CONTACT HEAT EVOKED POTENTIALS: NORMAL VALUES AND USE IN SMALL-FIBER NEUROPATHY

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ABSTRACT: *Introduction:* Contact heat evoked potentials (CHEPs) may be an objective, non-invasive diagnostic tool in small-fiber neuropathy (SFN). This study establishes normal CHEP values and examines their applicability in SFN patients. *Methods*: Standardized CHEPs were administered at the wrist and ankle. The N_2 and P_2 latencies and N_2–P_2 peak–peak amplitude were recorded by electroencephalography. We examined healthy subjects ($n = 97$), stratified by age and gender, and SFN patients with abnormal intraepidermal nerve fiber density ($n = 42$). CHEP reproducibility and interobserver values were also investigated. *Results*: CHEP normative values were determined. There was a 9–16% increase in latency per centimeter of height with increasing age. Amplitudes were higher in women than men, and decreased (17–71%) with aging. Test-retest reproducibility and interobserver values were >0.61 and >0.96, respectively. CHEPs were abnormal in 73.8% of the patients. *Conclusion*: In this study we have established normal values, reliability, and clinical applicability of CHEPs in SFN.

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In small-fiber neuropathy (SFN), small-caliber myelinated (Aδ) and unmyelinated (C) fibers are affected.[1,2] Interest in this syndrome has increased, particularly since the introduction of intraepidermal nerve fiber density (IENFD) assessment.[3] The diagnosis of SFN is based on sensory and autonomic symptoms not otherwise explainable, intact large-fiber function on examination (normal vibration sense and nerve conduction studies) with abnormal IENFD, and/or abnormal temperature threshold testing (TTT).[4,5] The sensitivity of IENFD for SFN depends largely on the chosen cut-off values,[6] and this technique is not widely available. Furthermore, TTT requires subject cooperation, is potentially vulnerable to malingering, and studies have shown great variability with regard to techniques and methods used.[7] In addition, autonomic function testing can be performed, but it may not be sufficiently sensitive.[2,8]

There is a need for additional objective, reliable, sensitive diagnostic tools for SFN. Cerebral electroencephalographic (EEG) responses to heat stimuli, conveyed by Aδ and C fibers, can be evoked and measured objectively by laser evoked potentials (LEPs) and CHEPs.[9–12] CHEPs have some advantages over LEPs, such as fewer safety precautions, better baseline temperature control, and negligible burn risk.[13] In SFN, CHEPs appear to be sensitive and to correlate with IENFD.[10,13–16] Previous studies have shown CHEP latency and amplitude values to be dependent on gender, age, and pain thresholds.[17–20] However, normative values are only available for an Asian population.[18] Therefore, we aimed to provide age- and gender-stratified normative values for CHEPs over a wide age range of healthy Dutch controls. In addition, we examined the reproducibility of the CHEP curves and interobserver readouts of researchers, and we determined the clinical applicability of CHEPs in patients with SFN and abnormal IENFD.

METHODS

Healthy Controls. This study took place between January 2009 and February 2013. All data were collected prospectively. Healthy participants were recruited from hospital volunteers and through advertisements. Examination of all participants took place at outpatient clinic departments in a comfortable, temperature-controlled room. All participants underwent a standardized interview and examination. Eligibility was based on the following:

Abbreviations: AUC, area under the curve; CHEP, contact heat evoked potential; IENFD, intraepidermal nerve fiber density; LEP, laser evoked potential; MUMC, Maastricht University Medical Center; ROC, receiver-operator characteristic; SFN, small fiber neuropathy; TTT, temperature threshold testing

Key words: contact heat evoked potentials; polyneuropathy; quantitative sensory testing; reference values; small-fiber neuropathy

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age ≥18 years; no sensory symptoms or signs on neurological examination; no diseases that may cause polyneuropathy [e.g., diabetes mellitus, renal failure, alcohol abuse (arbitrarily defined as drinking at least 4 international units per day), or systemic illnesses like sarcoidosis or malignancy]; no past or present use of neurotoxic drugs; and normal nerve conduction studies.

Patients. Patients with a distal sensory neuropathy compatible with SFN were recruited at the Maastricht University Medical Centre (MUMC). Exclusion criteria included: limb or spine deformities; other neurological diseases and symptoms or signs of large-fiber neuropathy, such as weakness, vibration threshold abnormalities as determined by use of a Rydel–Seiffer graduated tuning fork; or abnormalities on nerve conduction studies in either latency, amplitude, or velocity of at least 2 examined nerves.

In the absence of a "gold standard" for the diagnosis of SFN, patients with SFN complaints and abnormal IENFD, compared with reference values,[21] were selected as a validation cohort, as we aimed to use the abnormal IENFD as a surrogate gold standard to determine the sensitivity of the various CHEP parameters.

Assessments. ***Basic Requirements and Assessments.*** Basic characteristics (age, gender, and height) were assessed, and a standardized neurological examination was performed. Nerve conduction studies were performed with a Keypoint.NET (Medtronic, Minneapolis, Minnesota) or Synergy (Natus Medical, Middleton, Wisconsin) machine. One-sided measurements of compound muscle action potential (CMAP) or sensory nerve action potential (SNAP) amplitude and nerve conduction velocity of median motor and sensory nerves, fibular motor, tibial motor, and sural sensory nerves were conducted. F-responses of median, fibular, and tibial nerves were recorded as well as a tibial H-reflexes. In healthy subjects, only fibular CMAP amplitude and sural SNAP amplitude and nerve conduction velocity were measured to minimize subject discomfort. Heat pain (healthy subjects and patients) and warm and cool thresholds (patients) were determined at the thenar eminence and dorsum of the foot with an advanced thermal stimulator or a thermal sensory analyzer (Medoc, Ramat Yishai, Israel), in accordance with available guidelines, and compared with published normative data.[22–24] Heat pain threshold was determined by the method of limits. Warm and cool thresholds were considered abnormal if they were outside reference values for both the methods of levels and limits.

In patients, a skin biopsy was performed at the lower leg for IENFD determination, as previously described.[5,25]

Contact Heat Evoked Potentials. A CHEP stimulator (Medoc) was used to apply heat pulses to the distal volar side of the right forearm and 10 cm above the right lateral malleolus. The thermode consists of a heating thermo foil and a Peltier element with an active water cooling system; heating and cooling rates of 70°C/second and 40°C/second, respectively; and a thermode diameter of 29 mm. Baseline temperature was set at 35°C and peak temperature at 51°C. Evoked potentials were recorded using the Keypoint.NET (Medtronic) or by continuous EEG recording (Brainlab 4.0; OSG, Belgium), with the electrodes placed on Cz, Pz, and Fz according to the International 10–20 system, referenced to linked ears. The evoked potentials were filtered with a bandpass filter at 0.2–100 Hz. With the Keypoint.NET device, artifact rejection was automatic, based on the amplitude of the response. Two test stimuli were given to exclude startle phenomena (e.g., blink, muscle, and movement artifacts). A pilot study of 15 participants determined how many stimuli would be tolerable for a subject and could still be averaged for a reliable response. For each location (first wrist, then ankle) a series of 12 stimuli were given. The participants were instructed to keep their eyes open in a fixed, neutral position to avoid blink artifacts and EEG α-wave contamination during the time-frame of the stimulus. The interstimulus interval was set randomly between 10 and 18 seconds. To avoid habituation to the heat stimuli, the thermode was moved slightly after each stimulus, remaining within a predefined area (similar distance to recording EEG electrode), and maintaining optimal skin contact.[26,27] All subjects were tested twice within a visit with a time interval of approximately 15–30 minutes.

Protocol Approval, Registration, and Consent. The study was approved by the medical ethics committee of the participating hospital and the Central Committee for Human Related Research (identifier number p06.0066L/MEC 05–224), in accordance with the guidelines of the Declaration of Helsinki (amended October 2008, Seoul, Korea). All participants gave written informed consent prior to inclusion.

Statistical Analysis. ***Assessment of CHEP Parameters.*** Latency of the first negative (N_2) and positive (P_2) peaks and N_2–P_2 peak-to-peak amplitude of the late evoked responses (at Cz) were assessed in all participants per testing site and per test run. For all healthy subjects, the N_2 and P_2 latencies and N_2–P_2 amplitudes of the averaged recordings were determined by visual inspection by 2 observers (physicist and technician). The first run was used to calculate normative values using quantile

Table 1. General characteristics of healthy participants and patients with small-fiber neuropathy (SFN)

	Healthy participants ($n = 97$)	SFN patients with abnormal intraepidermal nerve fiber density ($n = 42$)	*P*-value (chi-square test or Student *t*-test)
Gender, *n* (%)			
Women	51 (52.6)	27 (64.3)	0.17
Men	46 (47.4)	15 (35.7)	
Age [mean in years (SD), range]	44.3 (14.5), 17–78	50.6 (12.6), 26–77	0.008
Height [mean in cm (SD), range]	176 (10.2), 150–198	174 (7.2), 158–194	0.13
CHEP N_2 latency [mean ms (SD) range]			
Volar forearm	399 (45), 314–604	427 (66), 290–668	0.002
Distal leg	469 (40), 322–569	512 (65), 418–708	<0.0001
CHEP P_2 latency [mean ms (SD), range]			
Volar forearm	522 (55), 370–754	534 (79), 378–872	0.16
Distal leg	589 (47), 477–714	613 (66), 466–882	0.008
CHEP N_2–P_2 amplitude [mean μV (SD), range]			
Volar forearm	29.5 (11), 9.2–61.7	19.4 (10), 3.7–43	<0.0001
Distal leg	28.9 (12), 9.6–70.9	14.4 (13), 1.9–60.7	<0.0001

regression analyses (95% chosen specificity) and for comparison purposes between the CHEP parameters. The possible influences of age, gender, and height were also examined. The 95th percentile upper limits and median values were estimated for the N_2 and P_2 latencies. The 5th percentile lower limit and median values were calculated for the N_2–P_2 amplitudes on both sides examined. Comparison of basic scores between healthy controls and patients with SFN was performed using a chi-square test or a Student *t*-test, depending on the type of data.

Reproducibility and Receiver-Operator Characteristic Curve Studies. Test-retest values were determined for the 2 test runs (test-retest of curve reproducibility) within each session in all healthy subjects and interobserver read-outs (the second assessment was performed without having access to the first data findings) were also calculated (by 1-way analysis of variance).

Patients with SFN and abnormal IENFD were selected as the "validation gold standard cohort" for the purposes of this study. Through univariate logistic regression studies and calculation of the corresponding areas under the receiver-operator characteristic (ROC) curves (ROC-AUC), the diagnostic accuracy of the various CHEP parameters was evaluated. Sensitivity (fraction of positive cases classified correctly) and specificity (fraction of negative cases classified correctly) were also calculated to determine which CHEP parameter (N_2 latency, P_2 latency, or N_2–P_2 amplitude at the wrist or ankle) best differentiated between healthy controls and the validation patient cohort, and whether acceptable AUC values could be obtained. The ROC-AUC demonstrates no discrimination if values are $\leq$0.5, acceptable discrimination for values 0.7–0.8, excellent discrimination if values are 0.8–0.9, and outstanding discrimination if values are $\geq$0.9.[28] The ROC-AUCs for the forearm and for the lower leg parameters were compared separately with each other through a test for equality.[29] Analyses were performed with Stata v11.0 for Windows XP (StataCorp LP, College Station, Texas). $P < 0.05$ was considered significant.

RESULTS

Participants. All healthy participants ($n = 97$) were examined in the outpatient and electrophysiology departments. The entire examination took 60–120 minutes. The CHEP procedure was well tolerated by all but 1 healthy participant. This subject withdrew from the study. The basic characteristics for the healthy volunteers are listed in Table 1.

Stratification according to age and gender led to 6 age groups: age 18–29 years (9 men, 9 women); 30–39 years (7 men, 12 women); 40–49 years (12 men, 9 women); 50–59 years (11 men, 10 women); 60–69 years (5 men, 7 women); and 70–79 years (3 men, 3 women). Most volunteers were white/Caucasian (98%). Mean pain thresholds at the foot were 45.9°C (SD 3.0°C, range 33.4–50.1°C), which is within the normal range.[23]

A total of 166 patients were examined at our outpatient clinic during the study period, and 42 (25.3%) had abnormal IENFD. There were more women in the patient group compared with controls, but this difference was not significant (Table 1). The mean age was higher in patients compared with controls. All CHEP parameters except P_2 latency at the forearm differed significantly between the control and patient groups (Table 1).

CHEP Results and Reproducibility. Evoked potentials could be recorded by stimuli applied to the volar side of the forearm and the lateral ankle (35°C baseline temperature) in all but 9 healthy

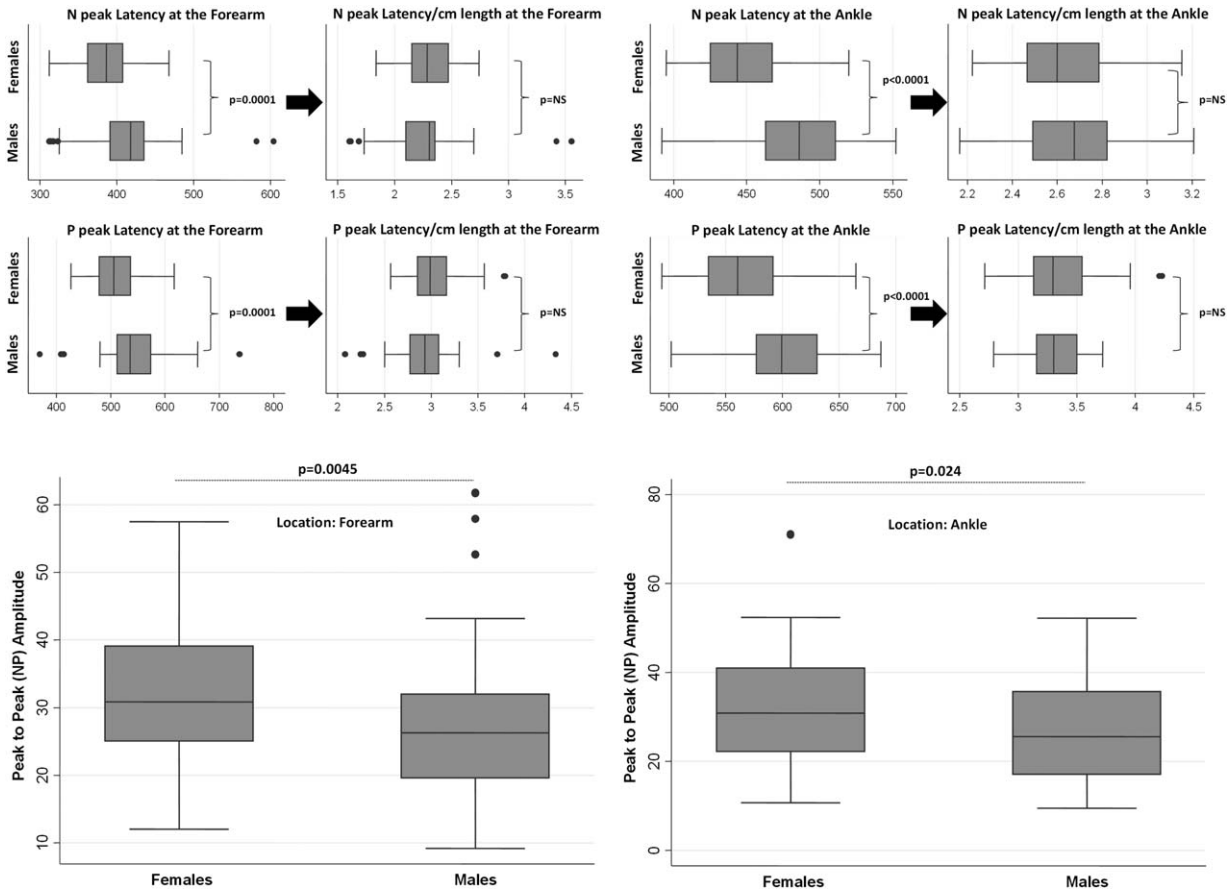

FIGURE 1. CHEP peak latency and amplitude findings in healthy controls. Boxplots show peak latencies and peak amplitudes in healthy men and women. NS = not significant.

subjects (9%). Overall, amplitudes were lower in the second session. A stimulus response could be recorded in all but 2 unresponsive subjects, aged 64 and 70 years, respectively, after having raised the thermode baseline temperature to 40°C. Latencies were significantly height-dependent, being longer in men than in women (Fig. 1). By dividing the latencies by height (cm) in each healthy control, we were able to allot for latencies/cm height values that were not gender-dependent; these values increased slightly with age. N_2–P_2 amplitude values were significantly higher in women than in men and decreased significantly with age (Table 2 and Fig. 1).

Normative clinically useful reference values (median and 95th percentiles for the N_2 and P_2 latencies/cm height and median and 5th percentiles for the N_2–P_2 amplitudes scores) were calculated from the recorded evoked responses for the 2 examination sites (Table 2). Acceptable high test-retest curve reproducibility and interobserver read-out reliability scores were obtained, with all scores being >0.6 up to 0.99 (Table 3).

Diagnostic Accuracy and Comparison between the Various CHEP Parameters. The 42 patients with abnormal IENFDs were considered the surrogate "gold standard" for calculation of sensitivity, specificity, and percentage of subjects correctly classified. Figure 2 shows the comparison of CHEP parameters (N_2 latency/cm height, P_2 latency/cm height, and N_2–P_2 amplitude) at the forearm and at the ankle, separately. The N_2–P_2 amplitude at the ankle demonstrated the highest ROC-AUC (AUC = 0.83; sensitivity 69.1%, specificity 87%; correctly classified 80.7%) followed by the N_2–P_2 amplitude at the wrist (AUC = 0.76; sensitivity 50%, specificity 88.5%; correctly classified 76%). For the remaining parameters, sensitivity, specificity, and correctly classified findings were lower (data not shown). Comparison between the N_2–P_2 amplitude values with the N_2 latency/cm and P_2 latency/cm at the ankle demonstrated higher values for the N_2–P_2 amplitudes (Fig. 2). Comparison between N_2–P_2 amplitude values at the ankle versus the values at the wrist showed a trend in favor of the N_2–P_2 amplitude at the ankle, but the difference was not significant ($P = 0.16$). Using normative values for the TTT and CHEPs, a diagnostic accuracy of 73.8% (31 of 42) was seen for both diagnostic techniques separately in patients with SFN with reduced IENFD. In 60% of patients (25 of 42), both TTT and CHEPs were abnormal and,

Table 2. Contact heat evoked potential normative values

A. Latency normative values for men and women

	Forearm				Lower leg			
	N_2 latency/cm height (ms/cm)		P_2 latency/cm height (ms/cm)		N_2 latency/cm height (ms/cm)		P_2 latency/cm height (ms/cm)	
Age (y)	Median	95th percentile	Median	95th percentile	Median	95th percentile	Median	95th percentile
< 30	2.2	2.5	2.9	3.3	2.5	2.9	3.2	3.6
30–39	2.2	2.5	2.9	3.4	2.6	3.0	3.3	3.7
40–49	2.3	2.6	3.0	3.6	2.7	3.0	3.3	3.8
50–59	2.3	2.7	3.0	3.7	2.7	3.1	3.3	3.8
60–69	2.3	2.7	3.0	3.8	2.8	3.2	3.4	3.9
70^+	2.4	2.8	3.1	4.0	2.9	3.3	3.4	3.9

B. Amplitude normative values

	Women				Men			
	Wrist		Ankle		Wrist		Ankle	
	N_2–P_2 amplitude (μV)		N_2–P_2 amplitude (μV)		N_2–P_2 amplitude (μV)		N_2–P_2 amplitude (μV)	
Age (y)	5th percentile	Median	5th percentile	Median	5th percentile	Median	5th percentile	Median
<30	20.6	33.7	17.6	38.2	11.8	27.8	18.6	32.4
30–39	16.4	32.5	15.2	32.4	11.4	26.8	15.2	29.1
40–49	12.8	31.4	13.2	27.4	11.1	26	12.2	26.3
50–59	9.3	30.5	11.1	22.4	10.8	25.1	9.2	23.4
60–69	6.1	29.5	9.3	17.8	10.5	24.3	6.5	20.9
70^+	1.2	28.1	6.5	11.1	10.1	23.2	2.5	17.1

when combining both techniques, 88% (37 of 42) of patients had an abnormal test result.

DISCUSSION

In this study we have provided clinically applicable normative values for various CHEP parameters recorded at the volar forearm and distal leg for normal Dutch volunteers. Observed amplitudes and latencies were comparable to reported values.[13,17,18,30] Subject age showed a slight increase in latency/cm height and a substantial decrease in N_2–P_2 amplitude. Similar findings have been reported in a smaller Asian cohort.[18] Because repeated stimulation at the same location may lead to amplitude reduction,[31] we randomized the stimulus interval and moved the thermode, as has been advised in previous studies.[26,27] This optimizes the "stimulus novelty," which has been shown to influence amplitudes in laser evoked potentials.[32] Nonetheless, amplitudes decreased in our subjects after repeated stimulation, suggesting central habituation[33] and/or nociceptor fatigue.[34]

We also found acceptable test-retest reproducibility for the curves and interobserver reliability values. Other investigators have shown reproducibility in subjects tested twice (on average, 1-month interval) to be "fair to substantial."[35]

Using our normative CHEP values versus reported normative TTT values,[23,24] both techniques capture 74% of patients with SFN and

Table 3. Test–retest and interobserver values of contact heat evoked potential parameters

	Wrist		Ankle	
	Test–retest curves (95% CI)	Interobserver values (95% CI)	Test–retest curves (95% CI)	Interobserver values (95% CI)
N_2 latency, observer 1	0.78 (0.69–0.87)	N_2 latency 0.96	0.66 (0.53–0.80)	N_2 latency 0.99
N_2 latency, observer 2	0.73 (0.63–0.84)	(0.95–0.97)	0.68 (0.54–0.81)	(0.98–0.99)
P_2 latency, observer 1	0.75 (0.65–0.85)	P_2 latency 0.97	0.62 (0.46–0.77)	P_2 latency 0.97
P_2 latency, observer 2	0.74 (0.63–0.84)	(0.96–0.98)	0.61 (0.46–0.77)	(0.95–0.98)
N_2–P_2 amplitude, observer 1	0.67 (0.55–0.80)	N_2–P_2 amplitude	0.68 (0.54–0.81)	N_2–P_2 amplitude
N_2–P_2 amplitude, observer 2	0.67 (0.55–0.80)	0.99 (0.99–1.0)	0.67 (0.53–0.80)	0.99 (0.99–1.0)

CI, confidence interval.

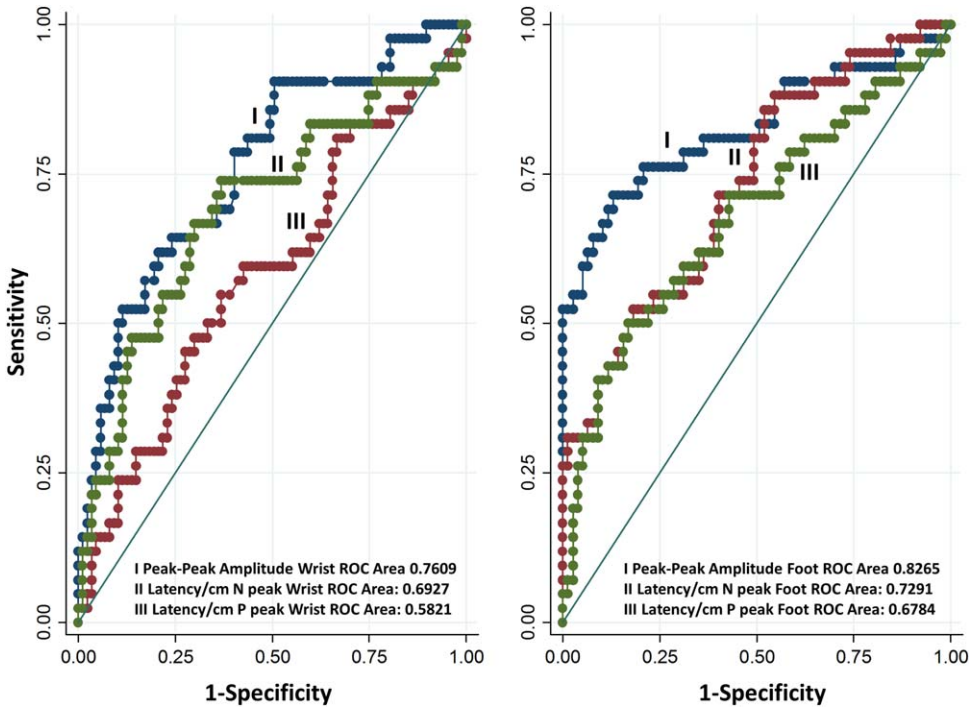

FIGURE 2. Comparison of CHEP parameters at the forearm and ankle separately, using the area under the ROC curves. **(A)** CHEP parameter comparison at the wrist: the N_2–P_2 amplitude ROC-AUC was larger (0.7609) when compared with the AUC for N_2 peak latency/cm (0.6927) and P_2 peak latency/cm height (0.5821), respectively. Through ROC comparison (using the Stata command "Roccomp"), we found a significant difference between the amplitude ROC-AUC values versus P_2 peak latency/cm height scores ($P = 0.012$). No significant difference ($P = 0.27$) was obtained between the N_2–P_2 amplitude versus N_2 peak latency/cm height values. **(B)** CHEP parameter comparison at the ankle: the N_2–P_2 amplitude ROC-AUC was greater (0.8265) compared with the AUC for N_2 peak latency/cm (0.7291) and P_2 peak latency/cm height (0.6784), respectively. The area under the ROC curve for N_2–P_2 amplitude yielded a favorable trend when compared with the other 2 methods (N_2–P_2 amplitude vs. N_2 latency/cm: chi-square = 3.63; $P = 0.056$; N_2–P_2 amplitude vs. P_2 latency/cm: chi-square = 4.83; $P = 0.028$). However, the differences were not significant after Bonferroni correction.

abnormal IENFD. In addition, CHEP N_2–P_2 amplitudes at the ankle, more than at the wrist, showed acceptable diagnostic accuracy in differentiating between our controls and SFN patients, with reasonable sensitivity and specificity scores. Several studies have demonstrated increased CHEP latencies and reduced amplitudes in various forms of peripheral neuropathies that involve small nerve fibers.[10,16,36,37] Our ROC findings (Fig. 2) and reproducibility values provide further support for CHEPs to be considered as a reliable, non-invasive technique to assess function of small nerve fibers, particularly as skin biopsy is not widely available.

There are some methodological issues that should be addressed. First, recordings were not obtained initially in 9 healthy subjects (9%). However, after a slight increase of baseline temperature to 40°C, there were only 2 subjects (2%) in whom no CHEPs were obtained. Similar observations were reported in previous studies.[17,27,38,39] A possible partial explanation for this effect could be that, at higher temperature, the ultra-late C-fiber response does not influence the occurrence of the late Aδ response.[40] Due to the burden of the CHEP examination, no additional studies were performed at 40°C for the responsive subjects already examined at 35°C. However, future studies should determine the best baseline temperature and optimum number of stimuli to improve test performance. Also, the question remains open whether some of these "healthy" subjects may have subclinical nerve fiber deficits as a form of subclinical polyneuropathy. Second, we had difficulty recruiting healthy subjects aged >70 years. Most potential participants in this age category were rejected due to comorbidity and/or abnormalities on examination. Some caution is therefore suggested when using the normative values in subjects >70 years old. Third, the first CHEP responses obtained after the test stimuli were often the clearest responses. On the other hand, subjects did not usually react with movements to the stimulus, and it was very easy to avoid blinking during the actual delivery of the heat pulse. So, giving test stimuli and discarding the results of these stimuli to exclude "startle effects" may have negatively influenced the results. Fourth, a diagnostic accuracy of 74% was obtained with the CHEPs in SFN patients diagnosed using abnormal IENFD as a surrogate gold standard, but a lower sensitivity could be

expected in an unselected SFN population, as skin biopsy does not capture all SFN patients.

Despite these shortcomings, we have demonstrated clinically applicable, reliable normative values for CHEPs in the distal leg and volar forearm and showed clinical utility in patients with SFN. The use of CHEPs is suggested as an additional non-invasive tool in the diagnosis of SFN.

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