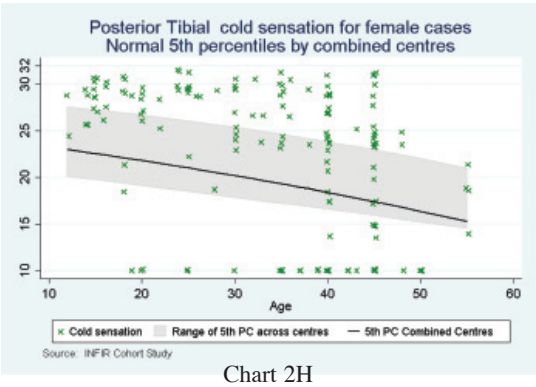

Chart 2H

for the subjects in the present study so are unable to comment on the effect of occupation. Since the observed difference between centres reached statistical significance we decided to apply centre-specific thresholds in our analysis of the cohort data.

In conclusion, this is the first published study presenting reference values for warm and cold sensation among men and women and by age group in a study population in UP State, northern India. Subject to issues relating to assessments of the foot lateral dorsum, the results are of value in the assessment of nerve function in a wide range of neurological conditions.

Applying the reference values to data from the leprosy cohort illustrates the high rates of impaired function among people affected by leprosy. Further analysis of data from the Cohort Study will determine if detecting change in warm or cold sensation during multi-drug therapy is an indicator for the onset of clinical changes in nerve function associated with leprosy reactions.

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