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Sural Nerve Biopsy in Leprosy Patients After Varying Periods of Treatment: Histopathological and Bacteriological Findings on Light Microscopy[1]

Redda Teckle Haimanot, Robert N. Mshana, A. Colin McDougall, and Joseph G. Andersen[2]

Publications in recent years ([1, 2, 4, 9–12, 18, 19]) have dealt with various aspects of neural involvement in leprosy, and the histopathological features have been particularly well reviewed by Weddell and Pearson ([21]). Many authorities consider that neural invasion occurs early; that it is invariable in all cases of leprosy, whatever the stage or classification; that bacilli may persist in nerves even after many years of apparently regular treatment at satisfactory dosage; and that in some cases, admittedly rare, the peripheral nervous system only is affected, giving rise to "pure neural leprosy". To the histopathologist, the neural element in the pathogenesis of this disease is not in doubt, but no convincing explanation has ever been forthcoming for what appears to be an exceptional predilection of *Mycobacterium leprae* for nerve tissues ([19]), including the Schwann cell. The numerous factors governing the granulomatous response have been described by Spector, *et al.* ([17]) and Ridley has reviewed the subject of macrophage stimulation and activity in lepromatous leprosy ([14]). Rea and Taylor ([13]) studied the mean serum lysozyme (muramidase) levels and the tissue distribution of this enzyme in the skin of patients affected by different types of leprosy and Cologlu, in a publication in Turkish ([3]), reported findings in skin biopsies, drawing attention to the presence of leprosy bacilli in structures such as endothelial lining cells of vessels, Schwann cells, and smooth muscle cells in arrector pili muscle—all of which failed to show any lysosomal activity on special stains. These observations, together with some uncertainty about the origin of mononuclear infiltrating cells within nerves affected by leprosy, led us to the present investigation in which biopsies were taken from the sural nerve (and the skin) of patients with various types of leprosy, including some with adverse reactions based on cell-mediated immune processes. The muramidase findings, using immunohistological techniques, are reported separately ([8]).

The routine findings on examination of these sural nerve biopsies with a hematoxylin and eosin (H&E) stain (for the infiltrative changes) and a modified Ziehl-Neelsen stain (for acid-fast bacilli) were, however, of considerable interest. Especially in view of the limited number of publications in the literature on sural nerve biopsy in leprosy, we report here the findings in a group of 18 patients from Ethiopia, after varying periods of treatment with antileprosy drugs.

PATIENTS AND METHODS

Eighteen Ethiopian leprosy patients attending the All-Africa Leprosy Rehabilitation and Training Centre in Addis Ababa were included in this study. The initial clinical examination included a classification of their type of leprosy, using the Ridley-Jopling scale ([16]). There was no evidence that any patient suffered from diabetes mellitus, amyloidosis, severe malnutrition, or alcoholism. A careful examination for anesthesia or hypesthesia, especially in the region of sensory supply of the sural nerves, was carried out in all patients prior to biopsy.

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[2] R. T. Haimanot, M.D., F.R.C.P.(C), Department of Internal Medicine, Faculty of Medicine, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. R. N. Mshana, M.D., Armauer Hansen Research Institute (AHRI), P.O. Box 1005, Addis Ababa, Ethiopia. A. C. McDougall, M.D., F.R.C.P., Department of Dermatology, The Slade Hospital, Headington, Oxford OX3 7JH, England. J. G. Andersen, M.D., D.M.Sc., M.Ch., All-Africa Leprosy and Rehabilitation Training Centre (ALERT), P.O. Box 165, Addis Ababa, Ethiopia.

TABLE 1. *Eight patients with lepromatous (LL) leprosy and borderline lepromatous (BL) leprosy.*

Case no.	Sex/ age	Clinical classification	Therapy		Bacilli		Histopathological findings	
			Drug(s)	Duration (yr)	Skin	Nerve	Skin	Nerve
1	M/31	BL	DDS[a]	10	few	0	LL, regressing	LL, regressing
2	M/12	LL (ENL)[b]	DDS/RMP[c]	2	0	0	LL, regressing	LL, regressing
3	M/31	LL	DDS	1	many	many	LL, active, relapsing	LL, active, relapsing
4	M/57	LL	DDS	7	few	0	LL, widespread pathology	LL
5	M/63	BL	DDS	12	many	many	LL, extensive pathology	LL
6	F/10	LL (histoid)	DDS/RMP	12	many	many	LL, active with histoid features	LL, active
7	M/38	LL	DDS/RMP	1	many	many	LL	LL, active
8	M/23	LL	DDS/RMP	1	many	many	BL, extensive pathology	BL/LL, grossly abnormal

[a] DDS = dapsone.
[b] ENL = erythema nodosum leprosum.
[c] RMP = rifampin.

At the time of intake, all patients had received treatment with either dapsone (DDS) alone or with dapsone plus rifampin (RMP), as shown in Tables 1 and 2, from which it can be seen that the period of treatment at the time of the biopsy varied widely, between extremes of one month and 12 years. The relevance of these periods in relation to the microscopic findings are discussed below.

Skin biopsies were taken from active lesions by standard techniques under local anesthesia. Where no active lesion could be detected, a biopsy was taken from the site considered, from examination of the records, to have previously shown a lesion. In no instance was a biopsy taken from an active lesion or from a site in close proximity to either sural nerve. Sural nerve biopsies were taken according to standard techniques ([5]) (but removing fascicles only about 10 mm in length) from either the right or the left leg, whether or not the sural nerve was enlarged and palpable. In fact, the nerves were palpable in all cases except numbers 16 and 17 (Table 2). In no instance was the nerve biopsy incision made either through or near a skin lesion. Specimens of skin and nerve were fixed in formaldehyde-mercuric chloride-acetic acid (FMA) solution for 2 hr, then transferred to 70% alcohol for 24 hr before embedding in paraffin. Sections were cut at 5 μm and stained with H&E and a modified Fite stain ([7]) for acid-fast bacilli. Sections were also stained for muramidase; technical details are to be described in a separate publication ([8]).

RESULTS

Our results are summarized in Tables 1 and 2. Although it is acknowledged that there is some clinical and immunological overlap in patients within the "borderline" or "dimorphous" group, we have combined in one table borderline-lepromatous (BL) and lepromatous (LL) patients, since in practice the treatment and management of these two groups is somewhat similar. Furthermore, they are both types of leprosy in which large, or very large, numbers of leprosy bacilli are widespread in the tissues. By contrast, Table 2 contains essentially mid-borderline (BB), and borderline-tuberculoid (BT) cases, including four cases of the latter classification who were at the time of biopsy in reversal (upgrading) reaction ([15]), albeit modified by treatment with steroids. The latter cases were methodically included in view of a previous

TABLE 2. *Ten patients with mid-borderline (BB) or borderline tuberculoid (BT) leprosy.*

Case no.	Sex/ age	Clinical classi-fication	Therapy		Bacilli		Histopathological findings	
			Drug(s)	Du-ra-tion (mo)	Skin	Nerve	Skin	Nerve
9	F/33	BB	DDS[a]	1	0	few	BB upgrading to BT	BB upgrading to BT
10	M/22	BB	DDS/RMP[b]	24	0	many	Nonspecific; not diagnostic of leprosy	BL, grossly abnormal
11	F/27	BB/BL	DDS/Pred[c]	2	few	few	BB upgrading to BT	BB upgrading to BT
12	M/16	BT in reaction	DDS/Pred	9	0	0	BT upgrading to TT[d]	BT upgrading to TT
13	M/17	BT in reaction	DDS/Pred	4	0	0	BT upgrading to TT	BT upgrading to TT
14	F/23	BT in reaction	DDS/Pred	24	0	0	Nonspecific; not diagnostic of leprosy	BT
15	M/28	BT in reaction	DDS/Pred	60	0	0	Nonspecific; not diagnostic of leprosy	BT
16	M/50	BT	DDS	36	0	0	Nonspecific; not diagnostic of leprosy	Normal
17	M/16	BT	DDS	24	0	0	BT/TT	Normal
18	F/40	BT	DDS/Pred	3	0	0	Borderline changes but further classi-fication not possible on material submitted	BT

[a] DDS = dapsone.
[b] RMP = rifampin.
[c] Pred = prednisolone.
[d] TT = tuberculoid leprosy.

observation ([13]) particularly of high serum levels of muramidase and intense granular reaction patterns in the tissues during such reactions.

In the case of the eight BL and LL patients in Table 1, infiltrative changes were often marked and widespread from one end of the biopsy to the other. The perineurium was frequently obliterated or heavily infiltrated and the normal structure of the endoneurial area destroyed; bacilli were widespread, including globi (Fig. 1). These changes, together with large numbers of bacilli, were also observed in Cases 5 and 6, in whom the period of treatment was more than 12 years. Cases 5 and 6 had many bacilli in all areas and Case 6 showed clinical and histopathological evidence of histoid leprosy, as described by Wade in 1963 ([20])—the nerve did not show histoid features. In Case 2, the patient clinically had erythema nodosum leprosum (ENL) on the skin, but no evidence of ENL was found on histopathological examination of the nerve.

In the ten patients with borderline-tuberculoid (BT) or mid-borderline (BB) leprosy in Table 2, the histopathology in skin was nonspecific and not diagnostic of leprosy in Cases 10, 14, 15 and 16. In Case 18, the changes were suggestive of borderline (dimorphous) leprosy, but a more precise classification could not be made on the material submitted. Nevertheless, in Case 10 the changes were gross, and in Cases 14, 15 and 18, there was very definite evidence of borderline-tuberculoid (BT) leprosy in the nerve. Possible reasons for the negative findings in skin in the above cases and the

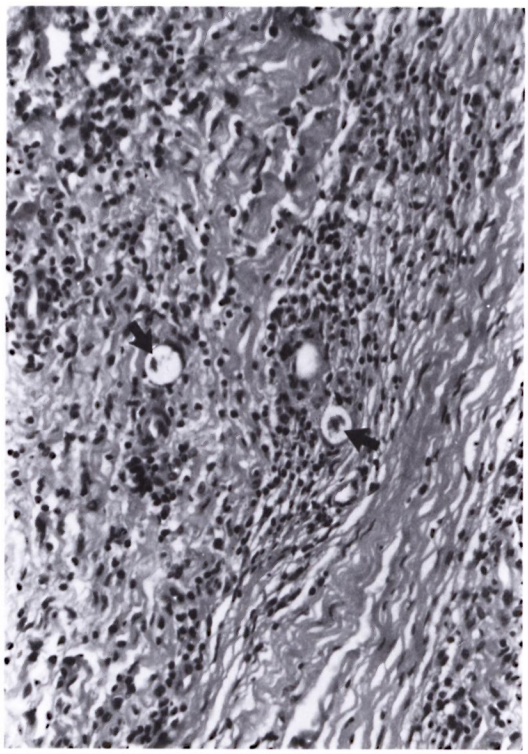

FIG. 1. Sural nerve biopsy; lepromatous (LL) leprosy, treated with daily dapsone for one year. Endoneurial area shows extensive fibrosis, together with infiltration by histiocytes and some lymphocytes. Bacilli are widespread and some lie in large globi in vacuoles within multi-nucleate cells (arrows). Bacillary material in these vacuoles was shown, on Fite staining, to be acid-fast and bacillary (H&E ×250).

normal findings in the nerve in Cases 16 and 17 are discussed below. In several cases it was remarkable that the entire area of the section was pathological (Fig. 2), all fascicles showing endoneurial and perineurial infiltration. Cellular infiltration by histiocytes, epithelioid cells, and lymphocytes (Fig. 3) was often intense and widespread, with virtually complete replacement of normal structure. Case 12 was of exceptional interest in that there was not only a massive replacement of nerve tissue by lymphocytes, but also extensive caseation necrosis in the endoneurial zone (Fig. 4).

DISCUSSION

The clinical records and history of drug intake were incomplete in some of these patients and in view of the fact that most of them were taking drugs as outpatients, it is possible that regularity of intake, especially in the long-treated LL and BL cases, was far from satisfactory. Those in Table 1 with extensive disease and many bacilli in their biopsies were referred for further investigations of drug resistance, notably to dapsone, an increasingly common phenomenon in Ethiopia and many other countries. The striking features on microscopic examination of the nerve tissues in this group of eight cases were the extent and severity of typically lepromatous changes; foamy macrophages were seen in all cases, and they often extended throughout the entire biopsy. The correlation between clinical and histopathological findings was close in this group, except that in Cases 1 and 5 the classification in nerve was lepromatous rather than borderline-lepromatous. The histoid findings in Case 6 were not present in the nerve and indeed this form of leprosy has to our knowledge so far been described in only one case, occurring in India (K. Ramanujam, personal communication, 1980).

The nonlepromatous cases in Table 2 are less straightforward in the matter of interpretation. The apparent lack of correspondence in some cases between the clinical findings and those in skin and nerve may, however, be inherent in the types of leprosy from which these patients suffered, since it has long been known that the borderline forms exhibit skin and nerve lesions which are asymmetric and "patchy" in distribution. The negative findings in nerve in Cases 16 and 17 may therefore be due to the fact that the sural nerve biopsied was not affected by the disease. In the case of the skin biopsies which showed only nonspecific, nondiagnostic changes, it is more than likely that long periods of drug treatment had resulted in the virtual disappearance of lesions, so that any selection of a skin site for biopsy was impossible. In the eight cases showing abnormal changes in nerve, the striking overall finding was again the extent and intensity of the pathology in the material submitted (Figs. 2–4), including caseation necrosis (Fig. 4).

There were no difficulties in locating sural nerves or in obtaining adequate tissue. All biopsy wounds healed by first intention and there was no evidence of an increase of neural deficit in the area supplied as a result of the procedure.

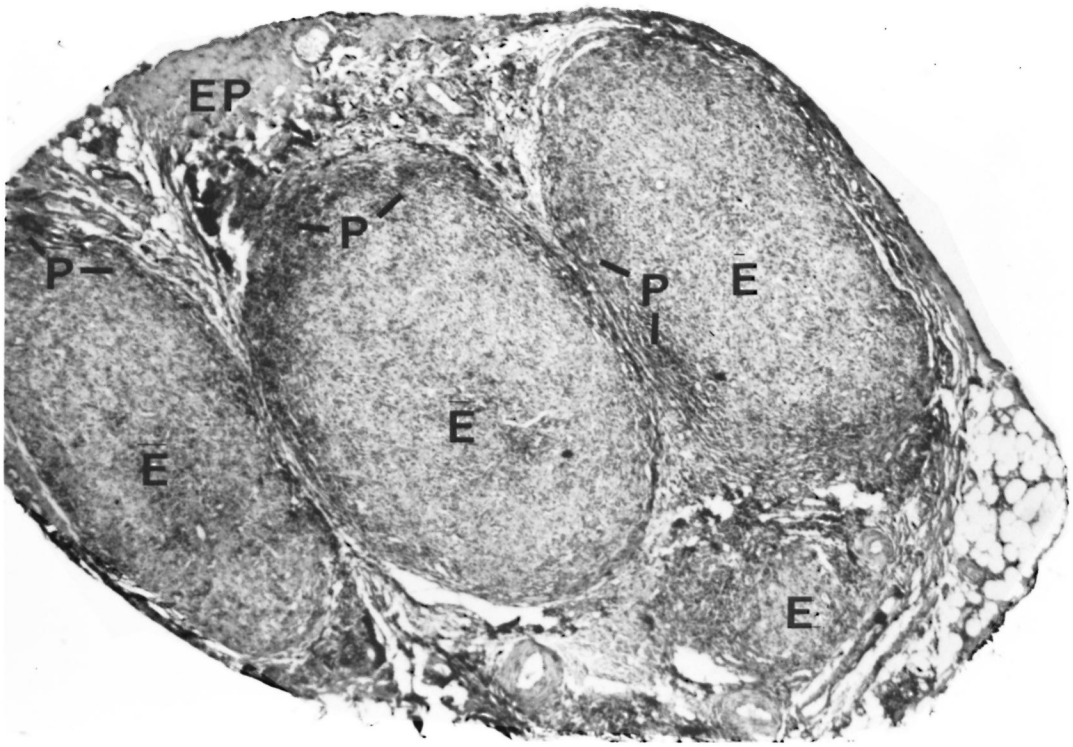

FIG. 2. Sural nerve biopsy; borderline tuberculoid (BT) leprosy. All four fascicles show marked infiltration of virtually the entire endoneurial area (E) with multi-layering and infiltration of the perineurium (P). EP = epineurium. (H&E ×20).

We conclude that sural nerve biopsy in experienced hands is safe and that it has revealed pathological changes of significance in this group of patients. Enna, *et al.* (6), in an evaluation of biopsy of this nerve in leprosy, commented that it had ". . . been found to show evidence of activity in some cases of borderline and lepromatous leprosy which have been inactive by the usual criteria for several years." This observation and the findings in the present study suggest that the procedure may be indicated in the further investigation of both treated and untreated patients with different types of leprosy. This, in our view, should be attempted only by experienced observers, including an operator who is trained in nerve biopsy. Furthermore, the procedure should certainly not replace the examination of well-selected and processed skin biopsies, together with the use of routine slit-skin smears in the diagnosis and classification of this disease. For special investigations, however, it is a potentially valuable procedure which may throw light on the pathogenesis of leprosy in untreated, treated, and relapsed patients.

SUMMARY

As part of a larger study of nerve biopsies from leprosy patients in Ethiopia for the presence of muramidase (lysozyme), sections were also examined by light microscopy after staining with hematoxylin and eosin for cellular infiltrate and a modification of the Ziehl-Neelsen stain for leprosy bacilli. The muramidase findings will be reported separately. This paper describes the infiltrative and bacterial findings in a group of 18 patients, including four with nonlepromatous forms of leprosy who were suffering from delayed hypersensitivity reaction at the time of biopsy. The findings were unexpectedly interesting and revealing. Lepromatous and borderline-lepromatous patients all showed endoneurial and perineurial infiltration of considerable extent and, in several instances, bacilli were wide-

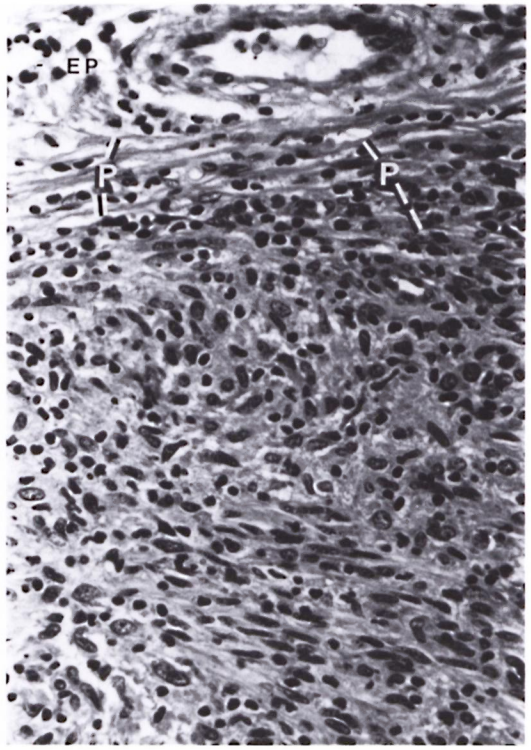

FIG. 3. Sural nerve biopsy; borderline tuberculoid (BT) leprosy. Endoneurial area is heavily infiltrated by histiocytes (many showing epithelioid change) and lymphocytes. Perineurium (P) is multi-layered and infiltrated, mainly by lymphocytes. EP = epineurium. (H&E ×400).

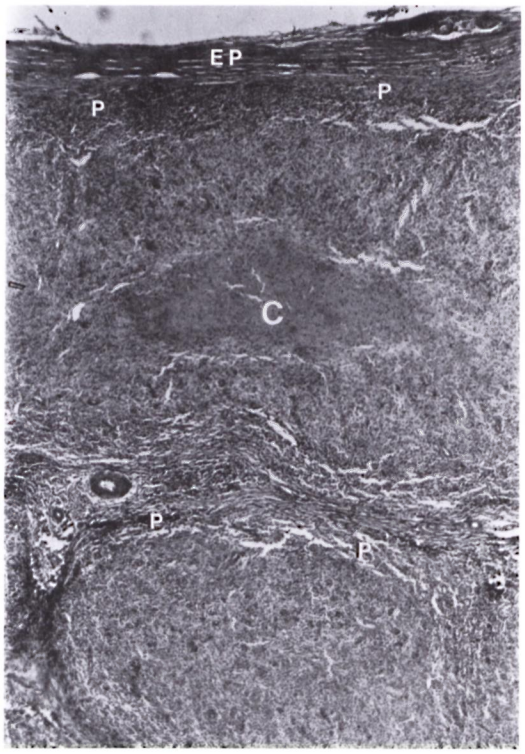

FIG. 4. Sural nerve biopsy; borderline tuberculoid (BT) leprosy. All fascicles are extensively infiltrated and there are large areas of caseation necrosis (C). P = perineurium; EP = epineurium. (H&E ×20).

spread from one end of the biopsy to the other; in two patients, solid-staining bacilli and globi were found, indicating relapse. In all except two of the nonlepromatous patients (mainly borderline-tuberculoid) there was an extensive and severe granulomatous infiltration, and in one case there was marked caseation in the endoneurial zone.

Within the limits of the present study, the findings indicate that biopsy of a peripheral nerve, even when it is not obviously associated with a skin lesion, may reveal pathological changes which are greater in degree than those suggested by skin biopsy or clinical examination. These observations in a somewhat heterogeneous group of patients treated for varying periods of time, and in a study which was not prospectively planned, suggest that similar observations in a larger group of untreated and treated patients, including those who have relapsed, may be of value.

Sural nerve biopsy should be performed only by a medically qualified and experienced operator, but it is not technically difficult, did not give rise to sensory deficit in this series, and is potentially highly revealing of pathological change.

RESUMEN

Como parte del estudio realizado en Etiopía para la búsqueda de muramidasa (lisozima) en biopsias de nervios de pacientes con lepra, los cortes histológicos de estos pacientes se tiñeron con hematoxilina-eosina y con el colorante de Ziehl-Neelsen, y se examinaron por microscopía de luz para localizar infiltrados celulares y bacilos de la lepra. Los hallazgos sobre la muramidasa se publicarán en forma separada. En este trabajo se describen los hallazgos sobre los infiltrados y los bacilos de la lepra en un grupo de 18 pacientes (4 con lepra no lepromatosa) que mostraban una reacción de hipersensibilidad retardada al momento de la biopsia. Los hallazgos fueron muy interesantes y reveladores. Todos los pacientes con lepra lepromatosa e intermedia mostraron infiltración endoneurial y perineurial de considerable extensión. En varios casos se encontraron bacilos dispersos de un extremo al otro de la

biopsia; en dos pacientes se encontraron bacilos sólidamente teñidos y globi sugerentes de recaídas. A excepción de 2 pacientes no lepromatosos (intermedios-tuberculoides), todos los demás mostraron una infiltración granulomatosa extensa y severa, en un caso hubo marcada caseificación en la zona endoneurial.

Dentro de las limitaciones del estudio, los hallazgos indican que la biopsia de un nervio periférico, aún cuando no esté asociado en forma obvia con una lesión de la piel, puede revelar cambios patológicos de mayor magnitud que los sugeridos por la biopsia de la piel o por el exámen clínico. Las observaciones hechas en este estudio que no se planeó en forma prospectiva, con pacientes más bien heterogéneos desde el punto de vista clínico y de tratamiento, sugieren la importancia de realizar un estudio similar en un grupo de pacientes más grande y mejor controlado desde diversos puntos de vista.

La biopsia del nervio sural, aunque técnicamente no es difícil, debe hacerse solo por un cirujano calificado y experto a fin de no ocasionar un déficit sensorial; el estudio de la biopsia neural puede, potencialmente, revelar los cambios patológicos.

RÉSUMÉ

Dans le cadre d'une étude plus large portant sur les biopsies nerveuses chez des malades de la lèpre en Ethiopie, concernant la présence de muramidase (lysosome), on a examiné des coupes par microscope optique après coloration par l'hématoxyline et l'éosine, en vue de déceler les infiltrations cellulaires, ainsi qu'une modification de la coloration de Ziehl-Neelsen pour les bacilles de la lèpre. Les observations se rapportant à la muramidase seront relatées séparément. Cet article décrit les observations se rapportant à l'infiltration et aux manifestations bactériologiques, dans un groupe de 18 malades, dont quatre atteints d'une forme de lèpre non lépromateuse, souffrant d'une réaction d'hypersensibilité retardée au moment de la biopsie. Les observations ont été particulièrement intéressantes et révélatrices. Tous les malades lépromateux et lépromateux-borderline présentaient une infiltration très étendue endoneurale et périneurale; dans plusieurs cas, les bacilles étaient largement répandus d'une extrémité de la biopsie à l'autre; chez deux malades, on a trouvé des bacilles solides à coloration uniforme et des globi, ce qui témoigne d'une récidive. Dans tous les cas de malades non lépromateux, et particulièrement chez les malades borderline-tuberculoides, sauf deux, on a constaté une infiltration granuloma teuse étendue et prononcée; dans un cas, on a noté une caséification notable de la zone endoneurale.

Dans les limites de cette étude, ces observations indiquent que la biopsie d'un nerf périphérique, même lorsqu'il n'est pas associé de façon manifeste avec une lésion cutanée, peut révéler des modifications pathologiques d'un degré plus prononcé que ne le suggère la simple examination clinique ou une biopsie. Ces observations, menées dans un groupe quelque peu hétérogène de malades, traités pour des durées variées, recueillies au cours d'une étude qui n'était pas planifiée pour être prospective, suggèrent que des observations semblables pourraient fournir des informations valables, si elles étaient recueillies chez un nombre plus élevé de malades, tant traités que non traités, y compris ceux qui ont présenté des récidives.

La biopsie du nerf sural ne devrait être pratiquée que par du personnel qualifié et expérimenté; cette biopsie n'est cependant pas difficile du point de vue technique; elle n'a pas entraîné de pertes sensorielles dans cette série de malades; elle peut fournir des renseignements fort intéressants quant aux modifications pathologiques.

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