International Journal of Leprosy Volume 35, Number 2
Printed in U.S.A.

A Logarithmic Index of Bacilli in Biopsies

2. Evaluation[1]

D. S. Ridley[2]

In this paper the logarithmic index of biopsies (LIB), which was described in the previous paper ([3]), is compared with the original biopsy index (IB) and the logarithmic bacterial index of smears (BI) ([2]). The use and limitations of the three indices are evaluated by the study of some results on patients before treatment and during the course of sulfone therapy.

METHOD

Patients at the Jordan Hospital, Redhill, England, are classified on the five-group TT-LL system ([4]). The groups in which bacilli are found are pure lepromatous (LL), borderline-lepromatous (BL), borderline (dimorphous) (BB), and a borderline-tuberculoid (BT). Biopsies are made and smears are examined as a routine before treatment, and thereafter every six months. The biopsies are usually made on two lesions on each occasion and the smears are taken from six to eight lesions. The BI and the IB are assessed, and it was easy from the records to calculate the LIB of former patients. The indices of the following patients were collected and analyzed: (a) all LL patients admitted during the last five years, and (b) all BL, BB, and BT patients admitted during the last 10 years; the shorter period of five years would not have provided adequate numbers of patients for these groups. All these patients were included in the analysis of indices which follows, provided that (1) the area of the granuloma of the initial biopsy (before treatment) was at least 0.2 (1/5th of the section), (2) the initial LIB was at least 1.0, and (3) the period of observation under treatment with sulfones was at least one year for LL and BL patients; BB and BT patients were accepted for six months' observation only because most of them were bacteriologically negative at the end of this time. These criteria were fulfilled by 19 LL patients, 10 BL, 6 BB and only 2 BT. Two patients who were intermediate between LL and BL were omitted from the analysis. The patients were of mixed racial groups: European, Eurasian, African and Indian.

RESULTS

There is only a poor correlation between the actual values of the LIB and those of the original biopsy index, IB, and since the first is purely logarithmic and the IB is partly arithmetic, it is not surprising that the rate of fall with treatment of the patient is much slower in the former than in the latter.

There is a closer correlation between the LIB and the BI of smears. The behavior of these two indices during therapy in some representative cases is illustrated in Figure 1. It is seen that (1) there is a broad correlation between the values of the two indices, (2) the initial pretreatment level of the LIB is usually higher than that of the BI, (3) the rate of fall of the LIB is usually higher than that of the BI, and (4) random fluctuations of the LIB are relatively infre-

[1]Received for publication 14 November 1966.
[2]D. S. Ridley, M.D., M.C. Path., Hospital for Tropical Diseases, 4 St. Pancras Way, London, N.W. 1, England.

quent and rarely exceed 0.5 units. These conclusions are as true for the series as a whole as they are for the seven cases illustrated. The BI and the LIB are both based on the same logarithmic scale, but there is no reason why the two should necessarily give the same values because of the different sampling technics used in making smears and sections. The general similarity of the levels of these indices therefore is partly fortuitous. But if the BI took full account of both the components of the biopsy formula it should fall at the same rate as the LIB, which it does not.

A more detailed analysis of the course of the three indices during treatment of the patient is only possible when the patients are first classified according to their TT-LL groups. The mean fall of each index for each six months of treatment is shown in Table 1. Much work has been done previously on the rates of fall of the IB in the different groups, the falls being expressed as a percentage of the index level at the

TABLE 1. *Mean rates of fall in indices according to classification; expressed as amount of fall per period of six months.*

Index	Length of treatment and classification						
	0-½ years				½-2 years		3-5 years
	LL	BL	BB	BT	LL	BL	LL
Biopsy formula:							
Bact. density	0.00	0.67	3.0	2.3	0.19	1.12	0.57
Granuloma	0.20	0.15	0.2	0.0	0.13	0.28	0.10
LIB (units)	0.20	0.82	3.1	2.1	0.32	1.40	0.67
BI (units)	0.18	0.26	1.3	1.6	0.20	0.89	0.44
IB (units)	0.80	0.79	1.0	1.0	0.36	0.68	0.26
IB %	24	41	82	100	23	35	27

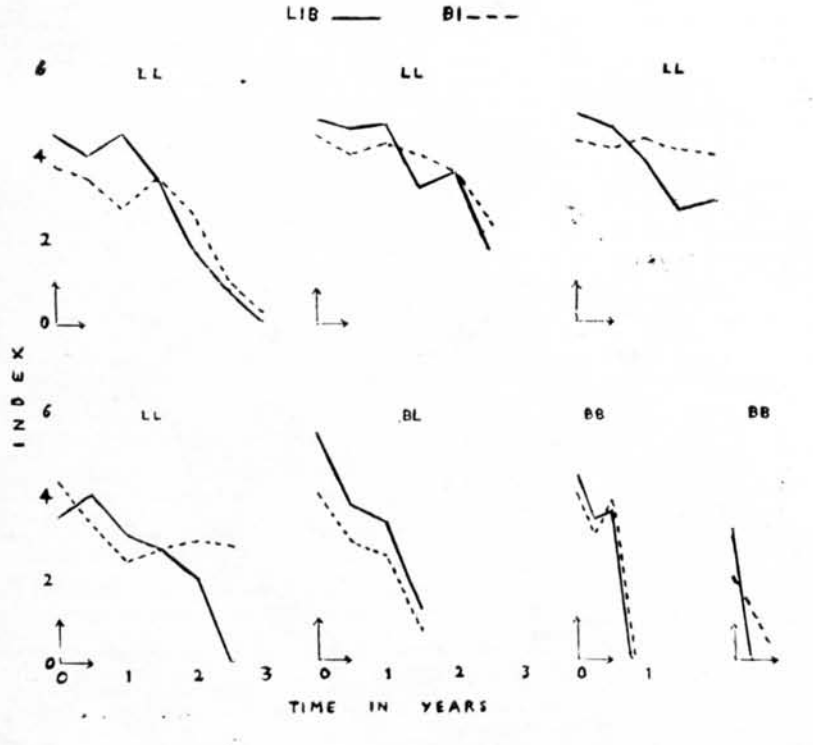

FIG. 1. Progress of LIB and BI in seven patients during treatment.

time. The rates of fall in the IB in this series for each of the four bacteriologically positive groups agree closely with previously published rates. In the case of the logarithmic indices, there is no justification for calculating the rates of fall on a percentage basis. A fall of 1.0 in the LIB or BI indicates a fall of 1/10th of the original level, whatever that level may have been. The rate of fall, therefore, is expressed as the fall in units per six months period.

In Table 1 the LIB values are broken down into the component parts of the biopsy formula (the LIB does not necessarily equal the sum of its parts; for example, when the bacterial density is zero the LIB also is zero, but granuloma may still be present). A comparison of the BI with the LIB shows that the BI gives a slower rate of fall than the LIB in all groups throughout the whole period of treatment up to five years. It is evident that the fall in the BI is sometimes a reflection of diminution of the granuloma and at other times of a reduction in the bacterial density. The relationship between these factors can be accounted for if it is assumed that the fall in the BI equals the fall in the granuloma plus half the fall in the bacterial density. On this basis the expected and the actual values of the BI in the seven columns of the table would be as follows:

	Expected						
BI	0.20	0.48	1.7	1.2	0.23	0.84	0.39
	Actual						
BI	0.18	0.26	1.3	1.6	0.20	0.89	0.44

The relationship between these indices is affected by a number of other considerations, but in the final outcome they prove not to be of great importance, and discussion of them therefore is relegated to an appendix. There is no constant relationship between the rate of fall in the IB and that of the other indices.

THE LIB AND THE ASSESSMENT OF BACTERIOLOGIC PROGRESS

The LIB, BI and IB agree in indicating that the bacteriologic response to therapy improves markedly as the immunologic scale is ascended from LL to BT (Table 1).

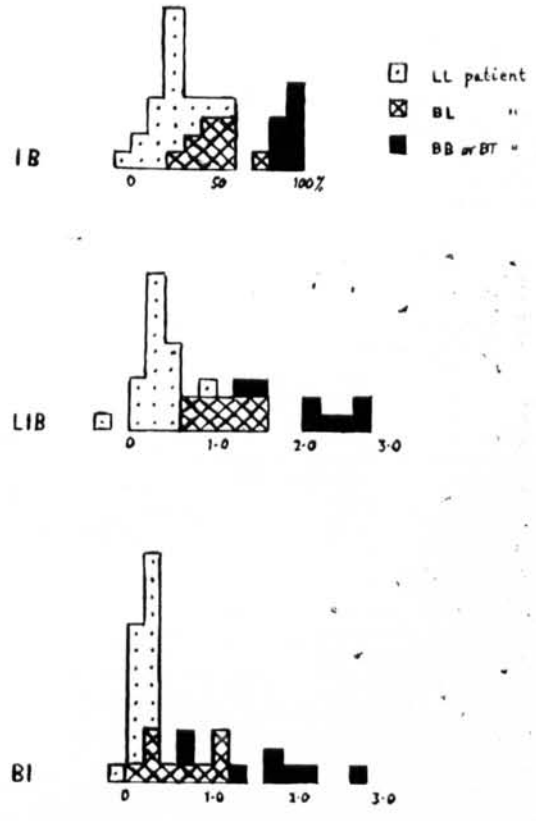

FIG. 2. Mean rates of fall of three indices according to classification of leprosy; distribution of patients.

Figure 2 shows the distribution of patients in the different groups according to the rates of fall of the three indices. The LIB is found to be more sensitive than the BI in separating the LL, BL and BB groups, and more sensitive than the IB in separating LL from BL. The BT group is not clearly separated because, although the indices usually fall to zero within six months, the fall may not be very great, since the initial index is often not very high. This applies also to a few patients of the BB group. The capacity of patients in these two groups to make a good bacteriologic response to treatment is proved by the large fall of all indices in those patients who present with high initial indices. But taking the groups as a whole this capacity is best demonstrated by indices in which the fall is expressed as a percentage (IB).

The rate of fall of the LIB increases very slowly in LL patients as treatment proceeds. In BL patients it increases more rapidly. In BB and BT patients it is impossible to measure the rate of fall after the first six months, as the level of the index is then usually close to zero. The rapid improvement of the bacteriologic response of BL patients is often accelerated by an immunologic improvement, which is associated with a change in classification to BB or BT.

A study of the biopsy formula shows that the higher rate of fall of the LIB in borderline than in lepromatous patients is due entirely to the fall in bacterial density. The rate of diminution of the granuloma is approximately equal in all groups (the BT group is based on only two patients), although it is possible that some of the absorption of granuloma in borderline patients might sometimes be masked by infiltration of inflammatory cells and edema, due to borderline reactions. At all events, the contribution made by the granuloma component of the biopsy formula to the total bacteriologic response is a relatively small one in all except LL patients. Even in them the relative importance of this factor declines as treatment is prolonged.

DISCUSSION

The LIB is the best assessment of the number of bacilli in a lesion short of actually making a count in a homogenate. The only possible objection to its use for the bacteriologic assessment of a patient is that the one or two lesions on which the LIB is based might not be representative. The similarity in the behavior of the LIB and the BI of smears during treatment proves that random errors are not a serious objection to the LIB, which can therefore be used as a yardstick by which to evaluate other indices.

It is found, first, that the LIB can usefully supersede the original biopsy index, IB. Nevertheless, the work done with the IB in assessing response to treatment as an aid to the definition of the TT-LL classification is fully confirmed by the present study.

The relationship between the LIB and the BI is not a straightforward one, and in spite of the greater accuracy of the LIB it is likely that the smear index will continue to be used for routine purposes because of its simplicity, and as a check on the LIB because it is based on multiple lesions. This is not to say that the LIB is not simple, also, once histologic sections have been prepared; and these are desirable for their own sake in any research project or therapeutic trial. The BI is seen to be a meaningful though not exact representation of bacillary numbers. The formula indicating that the fall in the BI represents the diminution of the granuloma plus half the fall in the bacterial density, would imply first that, in making a smear from a lesion with a small granuloma, the small amount of juice was spread out thinly so that the BI was only half the original bacterial density, and, secondly, that with larger granulomas the larger volume of juice allowed a correspondingly thicker film to be made. This relationship between biopsy and smear clearly depends on the technic of the operator who makes the smear.

The difference in the manner of the bacteriologic response to treatment of lepromatous and borderline patients, which is shown by a study of the biopsy formula, is probably fundamental. The bacterial density of LL patients does not show a sharp decline until about the third year of chemotherapy; during the first six months the decline is zero. These patients appear to be incapable of destroying bacilli *in situ*, and three years is presumably the time taken for bacilli to disintegrate to the point of becoming unstainable. By contrast, the rapid bacteriologic response to treatment of BB patients, which is a thousand-fold greater than that of LL patients, is due almost entirely to a fall in bacterial density brought about presumably by destruction of bacilli *in situ* in skin. Furthermore, BL patients, so close in many respects to LL, are found by this criterion to belong to the borderline groups; the rate of fall in bacterial density in the first six months of treatment is greater than that of the LL patients even after three years. It should be added that not all BL patients

show a very early decline in bacterial density, although this always occurs earlier than in LL patients, and when it comes, the decline is usually sharp.

The rate of absorption of the granuloma never alters greatly, but the granuloma is the only component of the biopsy formula to alter during the first six months of treatment of LL patients, when the rate of absorption is at a maximum. This is the situation in which preliminary therapeutic trials of new drugs are usually undertaken, and it is disappointing that the fall in the LIB is small (0.20), although arithmetically it represents a fall of 27 per cent, that being the percentage reduction in the granuloma while bacterial density remains constant. The fall in the BI is no greater than that of the LIB. There remains the morphologic index, which is the most logical index to employ at the commencement of treatment, since the test then is the death of bacilli rather than the decline in their numbers. There are three indices that might be specially recommended for short term therapeutic trials in LL patients: (1) the morphologic index expressed as a percentage; (2) a "granuloma index" expressed as a percentage; this would be more sensitive than the LIB; and (3) the LIB (viable), which was described in the preceding paper ([3]). For longer term therapeutic trials the ordinary LIB would be most valuable.

SUMMARY

The behavior of the modified biopsy index (logarithmic index of bacilli in biopsies, LIB) has been compared with that of other bacterial indices of biopsies and smears in patients under treatment.

It is shown that the random errors of biopsy indices are relatively small. The rate of fall of the LIB during treatment is about twice that of the smear BI, which does not fully reflect the bacterial content of lesions.

The independent assessment of alterations in bacterial density and size of granuloma, which is made possible by the LIB, shows some significant differences in the mode of response to treatment by lepromatous and borderline leprosy patients.

Previous work done with the original biopsy index in assessing response to treatment as an aid to definition of the TT-LL groups is fully confirmed.

RESUMEN

El comportamiento del index modificado para biopsia (index logarítmico de bacilos en biopsias, LIB) ha sido comparado con otros índices bacterianos de biopsias y frotis en pacientes bajo tratamiento.

Se demuestra que los errores al azar de índices de biopsias son relativamente pequeños. La tasa de disminución del LIB durante el tratamiento es dos veces, aproximadamente la que se observa en el frotis, BI, la cual no refleja totalmente el contenido bacteriano de las lesiones.

La evaluación independiente de los cambios en la densidad bacteriana y tamaño de granuloma, lo cual es posible realizar por el LIB, muestra algunas diferencias significativas en la manera de responder al tratamiento en pacientes con lepra lepromatosa y borderline.

Trabajos previos hechos con el index original de biopsias para evaluar la respuesta al tratamiento como una ayuda a la definición de los grupos TT-LL se confirman totalmente.

RÉSUMÉ

On a comparé, chez des malades en traitement, le comportement de l'index biopsique modifié (index logarithmique du nombre des bacilles dans les biopsies, LIB) avec le comportement d'autres indices bactériens utilisés pour les biopsies et les frottis.

On a montré que les erreurs dues au hasard dans les indices biopsiques étaient relativement faibles. Le taux de diminution du LIB au cours du traitement est environ deux fois plus élevé que le taux de diminution de l'indice bactériologique (BI utilisé pour les frottis, celui-ci ne reflétant pas complètement le contenu bactérien des lésions).

L'évaluation indépendante des modifications de la densité bactérienne et de la dimension du granulome, rendue possible par le LIB, montre qu'il existe des différences significatives dans la manière de répondre au traitement chez les malades atteints de lèpre lépromateuse et chez ceux atteints de lèpre borderline.

Ceci confirme donc entièrement les travaux antérieurs menés en utilisant l'index biopsique original pour estimer la réponse au traitement, considéré comme un appoint pour la définition des groupes TT-LL.

Acknowledgments. I am grateful to Dr. G. R. F. Hilson for some helpful comments, and I have to thank Dr. W. H. Jopling for the clinical assessment of the patients, and for taking the biopsies and smears: also Mrs. M. J. Ridley for the histologic preparations, and the research funds of the Hospital for Tropical Diseases for a grant for technical assistance.

REFERENCES

1. Ridley, D. S. The bacteriological interpretation of skin smears and biopsies in leprosy. Trans. Roy. Soc. Trop. Med. & Hyg. **49** (1955) 449-452.
2. Ridley, D. S. Therapeutic trials in leprosy using serial biopsies. Leprosy Rev. **29** (1958) 45-52.
3. Ridley, D. S. and Hilson, G. R. F. A logarithmic index of bacilli in biopsies. 1. Method. Internat. J. Leprosy **35** (1967) 184-186.
4. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five-group system. Internat. J. Leprosy **34** (1966) 255-273.

APPENDIX

Factors Affecting the Relationship Between the LIB and the IB

The original reason for introducing the biopsy index, IB, was that serial biopsies seemed to indicate that the BI of smears failed to take full account of the diminution of the granuloma with treatment, and hence its rate of fall was too slow. Another observation was that the BI often failed to reflect the highest densities of bacilli ([1]), which is confirmed in the present study by the finding that the initial pretreatment LIB is usually higher than the corresponding BI. Some apparent support for the view that the BI does not take account of the diminution of the granuloma can be found when one plots the LIB against BI values for different sizes of granuloma. The BI is then found to give relatively higher values, against the LIB, in small than in large granulomas. Another factor, however, is involved here. The smallest granulomas are found in long-treated patients and in them the bacterial density is relatively even lower than the volume of the granuloma.

One reason why the LIB is often higher initially than the BI is presumably that the latter is based on smears of several lesions and therefore less likely to reflect the largest lesions, which are often those chosen for biopsy. The faster rate of fall of the LIB would be explained if it could be shown that this rate was faster for large than for small lesions. An analysis of paired lesions, one being larger than the other, shows that the LIB of large lesions does fall at a faster rate than in small lesions in the same patient. For a series of lesions with a mean difference in LIB's of 1.4, the rate of fall was 40 per cent greater in the large than in the small lesions. In the group of patients now being considered, however, the LIB of the initial biopsy was on the average only 0.55 higher than the BI of the corresponding smears. This might account for the rate of fall being 15-20 per cent higher for the LIB than the BI. But the rate of fall of the LIB was almost 100 per cent greater than that of the BI. Further-

more, the higher rate of fall of the LIB was an almost constant factor, irrespective of the difference in the initial indices.

The rate of fall of the LIB is higher in large than in small lesions in the same patient. When rates of fall are analyzed according to size of lesions in different patients no correlation can be found. The initial level of the LIB therefore has no bearing on the differences in the progress rates of the different groups. Incidentally there is no difference between the LL and BL groups in the mean level of the pretreatment LIB values, although the level in the BB group is lower and in the BT group it is lower still.