

Cytodiagnosis of Primary Neuritic Leprosy¹

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In South India the incidence of Hansen's disease is high, affecting 4.3 per 1000 people in the South Indian state of Karnataka (⁶). Pure neuritic (primary neuritic) Hansen's disease makes up about 18% of these cases (²). Most of these patients first present to the neurologist or physician for this neuropathy and are rarely seen by the dermatologist as first contact physician due to the lack of anesthetic patches. The diagnosis of pure neuritic Hansen's disease is, hence, difficult since the only confirmatory test is a nerve biopsy. However, a nerve biopsy is not a simple office procedure and is not without its own complications, particularly when major nerve trunks are involved. Fine needle aspiration (FNA) cytology, on the other hand, is a simple office procedure which enables one to obtain material for diagnosis without causing (unlike in a biopsy) a breach in the continuity of the nerve. This procedure has been used in one particular study for the demonstration of acid-fast bacilli (AFB) in primary neuritic leprosy (PNL) (⁵).

To the best of our knowledge FNA has not been used as a diagnostic measure for the morphological study of PNL. This paper attempts to categorize the histology of inflamed nerves in order to find out the validity of this technique with regard to the diagnosis, classification and AFB status of a clinically suspected case of primary neuritic leprosy.

MATERIALS AND METHODS

All consecutive cases of motorsensory neuropathy over a 9-month period who pre-

sented to the Dermatology Department of the St. John's National Academy of Health Sciences, Bangalore, India (to rule out Hansen's disease) were included in the study. The criteria for selection of the cases were: a) clinically established sensory neuropathy with or without deformity, b) absence of anesthetic patches, and c) absence of AFB in slit-skin smears.

The thickened nerves were graded clinically as per the following criteria: Grade (G) 0 = normal; Grade (G) 1 = palpably thickened; Grade (G) 2 = thickened and visible to the naked eye; and Grade (G) 3 = nerve abscess.

In mononeuropathy the concerned nerve was aspirated. In the case of polyneuropathy the most thickened nerve was aspirated. All patients were followed up for a period of 1 year after the aspiration. Histopathology was not used as the "gold standard" for comparison in order to avoid the unnecessary complication of a nerve trunk biopsy.

Cases presenting with polyneuropathy were screened for diabetes by fasting and postprandial glucose levels. Electroneuromyogram (ENMG) studies, collagen vascular disease workups, and a cutaneous nerve biopsy were done whenever necessary.

Method of aspiration

The nerve was palpated and the most prominent site noted. The area to be aspirated was cleaned with an alcohol swab. A nerve block was given using 2% lignocaine 0.5 cc–1 cc injected about 1 cm proximal to the site of aspiration. A 22-gauge 4-cm needle fixed to a 10-cc disposable syringe was used with a syringe holder, and aspiration was performed using a single-puncture, multidirectional technique. The direction of the needle was always kept parallel to the length of the nerve so as to cause minimal damage to the nerve. The material aspirated was smeared on glass slides which were fixed in 95% ethyl alcohol. Hematoxylin and eosin (H&E) and Fite-Faraco stainings for acid-fast organisms were performed.

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THE TABLE. *Details of patients included in the study.*

S. no.	Sex/age	Poly/mono ^a	Grade	Nerve	Cytologic aspirated appearance ^{b,c} + AFB stain	Diagnosis
1.	M/35	Poly	3	Superficial peroneal	Schwann cells ++ Lymphocytes ++ Macrophages ++ Negative ^d	Leprous neuritis
2.	F/27	Mono	1	Left lateral popliteal	Hemorrhagic Negative	Collagen vascular disease
3.	M/61	Poly	0	Right posterior tibial	Hemorrhagic Negative	Alcoholic neuropathy
4.	M/26	Mono	2	Left ulnar	Granuloma + Positive ^e	Leprous neuritis
5.	M/27	Mono	2	Left ulnar	Hemorrhagic Negative	Treated as HD
6.	M/21	Poly	0	Left popliteal	Hemorrhagic Negative	Toxic neuropathy
7.	F/10	Mono	2	Lateral popliteal	Schwann cells + Macrophages + Lymphocytes + Negative	Leprous neuritis
8.	M/60	Poly	0	Left lateral popliteal	Hemorrhagic Negative	Diabetic
9.	M/23	Mono	0	Left lateral popliteal	Schwann cells + Macrophages + Lymphocytes + Negative	Leprous neuritis
10.	M/40	Mono	2	Right ulnar	Schwann cells + Lymphocytes + Macrophages + Negative	Leprous neuritis
11.	F/25	Mono	2	Posterior tibial	Hemorrhagic Negative	Treated as HD
12.	M/30	Mono	2	Radial cutaneous	Hemorrhagic Negative	Leprous neuritis
13.	M/16	Mono	2	Left ulnar	Schwann cells + Macrophages + Lymphocytes + Negative	Leprous neuritis
14.	M/22	Mono	1	Right ulnar	Macrophages + Lymphocytes + Negative	Leprous neuritis
15.	M/35	Mono	0	Right ulnar	Macrophages + Lymphocytes + Negative	Leprous neuritis
16.	F/39	Mono	0	Right ulnar	Hemorrhagic Negative	Leprous neuritis
17.	M/12	Mono	2	Left ulnar	Granuloma +	Leprous neuritis
18.	M/57	Mono	1	Right ulnar	Schwann cells + Lymphocytes + Macrophages + Negative	Leprous neuritis
19.	M/26	Mono	3	Right ulnar	Granuloma + Positive	Leprous neuritis
20.	M/30	Poly	2	Left ulnar	Macrophages ++ Lymphocytes ++ Negative	Leprous neuritis
21.	M/16	Mono	2	Right ulnar	Granuloma + Negative	Leprous neuritis
22.	F/28	Mono	3	Right ulnar	Granuloma + Negative	Leprous neuritis
23.	F/25	Mono	2	Left ulnar	Granuloma Negative	Leprous neuritis
24.	M/12	Mono	2	Left ulnar	Schwann cells ++ Macrophages ++ Lymphocytes + Negative	Leprous neuritis
25.	M/65	Mono	2	Right ulnar	Lymphocytes ++ Macrophages + Negative	Leprous neuritis
26.	M/30	Poly	2	Left ulnar	Granuloma + Positive	Leprous neuritis
27.	F/86	Mono	1	Right ulnar	Hemorrhagic Negative	Treated as HD

^a Poly = Polyneuropathy; Mono = mononeuropathy.^b ++ = Numerous.^c + = Present.^d Negative = Negative for AFB^e Positive = Positive for AFB.

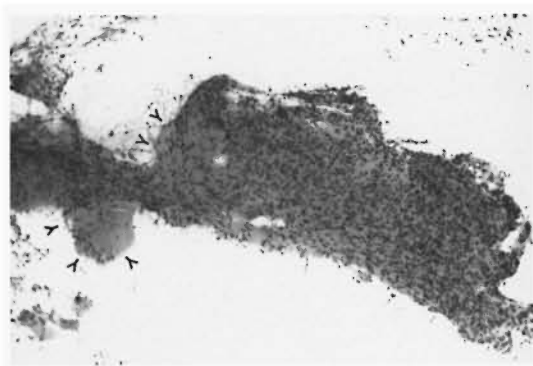


FIG. 1. Aspirate showing portion of a nerve with several Schwann cells extensively involved by granuloma (arrows) with epithelioid cells and Langhans' giant cells (H&E $\times 100$).

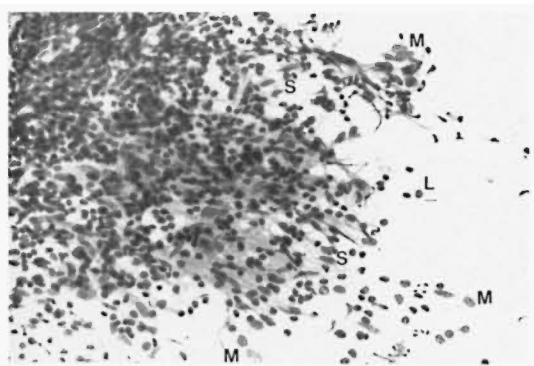


FIG. 3. Aspirate showing sheets of Schwann cells (S) amid which are identified several macrophages and lymphocytes (H&E $\times 250$).

Cytologic criteria for cell identification

Macrophages. Macrophages were cells which measured 20–80 μm in size, with a vesicular, light staining, peripheral or central nucleus with a clearly visible nuclear membrane. The nucleus was either oval or kidney shaped. The cytoplasm was abundant and sometimes showed vacuolation.

Epithelioid cells. An epithelioid cell was identified as a cell with a pale cytoplasm and a vesicular elongated, drawn out, indented or folded nucleus, producing a shape reminiscent of a footprint. The nuclear chromatin was fine and nucleoli were usually inconspicuous. The cytoplasmic margins were indistinct. One of the consistent features of epithelioid cells was the virtual absence of recognizable endocytosed material in their cytoplasm. These cells could be seen singly or in loose clusters.

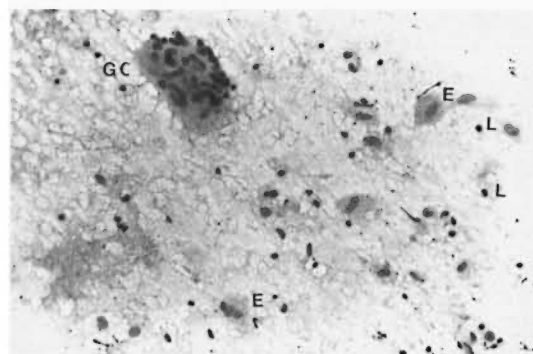


FIG. 2. Dispersed multinucleate Langhans' giant cells (GC) with peripheral arrangement of nuclei epithelioid cells (E) and lymphocytes (L) (H&E $\times 250$).

Apart from the above differences, the criteria adopted by the authors in differentiating the kidney-shaped nucleus of a macrophage from a "footprint" nucleus of an epithelioid cell were: a) The width of a macrophage nucleus was much more than that of an epithelioid-cell nucleus. b) Both ends of a kidney-shaped nucleus of a macrophage were of equal thickness; whereas one end of the elongated nucleus in an epithelioid cell was thinner as compared to the other. c) The indentation in the kidney-shaped nucleus of a macrophage was concave and smooth, and this was not the case in an epithelioid-cell nucleus.

Schwann cells. Schwann cells were spindle-shaped cells of varying sizes with abundant, pale-staining cytoplasm with pulled out ends and with oval, centrally or eccentrically placed vesicular nuclei with ill-defined nucleoli.

The presence of granulomas was considered a sure diagnostic indication of Hansen's disease in a nerve. Inflammation in a nerve is not normally seen and the presence of macrophages and lymphocytes, even without well-formed granulomas, was also taken as criteria for leprous neuritis.

RESULTS

Twenty-seven consecutive cases of motor sensory neuropathy were observed in our Dermatology Outpatient Department over a 9-month period. Of these, 20 were males and seven were females. Their ages ranged from 10 years to 86 years. The details on these patients are given in The

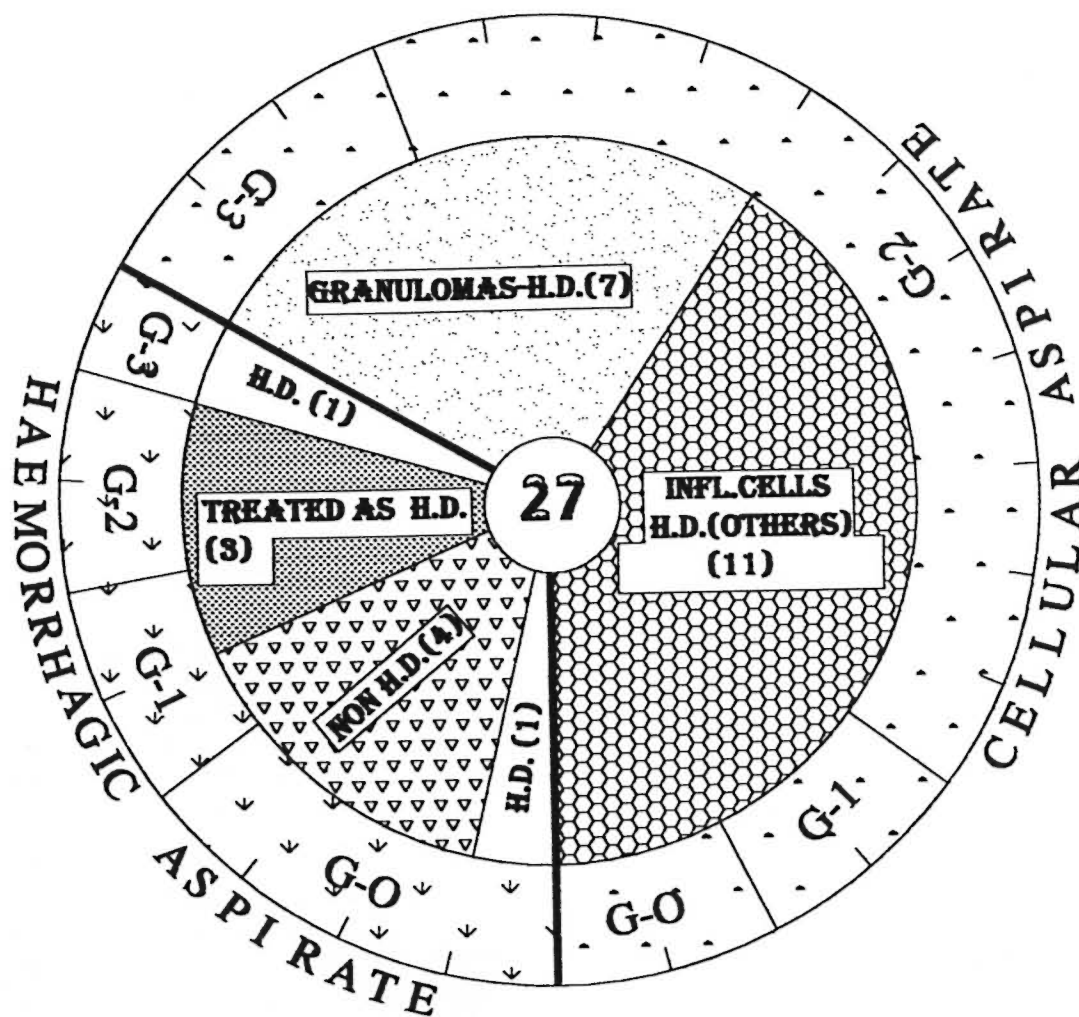


FIG. 4. Pie chart showing grading of nerve, type of aspirate, and diagnosis.

Table. Twenty-one patients presented with mononeuropathy and six with polyneuropathy. With regard to the clinical grading of nerves, 6 cases were grade 0, 4 were grade 1, 13 were grade 2, and 4 cases were grade 3.

At cytology, 18 out of the 27 (67%) cases showed an inflammatory aspirate of lymphocytes, macrophages, epithelioid cells and Langhans' type of giant cells. Seven out of the 18 showed well-formed granulomas. These granulomas had typical epithelioid cells. Schwann cells arranged in a parallel fashion could be seen intimately mixed with these granulomas (Fig. 1). Epithelioid cells, lymphocytes, and Langhans' type giant cells were seen splayed in the

background (Fig. 2). Three of these seven cases showed AFB.

Eleven out of the 18 cases showed only macrophages and lymphocytes amid the Schwann cells (Fig. 3).

Nine out of the 27 (33.4%) aspirates were considered hemorrhagic, although a few Schwann cells could be identified in three of the aspirates. These cases were further investigated. There were three polyneuropathy cases and six mononeuropathy cases. All three polyneuropathy (G0) cases were nonHansen's (diabetes, alcoholism and organophosphorus poisoning). Two mononeuropathy cases (G0 and G3) were biopsied and proved to be Hansen's disease.

The other four cases (G1 and G2) were given a therapeutic trial for Hansen's disease, and responded well to therapy (Fig. 4).

No untoward sequelae of the aspiration were observed on follow up for a period of 1 year.

DISCUSSION

Fine needle aspiration cytology has several applications in various areas. Most of the results, particularly on breast, thyroid and lymph nodes have shown accuracy rates ranging from 50% to 95% (³). This procedure has several advantages, including the ease of obtaining material and, if necessary, repeating the procedure a second or even a third time. The efficacy of this study in Hansen's disease has, however, not been exploited to its fullest. Although recent reports have appeared in the literature regarding the utilization of this technique on the cutaneous lesions of lepromatous leprosy (⁴). It was also used to demonstrate acid-fast organisms by Theuvenet, *et al.* in 1993 in 7 out of 11 cases of primary neuritic leprosy (⁵). To date, the diagnosis and classification of pure neuritic leprosy has been chiefly based on performing a cutaneous peripheral nerve biopsy (¹). Nerve trunk biopsies are generally not performed in the diagnosis of primary neuritic leprosy. Therefore, the present paper is the only large series of fine needle aspirations being used as a technique in the diagnosis of this disease based on the morphology of the aspirate and the presence and absence of acid-fast organisms. Eighteen of the 27 cases aspirated in our study yielded positive results, constituting a 67% sensitivity in diagnosing the disease. Of the 27 cases, four were found to be non-Hansen's disease, thereby raising the sensitivity to 75% in diagnosing leprosy neuritis.

Seven aspirates showed well-formed granulomas and three of these cases had acid-fast organisms. Unlike cutaneous leprosy, acid-fast bacilli could be picked up in neuritic leprosy with granulomas.

Nine aspirates proved to be hemorrhagic. These were mostly in the initial part of the study. It has been observed that as the study progressed, aspirations gave a better yield of material enabling proper interpretation (The Table). This could be due to better experience in the method of aspiration with more practice. Pain at aspiration, aspiration

of a relatively uncommon site (other than solid parenchymatous organs), and aspiration of a normal (Grade 0) nerve may have contributed to this.

Although a diagnosis of pure neuritic leprosy could be made in 18 cases, an attempt at morphological categorization was not undertaken with regard to the type of Hansen's disease due to this being a pilot study.

No untoward results were observed with this procedure. On comparing the efficacy of FNA cytology to a nerve biopsy, it is found that in 67% of the cases in this series FNA proved helpful in making a diagnosis of Hansen's disease whereas in biopsying a representative cutaneous nerve from the site of neurological deficit Hansen's disease was confirmed in only 50% of the cases (¹). Hence, the FNA technique is an easily adaptable and sensitive investigative tool since the lesion represents a nerve trunk pathology in contrast to equivocal results so far obtained in cutaneous nerve biopsies in the literature.

The authors, therefore, feel that fine needle aspiration cytology in a case of motor sensory neuropathy is a useful technique for the diagnosis of primary neuritic leprosy and also to elucidate the acid-fast bacilli status in such cases. Singh, *et al.* in their study (⁴) have found morphological discordant results between cytological and histological results in seven cutaneous cases which could only be rectified by using the bacterial index in Fite-stained sections. More extensive studies may, however, enable a morphological classification at cytology of this disease entity.

SUMMARY

The diagnosis of primary neuritic leprosy (PNL) and its differentiation from other causes of peripheral neuropathy is difficult since acid-fast bacilli (AFB) smears and skin biopsy are negative from anesthetic areas. A biopsy of the involved nerve is the only conclusive method of diagnosis. Such a biopsy may not necessarily be free of complications when a large nerve is involved. However, fine needle aspiration has in this study proved to be a simple technique to demonstrate inflammation granulomas and AFB from these involved nerves in 18 of the 27 cases suspected to have PNL.

The validity of the cytological classification into morphological subtypes may have to be supplemented by a large series of studies.

RESUMEN

No es fácil hacer el diagnóstico de la lepra neurítica primaria (LNP) ni su diferenciación de otras causas de neuropatía periférica porque usualmente no se encuentran bacilos ácido resistentes (BAAR) ni en la linfa cutánea, ni en las biopsias de piel de las áreas anestésicas. La biopsia del nervio afectado es el único método conclusivo de diagnóstico, aunque esta biopsia puede presentar complicaciones cuando se trata de una rama nerviosa grande. En el presente estudio encontramos que la aspiración con aguja fina resultó ser una técnica simple para demostrar inflamación, granulomas y BAAR en los nervios afectados, en 18 de 27 casos sospechosos de tener LNP. La validez de la clasificación citológica en los subtipos morfológicos requiere ser confirmada en estudios más extensos.

RÉSUMÉ

Le diagnostic de lèpre purement neuritique (LPN) et la différenciation de celle-ci des autres causes de neuropathies périphériques est difficile car il n'y a pas de bacilles alcool-acido-résistants (AAR) à l'examen du suc dermique et de lésions à l'examen des biopsies cutanées. Une biopsie du nerf atteint est la seule méthode permettant de conclure au diagnostic. Une telle

biopsie n'est pas sans risque de complications si un nerf majeur est lésé. Cependant, cette étude a démontré la valeur de la cytoponction à l'aiguille fine comme technique simple pour mettre en évidence une inflammation granulomateuse et des bacilles AAR à partir de nerf atteints chez 18 des 27 cas suspectés de souffrir de LPN. La classification cytologique des sous-types morphologiques devra certainement être validée et complétée par des études comprenant un nombre supérieur de cas.

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