Bacteriological status (point prevalence) of lepromatous outpatients under sulfone treatment

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The study concerns 337 outpatients with lepromatous leprosy who had been receiving dapsone treatment for 1–26 years—69% of them regularly and 31% irregularly. Routine bacterial examination (10 min per slide) by a paramedical technician showed only 50% of these patients to be bacteriologically positive. This rate attained 99% when each slide was examined for 30–60 min by a qualified bacteriologist. Other factors—apart from the limited action of sulfones—might account for these unexpected results: the material was collected by an experienced leprologist; the disease was still clinically active in 75% of the patients; about 25% of them had been treated for only 1–4 years; and the intake of dapsone in long-term treatment gradually decreases.

In an earlier study based on the long-term follow-up of lepromatous outpatients who had been receiving sulfone treatment for up to 20 years, Quagliato et al. (1) set out to determine the time required for bacteriological negativity (inactivity) and the cumulative coefficient of bacteriological reactivation (relapse) after inactivity had been attained. Other related aspects were also studied: the time required for reactivated patients to become bacteriologically negative and the cumulative coefficient of cases reactivated twice.

The object of the present study was to determine, at a stated moment (point prevalence), the bacteriological status of lepromatous (L) outpatients under sulfone therapy. This report refers to part of our material (337 out of 646 cases). The results and conclusions are therefore presented with reservations.

Whereas the previous investigation was a retrospective study based on routine clinical and bacterial examinations, the present one constituted planned research in which the above-mentioned examinations were performed under experimental conditions.

MATERIAL AND METHODS

The study encompasses 337 lepromatous outpatients who had been treated and followed up for 1–26 years at the health centre of Campinas, São Paulo, Brazil. The patients were classified according to the results of clinical and bacteriological examinations. Most of them had had a repeatedly negative lepromin reaction, and a substantial proportion also had been subjected to histopathological examination.

According to the severity of their disease at the beginning of treatment, patients were divided into three groups—L^1, L^2, and L^3 (early, moderate, and advanced lepromatous cases, respectively)—as follows: L^1, 80 patients (24%); L^2, 119 (35%); and L^3, 138 (41%). The treatment schedule and criteria for regular and irregular treatment were the same as in the study reported by Quagliato et al. (1).

When the bacteriological examination was performed, the distribution of patients according to the duration of treatment was: 1–4 years, 79 (23%); 5–9 years, 59 (18%); 10–14 years, 58 (17%); 15–19 years, 52 (16%); and $\geqslant$20 years, 89 (26%).

Of the 337 patients, 232 (69%) had attended the periodic examinations regularly and 105 (31%), irre-

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Table 1. Severity of lepromatous leprosy and bacteriological status

Severity	No. of cases	Bacteriological status [a]										
		negative		1 +		2 +		3 +		4 +	5 +	6 +
		No.	%	No.	%	No.	%	No.	%			
L^1	80	1	1.2	12	15.0	37	46.2	30	37.5	0	0	0
L^2	119	2	1.7	19	16.0	65	54.6	33	27.7	0	0	0
L^3	138	1	0.7	21	15.2	60	43.5	56	40.6	0	0	0
Total	337	4	1.2	52	15.4	162	48.1	119	35.3	0	0	0

[a] According to the Ridley reading criteria: 6 + = many clumps of bacilli in an average microscopic field (over 1000 bacilli); 5 + = 100 – 1000 bacilli in an average microscopic field; 4 + = 10 – 100 bacilli in an average microscopic field; 3 + = 1 – 10 bacilli in an average microscopic field; 2 + = 1 – 10 bacilli on the average in 10 microscopic fields; 1 + = 1 – 10 bacilli on the average in 100 microscopic fields.

gularly. When the regularity of treatment and the severity of the disease are related, the following pattern appears:

	Regular		Irregular	
	No.	*%*	*No.*	*%*
L^1	52	65	28	35
L^2	79	66	40	34
L^3	101	80	37	20

Patients were examined monthly, quarterly, or half-yearly, but only the examinations performed from January to March 1973 were taken into account. One of us (R.Q.) carried out the follow-up examinations and collected material from nasal mucosa and at least two skin lesions; in apparently inactive cases, material was collected from the ear lobes and elbows. One slide from each patient was kept in the health centre, stained by the Ziehl–Neelsen method and examined for 10 min by a paramedical technician. Two slides were sent to the Department of Microbiology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, one being stained by the Ziehl–Neelsen method and the other by the Nyka (periodic acid) method. These slides were examined by an assistant professor (A.A.). At first, 1 h on the average was spent on examining each such slide (up to case No. 200); thereafter, the time for each slide was reduced to ½ h. Ridley's criteria were used for grading bacteriological positivity.

The main purpose of the examination was to determine bacteriological negativity or positivity in each case. To that end, the smears from the nasal mucosa and from the skin were first rapidly examined. If bacilli were easily visible, the better smear was selected in order to count the bacilli and study their morphology. When bacilli were not found at the initial examination, each smear was examined at length. If a few bacilli were found in two or three smears, the smear with the largest number of bacilli was selected.

The findings were analysed by computer at the University of Campinas. The results relate only to the Ziehl–Neelsen staining method.

FINDINGS AND DISCUSSION

Of the 337 patients, 167 (50%) showed bacteriological positivity on routine examination. In the Department of Microbiology, 333 (99%) were found to be positive.

The data on (*a*) the severity of lepromatous leprosy (L^1, L^2, or L^3); (*b*) the regularity of treatment (regular or irregular); (*c*) the duration of treatment; and (*d*) the bacteriological status (negative or positive) were fed into the computer. However, only 337 patients were studied and, when such a detailed breakdown was attempted, the number of cases in each of the subcategories became too small for significant conclusions. Quagliato et al. (1) had been able to undertake a detailed analysis, their study including 815 patients. In view of the foregoing we studied the data as described below.

When the bacteriological status was related to the severity of the disease (Table 1), the proportion of negative patients (below 2%) was similar in groups L^1, L^2, and L^3. Also, there was no substantial difference between these groups as regards the total proportion and degree of positivity. The proportion of patients positive on routine examina-

Table 2. Degree of positivity and duration of treatment

Years of treatment	Degree of positivity											Total
	negative		1 +		2 +		3 +		4 +	5 +	6 +	
	No.	%	No.	%	No.	%	No.	%				
1 – 4	1	1.3	10	12.7	43	54.4	25	31.6	0	0	0	79
5 – 9	1	1.7	12	20.3	27	45.8	19	32.2	0	0	0	59
10 – 14	0	—	5	8.6	32	55.2	21	36.2	0	0	0	58
15 – 19	1	1.9	8	15.3	28	53.8	15	28.8	0	0	0	52
⩾ 20	1	1.1	17	19.1	32	36.0	39	43.8	0	0	0	89
Total	4	1.2	52	15.4	162	48.1	119	35.3	0	0	0	337

tion (50%) was high, but the results of examinations in the Department of Microbiology (99% positive) were unexpected. Only the latter will be the object of further analysis and consideration.

Souza Lima et al. (2) observed that, in 150 biopsies of apparently normal skin previously affected by lepromatous lesions in patients with early and moderate forms, 50% contained no bacilli; in 45%, a small number of bacilli with modified morphology or only granulations were found inside the nerve fibres and muscles; and, in 5%, besides the bacilli with modified morphology, there were rare homogeneous bacilli. These authors also performed biopsies on 64 leproma scars: in only 10% of them were bacilli not found.

Quagliato et al. (1) reported that, among 206 cases that had been inactive for 2 or more years and treated for at least 5 years, 35 were still bacteriologically positive and 36 had a regressing lepromatous structure in skin lesions. Of 159 patients with chronic inflammatory infiltrate and negative bacteriological examination by skin sections, 20 later underwent bacteriological reactivation despite apparently continuous sulfone therapy.

Karat et al. (3) observed that, even though bacilli were no longer visible in skin smears, as shown by the bacteriological index, 14% of patients had easily demonstrable acid-fast bacilli in biopsy sections of the liver.

The findings shown in Table 1 should be considered in the light of several factors. One element that could partly explain the findings is that the bacteriological examination of each slide initially took 1 h and later ½ h, and that it was performed by a medical bacteriologist. In cases with only a few bacilli (1+, according to Ridley's grading), examination of a slide for 15 min was often negative. Furthermore, the clinical examination and collection of material were performed by a leprologist (R.Q.) and about 25% of the cases had been treated for only 1–4 years.

Theoretically, the proportion and the degree of positivity should be lower the longer the duration of treatment, provided that this is carried out regularly. However, this was not observed in our material, although the degree of positivity was related to the duration of treatment (Table 2).

Though almost all patients were bacteriologically positive regardless of the duration of treatment, the degree of positivity decreased substantially in relation to that observed at the initial examination, when the lepromatous cases were detected and registered: in fact, groups L^2 and L^3 were strongly positive (3+ and 4+ according to the reading criteria of the 1947 Pan American Conference). It is assumed that the load of infectiousness decreased with treatment over the years, even if the results were far from being brilliant.

The progressive decrease in the proportion of lepromatous patients under regular treatment (from 83% after 0–4 years of treatment to 56% after >20 years) is a common observation and is another important factor to be considered. These and other findings (mainly regarding the clinical status, regularity of treatment, and degree of severity of the disease) suggest that many patients under what is considered to be " regular " treatment were not as " regular " as they should have been, even if they had a higher intake of dapsone than those considered to be irregular.

Table 3. Degree of positivity and morphological index

Degree of positivity	No. of cases	Morphological index								
		0	1	2	3	4	1–4	5–9	10–14	15–19
1+	52	51	—	—	—	—	—	—	—	1
2+	162	151	3	5	1	1	10	—	—	1
3+	119	65	22	16	2	4	44	4	4	2
Total	333	267	25	21	3	5	54	4	4	4

The degree of positivity is related to the morphological index in Table 3. Cases classified as 1+ (Ridley's criteria) had only a small number of bacilli—very often hardly any. The morphological index is therefore given only with great reservations. Of those 52 cases, 51 had a morphological index of zero. It appears that 77% of the treated cases classified as 2+ and 3+ also had zero. A higher morphological index seems to be related to a higher degree of positivity.

In our material there was a certain correlation between the degree of positivity and the morphological index. In the light of our findings and on the assumption that the load of infectiousness is a function of the number of bacilli and probably of their morphology, it is probable that, even if the morphological index is not determined, the load of infectiousness increases with the number of bacilli in treated and untreated cases. From the practical point of view, in mass campaigns with limited resources, an idea of the load of infectiousness could be gained by merely determining the degree of positivity.

The clinical status of the treated cases may also help to explain the unexpected findings. It appears that, among lepromatous patients treated for 1–4 years, the proportion of those clinically inactive is low (9%). In subsequent quinquennial periods this proportion increases substantially—to around 30%. The high proportions of clinically active cases, even among patients treated for 10 years or more, i.e., 64%, 75%, and 71% among those treated for 10–14, 15–19, and >20 years, respectively, make it easier to understand the 99% point prevalence of bacteriological positivity. On the other hand, this positivity also explains the high proportion of clinically active cases. The findings confirm the value of a follow-up examination undertaken by an experienced leprologist, especially when lepromatous patients have to be released from supervision.

Among irregularly treated patients in groups L^1, L^2, and L^3, the proportion of clinically active cases was 90% or more (maximum: 97%). The proportion of inactive cases was higher among those treated regularly; the best results were seen in patients of group L^1 under regular treatment (58% of clinically inactive cases). The clinical status was worse in 11 (5%) of 232 regularly treated patients and in 31 (30%) of 105 patients under irregular treatment.

These and other data provide evidence that, although one cannot be sure about the regular intake of dapsone, regularly treated patients are likely to have taken a higher amount of dapsone than those under irregular treatment. In our first study (1) it appeared that the regularity of treatment determined the bacteriological negativity of patients in groups L^1, L^2, and L^3—i.e., the probability of negativity was higher in the group regularly treated. On the other hand, the cumulative coefficient of reactivation was much higher among irregularly treated patients.

When the clinical status of L^1, L^2, and L^3 cases is related to their bacteriological status, one generally tends to find fewer bacilli in clinically inactive cases, especially in L^2 patients. The few bacteriologically negative cases were clinically inactive.

In L^1 and L^3 patients under regular treatment, the morphological index tended to be 0 in a greater proportion, and >5 in a smaller proportion, compared with irregularly treated patients (Table 4). In L^2 patients, the index was similar regardless of the regularity of treatment. Furthermore, it was 0 in 91% of clinically inactive lepromatous cases. About 25% of patients with clinically active disease showed a morphological index of 1–4 or even higher.

A comparison of the results with smears stained by the Ziehl–Neelsen and Nyka methods showed that a greater number of bacilli is seen with the latter.

CONCLUSIONS

Of 337 lepromatous outpatients who had been under dapsone treatment for 1–26 years (69% regularly and 31% irregularly), 50% showed bacteriological positivity on routine examination by a paramedical technician (10 min per slide). The proportion of positive results reached 99% when the slides were examined for 30–60 min by a qualified bacteriologist. Other factors that help to explain the unexpected results—apart from the limited action of sulfones—are as follows: the material was collected

Table 4. Severity of lepromatous leprosy, regularity of treatment, and morphological index

Severity	Treatment	Morphological index												Total
		0		1	2	3	4	1–4		5–9		10–16		
		No.	%					No.	%	No.	%	No.	%	
L[1]	regular	44	84.6	3	4	0	0	7	13.5	1	1.9	0	—	52
	irregular	16	57.1	4	3	1	1	9	32.1	1	3.6	2	7.1	28
L[2]	regular	66	83.5	6	4	1	2	13	16.5	0	—	0	—	79
	irregular	35	87.5	3	0	0	1	4	10.0	0	—	1	2.5	40
L[3]	regular	86	85.1	4	6	1	0	11	10.9	2	2.0	2	2.0	101
	irregular	24	64.9	5	4	0	1	10	27.0	0	—	3	8.1	37
	Total	271		25	21	3	5	54		4		8		337

by an experienced leprologist; 75% of cases were still clinically active; about 23% of the patients had been treated for only 1–4 years; and the intake of dapsone in long-term treatment is gradually reduced.

It is to be expected that, in a study carried out as far as possible under experimental conditions, the proportion of cases found to be bacteriologically positive is much higher than that detected in routine examinations. Therefore, in mass campaigns, bacterial inactivity—the criterion for releasing lepromatous patients from control—should be checked by a well-trained bacteriologist and the material should be collected by an experienced leprologist.

The findings reported may explain the high frequency of reactivation or relapse in lepromatous patients who are considered as inactive or have even been released from control when in fact they still had bacilli in the skin and possibly in other tissues (nerves, liver, spleen, and bone marrow).

ACKNOWLEDGEMENTS

We are grateful to J. F. Costa Meyer and E. F. Takahasi, University of Campinas, for the analysis of the data on the computer.

This study was carried out with support from the World Health Organization and the Order of Malta.

RÉSUMÉ

ÉTAT BACTÉRIOLOGIQUE DE SUJETS ATTEINTS DE LÈPRE LÉPROMATEUSE SOUS TRAITEMENT PAR LES SULFONES

Etudiant 337 cas de lèpre lépromateuse (cas L) traités par la dapsone depuis 1 à 26 ans (69% de sujets ayant suivi régulièrement le traitement et 31% l'ayant suivi de façon irrégulière), les auteurs ont constaté que le taux de positivité bactériologique s'établissait à 50% lorsque l'examen de routine était fait par un technicien paramédical (10 min par lame) mais atteignait 99% lorsque les lames étaient examinées pendant 30 à 60 min par un bactériologiste qualifié. Outre l'action limitée des sulfones, d'autres facteurs peuvent expliquer ces résultats inattendus, à savoir: le fait que le matériel d'étude a été recueilli par un léprologue expérimenté, le fait que chez 75% des sujets la maladie était encore cliniquement active (25% d'entre eux n'étaient traités que depuis un à quatre

ans), enfin la réduction graduelle de la prise de dapsone dans les traitements de longue durée. Dans le matériel étudié, il semble qu'il y avait corrélation entre l'indice morphologique et le degré de positivité.

Ces constatations pourraient expliquer la fréquence élevée des épisodes de réactivation ou des rechutes parmi les cas L dans lesquels on considère que la maladie est devenue inactive ou qui sont même soustraits au régime de surveillance.

Il semble donc que, dans les campagnes de masse, il ne faut renoncer à surveiller les cas L que si l'inactivité bactériologique est vérifiée par un bactériologiste expérimenté et si le matériel d'étude a été recueilli par un léprologue chevronné.

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