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ORIGINAL COMMUNICATION

Criteria for diagnosis of pure neural leprosy

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Abstract The clinical diagnosis of pure neural leprosy (PNL) remains a public health care problem mainly because skin lesions – the cardinal features of leprosy – are always absent. Moreover, the identification of the leprosy bacillus is not easily achieved even when a nerve biopsy can be performed. In an attempt to reach a reliable PNL diagnosis in patients referred to our Leprosy Outpatient Clinic, this study employed a variety of criteria. The nerve biopsies performed on the 67 individuals whose clinical, neurological, and electrophysiological examination findings strongly suggested peripheral neuropathy were submitted to *M. leprae* identification via a polymerase chain reaction (PCR). Mononeuropathy multiplex was the most frequent clinical and electrophysiological pattern of nerve dysfunction, while sensory impairment occurred in 89 % of all cases and motor dysfunction in 81 %. Axonal neuropathy was the predominant electrophysiological finding, while the histopathological nerve study showed epithelioid granuloma in 14 % of the patients, acid fast bacilli in 16 %, and nonspecific inflammatory infiltrate and/or fibrosis in 39 %. PCR for *M. leprae* was positive in 47 % of the nerve biopsy samples (n = 23). PCR, in conjunction with clinical and neurological examination results, can be a powerful tool in attempting to identify and confirm a PNL diagnosis.

Key words pure neural leprosy (PNL) · leprosy neuropathy · histopathology · polymerase chain reaction (PCR)

Introduction

In developing countries, leprosy is the most common cause of peripheral neuropathy, and any leprosy patient is susceptible to the development of neuropathy during the course of the disease. As an isolated clinical entity, it is characterized by clinical evidence of nerve deficit and nerve thickening with or without tenderness in the absence of any sign of skin inflammation or history of skin patches and is commonly referred to as *neuritic* or *polyneuritic* leprosy [3] as well as *pure neural, primary neural, pure neuritic,* and *primary neuritic* [7] leprosy. In 1952, Wade [17] named this form of the disease as a separate subgroup, a finding ultimately accepted by both the Madrid (1953) [8] and Indian classifications (1955) [4].

Sensory cutaneous impairment, numbness, paresthesia, nerve pain, and nerve thickening are the most common symptoms and signs of pure neural leprosy (PNL) [11]. Some authors claim that a definitive PNL diagnosis can only be achieved by performing a biopsy of the peripheral nerve [5, 7, 11]. However, in most cases, detection of the bacteria is extremely difficult; and, for the most part, histological findings are nonspecific. It is clear that the availability of a more specific and sensitive

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method for detecting *M. leprae* is of the utmost importance. For this reason, several laboratories have recently reported the identification of *M. leprae* DNA directly from nerve biopsy specimens [1] via the polymerase chain reaction (PCR).

Therefore, for purposes of classification, this study attempted to elaborate a more precise definition of PNL based on histological features and the detection of the bacteria or the *M. leprae* DNA in the nerve biopsy specimens obtained from patients who presented with peripheral neuropathy without skin lesions. The neuropathy was previously identified by clinical and electrophysiological examinations that pointed to leprosy as its cause. Hopefully, in clinical practice, such an approach will facilitate the evaluation of each feature to obtain a prompt diagnosis of this challenging neurological disease.

Patients and methods

Clinical and electrophysiological evaluation

Patients suspected of PNL were followed up from October 1998 to May 2001. Ninety six patients with a suspected PNL diagnosis were evaluated at the Leprosy Outpatient Clinic, Oswaldo Cruz Foundation, Rio de Janeiro, R. J., Brazil. Clinical and electrophysiological examinations were performed prior to initiating the study. Excluded from the study were patients with evidence of skin patches, infiltration or a history of skin lesion(s) as well as those presenting with such associated diseases as diabetes mellitus, alcoholism, hepatitis B or C, HIV or HTLV-I infections, rheumatoidal/rheumatic diseases, in addition to toxic, drug-induced or hereditary neuropathies. The research was carried out in strict compliance with the international norms on ethics in human research, having been previously approved by the Ethics Committee of the Oswaldo Cruz Foundation. All patients provided their written consent.

A detailed evaluation was performed on all individuals to record the number and distribution of affected nerves. Nerve thickening was ascertained by comparing one nerve with its counterpart on the contralateral side. Palms and soles were examined for the presence of cyanosis and/or erythrodermia; nerve pain was evaluated when it occurred spontaneously or on touch. Sensory impairment, motor deficit, and disability/deformity status were assessed by using standard methods. In brief, tactile sensation was tested by way of an aesthesiometer (the Semmes-Weinstein technique); thermal sensation was tested by using cold and warm metal objects; and a safety pin was used to ascertain pain perception. Individual muscle power was graded according to the method described by the Medical Research Council (MRC) of London [10]. Disability was recorded in accordance with the standard World Health Organization [18] grading scheme. Electrophysiological testing by way of the Nihon-Koden – Neuropack 2 EMG system followed standard procedure.

As a result of the preliminary clinical and electrophysiological evaluation of the 96 patients suspected of PNL, 29 individuals were excluded from the study either because they featured exclusion criteria or because peripheral neuropathy could not be confirmed.

Laboratory studies

Slit smears to determine the systemic bacillary load were taken from six standard sites (earlobes, elbows and knees).

Nerve biopsies and histological evaluation

Nerve biopsies were performed on a total of 67 individuals whose neurological and electrophysiological examinations confirmed the existence of peripheral neuropathy pointing to leprosy as its cause. The site of the surgical procedure (sensory nerves shown to be affected either clinically or electrophysiologically) depended on the nerve chosen, as follows: dorsal cutaneous ulnar nerve on the dorsum of the hand [2], sural nerve at the ankle level, and superficial peroneal nerve above the ankle. Nerve samples were taken and cut into two fragments. One fragment was immediately immersed into Carson's fixative solution (Millonig-buffered formalin) for 72h followed by the paraffin-embedding routine, and the other was frozen for PCR. Wade's modification of the Ziehl-Nielsen method was used for detection of acid fast bacilli (AFB).

PCR assay

For PCR detection of *M. leprae* DNA, nerve samples were processed according to the recommendations of Chemoulli et al. [1] with few modifications. Briefly, a small piece (1 mm^3) of the biopsy sample was incubated with 50µl NaOH at room temperature for 10min, neutralized with 1M NaH_2PO_4, and centrifuged. The supernatant was removed and the pellet suspended in 100µl of 60mM Tris-buffer pH 8.8. The samples were treated with 60µg of proteinase K at 50°C for 30min, followed by enzyme inactivation at 95°C for 5min. Samples were then submitted to a thermal shock procedure consisting of three consecutive cycles of 10min of boiling and snap-freezing. Amplification of *M. leprae*-specific DNA and hybridization conditions were performed as described elsewhere [12].

Results

Clinical and Electrophysiological evaluation

Notwithstanding the performance of nerve biopsies in the 67 suspected patients, diagnosis of PNL was only confirmed in 49 (73%) patients who presented with at least some of the histological and/or molecular biology criteria adopted in this study or were shown to have very strong clinical and electrophysiological indications of leprosy neuropathy. The PNL patients consisted of 38 males and 11 females from 17 to 79 years of age (mean ± SD = 42.4 ± 17.2). All were treated with multidrug therapy according to WHO recommendations: 46 of whom were for 6 consecutive months (PB leprosy) and the remaining 3 for 1 year (multibacillary leprosy). The duration of the symptoms ranged from 1 to 120 months (mean ± SD = 23.7 ± 26.1). Their most frequent presenting symptoms were paresthesia (55%), motor impairment (24%), nerve pain (12%), and sensory impairment (8%).

The mononeuropathy multiplex clinical form was the most frequently-detected pattern of nerve dysfunction in 61% of the patients, followed by mononeuropathy, in 33%. Only 3 patients presented with polyneuropathy as a clinical form.

When the number of damaged sensory and motor nerves was evaluated, it was found that sensory nerves

were the most frequently affected. The ulnar nerve was the most frequently affected sensory and motor nerve.

According to the conduction studies used to determine neurophysiological dysfunction, axonal lesions were predominant in all patients. Four patients had demyelinating lesions.

Laboratory studies

Forty-six patients (94%) were negative for acid-fast bacilli according to their lymph smear evaluations. Epithelioid granuloma, *M. leprae*-loaded macrophages, and caseous necrosis were found in only 7 patients; and the presence of AFB was detected in the sample nerves of 8 patients. These features defined the PNL diagnosis, and/or the detection of positive PCR, that occurred in the nerve biopsy specimens of 23 patients (82%) (Table 1). Twelve of the aforementioned (42%) had no inflammatory infiltrate in the nerves or AFB or any other finding that could lead to a final diagnosis of leprosy.

In 16 of the sample nerve biopsies, inflammatory infiltrates were detected, strongly suggesting the diagnosis of PNL in an endemic state (probable PNL). This group showed nonspecific, predominantly perineurial (34%) and endoneurial (34%) infiltrate (58%). Nerve fibrosis (mainly perineurial) was the second most common finding (40%). Nine of these patients (47%) showed nonspecific, inflammatory infiltration accompanied by nerve fibrosis, whereas twelve patients (62%) had nonspecific inflammatory infiltration, and 11 (57%) had nerve fibrosis alone.

The diagnosis of PNL in 2 other patients was based solely on clinical and electrophysiological features.

Final diagnosis of the 12 (42%) patients was, therefore, only possible thanks to the positive PCR finding (Table 1). In this group, nonspecific inflammatory infiltration was less frequent than positive PCR, affecting only 46% of the patients (n = 16). Among these 16 patients, 11 were found to have a positive PCR result. On the other hand, five patients who had epitheliod granuloma also had a negative PCR; and one patient with AFB had a negative PCR.

Table 1 Parameters: Definite group

Patient	PCR	Histopathology				
		Inflammatory Infiltration	Granuloma	AFB	Fiber loss	Fibrosis
1.	–	+	+	–	+++	+++
2.	+	–	–	–	++	++
3.	+	+	+	–	+	+
4.	+	–	–	–	–	–
5.	+	–	–	–	NA*	+
6.	+	+	–	–	–	+
7.	+	–	–	–	–	–
8.	+	+	–	+	+++	–
9.	+	+	–	+	+++	++
10.	–	+	+	+	+++	+
11.	+	+	–	+	+	+
12.	–	+	+	–	+++	+++
13.	+	–	–	–	++	+/–
14.	+	+	+	–	–	–
15.	+	+	–	–	–	+
16.	–	+	+	–	+++	+++
17.	+	–	–	–	+++	++
18.	+	–	–	–	+++	–
19.	+	–	–	–	+/–	–
20.	+	–	–	–	–	+
21.	+	–	–	–	–	+
22.	+	++++	–	+	++	++
23.	+	+	–	+	–	++
24.	+	–	–	–	–	–
25.	+	+	–	+	++++	+
26.	+	–	–	–	–	–
27.	+	++	–	+	+++	+
28.	–	++++	+	–	++++	+++

* Not available

Discussion

Leprosy neuropathy almost always occurs in conjunction with a certain type of skin lesion. The presence of nerve deficit in patients from endemic areas who did not have skin lesions is considered sufficient reason for a PNL diagnosis [7, 9, 16].

The proportion of leprosy patients with PNL will ultimately depend on the population in question, as, for example, in India, where its incidence has been reported to range from 5.5 to 17.7% of all leprosy cases [14]. In Brazil, an average PNL frequency rate has not yet been determined. In this study, the 49 patients diagnosed as PNL accounted for 17.1% of the total 286 patients diagnosed with leprosy in the Leprosy Outpatient Clinic at FIOCRUZ during the period of the study. This number, however, does not represent the actual incidence rate of the disease in Brazil since the clinic is a national referral center for leprosy treatment.

The electrophysiological data resulting in our study corroborate previous reports in that 91% of the patients examined demonstrated axonal nerve dysfunction. Likewise, Tzourio et al. [15] described patients with acute neuritis who exhibited motor conduction abnormalities suggesting demyelination. Although this is an uncommon finding in PNL, 8% of our patients showed such a pattern.

Also useful in choosing the most appropriate nerve

for biopsy, these electrophysiological findings made it possible to confirm or not the diagnosis of peripheral neuropathy. Furthermore, the defining histological criteria for leprosy neuropathy included the presence of AFB and epithelioid granuloma. The observation of a nonspecific inflammatory infiltrate (mononuclear cells with no differentiation as to Virchow or epithelioid cells) was found to be a less specific sign of leprosy neuropathy than the presence of an epithelioid granuloma. This finding was observed in thirty-five (71 %) of the patients in this study, 14 (40 %) of whom had AFB or epithelioid granuloma or both.

In recent years, the PCR technique has been successful in demonstrating the presence of AFB in leprosy samples [6]. In the present investigation, PCR contributed to the diagnosis of cases in which the clinical and histopathological data were not conclusive evidence of PNL. Moreover, the findings of this study were consistent with the results of Chemoulli et al. [1] in which PCR in nerve specimens increased by at least twice the frequency of detecting *M. leprae*. One patient showed the presence of AFB with an unexpected negative PCR, which could be considered a false negative result. A possible explanation is the presence of inhibitors in the sample that could be detected by using an internal control during amplification or by reconstituting the original sample with purified *M. leprae* DNA before the PCR.

The present data also confirm the findings of other researchers who have reported on the high sensitivity and near-perfect specificity of PCR studies to detect *M. leprae* [6, 12, 13]. As a result, in detecting *M. leprae* [6], the PCR study appears to be more sensitive than histopathological examination. PCR thus has considerable potential as a laboratory aid in diagnosing PNL. Nonetheless, it must be kept in mind that the results obtained via PCR should always be analysed in conjunction with clinical and histopathological findings.

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