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Reproducibility of Histology in Leprosy Lesions[1]

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Examination of the histology of skin lesions in leprosy is important for diagnosis and for classification of patients into groups within the clinico-pathological spectrum described by Ridley and Jopling ([6]). Sequential skin biopsies during treatment can be used to follow the course of the disease and are particularly useful in therapeutic trials ([4]). Parameters such as the bacterial index (BI) and the granuloma fraction (GF: the proportion of the dermis occupied by granulomas) decrease during effective chemotherapy and increase if relapse occurs ([5]). Changes in histological classification during treatment are also common ([4]), and it is thought that this indicates alterations in the patients' immune response to *Mycobacterium leprae*.

It is customary to estimate the BI and GF semi-quantitatively during microscopy of histological sections, but recently a precise quantitative method of estimating the GF in tissue sections has been established ([2]). The potential value of this semiautomated method of measurement of GF in clinical practice and research investigation can only be decided when the extent of variation of the GF within the same leprosy lesion and between lesions on the same patient is known.

This investigation was undertaken to determine the extent of GF variation at the edge of established skin lesions and between the edges of different lesions on the same patient. The opportunity was taken to examine the reproducibility of observed estimates of the BI and histological classification on the Ridley-Jopling scale ([4, 6]).

MATERIALS AND METHODS

Biopsy method

Biopsies were taken from lesions on the trunk or limbs, but never on the face or hands. Verbal informed consent was obtained from all of the patients. Following cleansing of the skin with Betadine® antiseptic, a small amount of local anesthetic (either plain 1% Lignocaine or 1% Lignocaine + adrenalin) was infiltrated around a suitable biopsy site at the edge of the chosen lesion. After a few minutes, the infiltrated area was tested for loss of sensation using a sterile needle. The biopsy was then taken using a sterile disposable 4-mm diameter skin-biopsy punch (Stiefel Laboratories, Slough, England). After securing hemostasis by pressure, the biopsy site was cleansed with antiseptic (Betadine®), and a sterile self-adhesive dressing was applied. This dressing was removed 24 hr later by the patient or a paramedical worker, and the wound was then allowed to granulate, thus avoiding the need for stitches. The biopsy site healed in 7–10 days and no significant complications were encountered.

The biopsies were fixed in buffered Formalin (4% formaldehyde). Diagnostic histopathology reports were sent to the relevant medical officers within 1 month of receiving the biopsies in Dundee.

Biopsy series 1. The first group of biopsies were taken by TS and SARK from patients attending the Leprosy Control, Training and Research Centre at Zaria in northern Nigeria. In each of the 44 cases, 4-mm skin-punch biopsies were taken from opposing edges of the same lesion to investigate vari-

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ation of leprosy histology at different positions on the edge of the lesion. The average age of the patients in this group was 29 years (S.D. 12.4 years); 24 were males and 20 females. Seventeen patients had not received treatment at the time of biopsy, 5 cases were receiving multidrug therapy (MDT) (WHO regimen), all for less than 3 months, and the remaining 22 cases were on dapsone monotherapy. Of the dapsone-treated patients, one patient had been treated irregularly with dapsone for 2 years, 20 had been treated for less than 3 months, and one for 5 months. Two cases were excluded because of diagnostic uncertainty. The remaining cases were classified clinically on the Ridley-Jopling scale ([4, 6]) as 2 indeterminate (Idt), 4 tuberculoid (TT), 19 borderline tuberculoid (BT), 5 borderline lepromatous (BL), and 12 lepromatous (LL).

Biopsy series 2. The second series of biopsies included some obtained at Pune, India, by CAG, and some from Bangladesh, obtained by IAC and CAHF at the HEED Leprosy Hospital, Kamalganj, Bangladesh. In each case, two 4-mm skin-punch biopsies were taken as described above from the edge of two separate lesions of differing size to study variation in histology between different lesions. An effort was made to choose lesions of disparate size and appearance where possible to determine the maximum variation which might occur. The biopsies were fixed in 4% buffered neutral formaldehyde, returned to Dundee, and processed in the same manner as those in the first series. A total of 60 patients (49 males, 11 females) with an average age of 35.5 years (S.D. 14.6) were included in this series, but three were subsequently excluded due to diagnostic uncertainty. The remaining cases were classified clinically on the Ridley-Jopling scale ([4, 6]) as 19 LL, 4 BL, 10 BB, 23 BT, and 1 TT. No indeterminate cases were examined. All were untreated or had received less than 1 month's treatment (MDT, WHO regimen).

Histological assessment

The position of the biopsy on the Ridley-Jopling scale and the BI were estimated by one observer (IAC) without referral to the clinical data supplied with each pair of biopsies.

Image analysis

The GF was measured by planimetry as previously described ([2]), using an Imagan planimeter (Graphic Information Systems Ltd., Blairgowrie, Scotland) and SM-Lux microscope (Leitz). The image of the pointer lying on the graphic tablet is superimposed on the eyepiece image by means of a tracing device (Leitz). This allows movement of a projected point of light from the tip of the pointer around the edge of each granuloma or dermal area. Granulomas were defined as collections of more than 10 mononuclear cells (MNC) lying together within the dermis; scattered MNC in the dermis were not included. When the perimeter of each granuloma was complete, the computer calculated the area encircled. The area of the dermis was similarly determined and the GF was calculated as:

$$GF = \frac{\text{Sum (area of granulomas)}}{\text{Area of dermis}}$$

Analysis of results

The degree of concordance between GF measurements made by different observers and by planimetry was estimated by linear regression analysis. Intra- and interlesional agreement in the GF between biopsies was also assessed by this method.

RESULTS

Biopsy series 1. The results of the GF measurements in biopsies from opposing edges of the same lesion (Fig. 1) show reasonable correlation ($r = 0.915$). There is a GF difference of more than 10% in seven cases, all but one of which are lepromatous and in two of these the difference in the GF is greater than 20%.

There was very good agreement in histological classification on the Ridley-Jopling scale (Table 1). Five patients showed indeterminate histology in one biopsy and BT leprosy in the other. There was surprisingly little disagreement in the BI assigned to each biopsy: a BI difference of 1 or more occurred in 13 cases (Table 2), but in only one case was there a difference of 2 points on the scale (a BT patient with a BI of 1+ in one biopsy and 3+ in the other).

Biopsy series 2. The paired biopsies taken from the edges of different lesions on the

TABLE 1. *Intralesional variation in Ridley-Jopling classification between biopsies taken from opposite edges of the same lesion (Biopsy series 1).*

Biopsy A	Ridley-Jopling classification—Biopsy B						ND[a]	Total
	Idt	TT	BT	BB	BL	LL		
Idt	2	0	5	0	0	0	0	7
TT		4	0	0	0	0	0	4
BT			14	0	0	0	0	14
BB				0	0	0	0	0
BL					5	0	0	5
LL						12	0	12
ND							2	2
Totals	2	4	19	0	5	12	2	44

[a] ND = Not diagnostic of leprosy.

same patient show considerable variation in GF (Fig. 2), and there was poor correlation between them (r = 0.466). The difference between them exceeded 10% in 22 cases (7 paucibacillary and 15 multibacillary). In 16 cases the GF difference between biopsies was greater than 20%.

Histological agreement between biopsies taken from the edge of different lesions is generally good (Table 3): there was a difference of one point or more on the Ridley-Jopling scale in only five cases. However, in a further six cases, the histology of one biopsy was not diagnostic of leprosy and these cases were excluded from the assessment of GF reproducibility. The BI differed between biopsies in 22 cases (Table 4), although in seven of these one biopsy showed nondiagnostic histology. In seven of the cases, the BI differed by 2 points between biopsies.

DISCUSSION

The value of the greater sensitivity of this technique for trials of antileprosy chemotherapy regimens and other research projects depends upon the extent of intra- and interlesional variation in the GF. The previous histometric studies have shown that there is little variation at various levels within single biopsies: the observer error in visual grading of GF is very much greater and results in overestimation of the GF ([2]). The results of GF measurement in biopsies from the center and edge of the lesion, and from clinically unremarkable skin 2 cm outside the lesion, confirm that biopsies taken from the edge of established skin lesions contain the greatest amount of granuloma and are, therefore, most suitable for diagnostic purposes ([1]). However, the GF was greater in central biopsies from the four early BT lesions which were characterized by recognizable BT features in the center of the lesion, but not at the edge ([1]).

Intralesional variation in GF has been investigated further in this study by taking biopsies from the opposing edges of the same lesion (Biopsy series 1) in a series of Nigerian patients (Fig. 1). There was little vari-

TABLE 2. *Intralesional variation in bacterial index (BI) between biopsies taken from opposite edges of the same lesion (Biopsy series 1).*

Biopsy A	Bacterial index—Biopsy B							Total
	0	1	2	3	4	5	6	
0	11	5	0	0	0	0	0	16
1		6	2	1	0	0	0	9
2			2	0	0	0	0	2
3				0	1	0	0	1
4					3	2	0	5
5						1	2	3
6							8	8
Totals	11	11	4	1	4	3	10	44

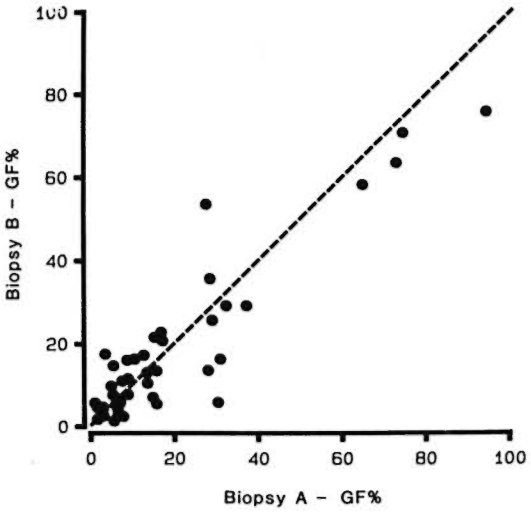

FIG. 1. Granuloma fraction (GF) measurements for biopsies from opposing edges (A and B) of the same lesion. Dotted line indicates expected distribution for complete agreement.

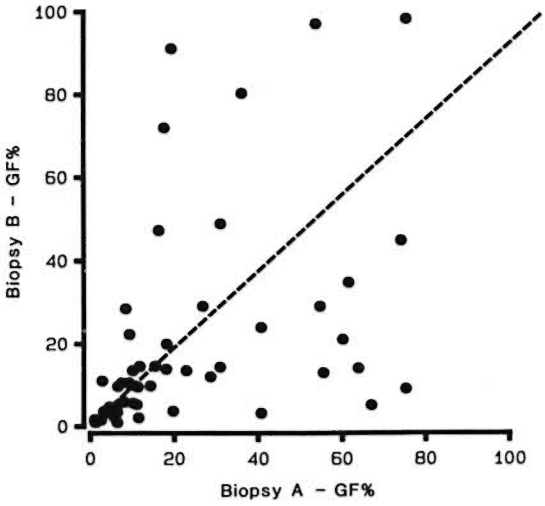

FIG. 2. GF measurements from the edges of two separate lesions (A and B) from the same patient.

ation between paucibacillary (TT-BT) patients, but several of the lepromatous patients showed differences in their GF of up to 15% of the dermal area. There was no concomitant difference in histological classification on the Ridley-Jopling scale between biopsies. Therefore, it seems likely that the observed variation in GF was due to sampling error, since the edges of lepromatous lesions are often indistinct. This problem apart, the results of GF measurement suggest that optimum intralesional reproducibility of the GF can be obtained by taking biopsies from the well-defined edges of established leprosy lesions.

The second series of biopsies were obtained in order to assess variation in leprosy histology between different lesions on the same patient. The results (Fig. 2) show relatively poor correlation in the GF ($r = 0.425$) between biopsies. The paired biopsies were obtained from lesions that differed in size and appearance whenever possible to assess the full extent of histological variation. Nevertheless, there was agreement in the Ridley-Jopling classification in 49 of the 60 cases. In the 11 cases in which there was disagreement, one of the biopsies showed either indeterminate or mild chronic inflammatory changes. This suggests that small lesions which are only just appearing have indeterminate histology, while histological differences between established lesions are unusual in untreated leprosy. A similar position does not seem to exist for the BI, which showed rather more variation be-

TABLE 3. *Interlesional variation in Ridley-Jopling classification between biopsies taken from edges of two different lesions on the same patient (Biopsy series 2).*

Biopsy A	Ridley-Jopling classification—Biopsy B						ND[a]	Total
	Idt	TT	BT	BB	BL	LL		
Idt	5	0	3	1	0	0	1	10
TT		1	0	0	0	0	0	1
BT			20	0	0	0	5	25
BB				1	0	0	0	1
BL					2	1	0	3
LL						17	0	17
ND							3	3
Totals	5	1	23	2	2	18	9	60

[a] ND = Not diagnostic of leprosy.

TABLE 4. *Interlesional variation in bacterial index (BI) between biopsies taken from edges of two different lesions on the same patient (Biopsy series 2).*

Biopsy A	Bacterial index—Biopsy B							Total
	0	1	2	3	4	5	6	
0	13	8	2	0	0	0	0	23
1		7	3	2	0	0	0	12
2			4	0	1	0	0	5
3				0	0	1	0	1
4					0	2	1	3
5						3	2	5
6							11	11
Totals	13	15	9	2	1	6	14	60

tween biopsies from different lesions. This may be determined by the size of the granuloma, which Ridley (4) suggests should be taken into account when calculating the Bacteriological Index of Granuloma.

The results of the present study are in keeping with previous qualitative studies of the histology of leprosy lesions by Ridley (4) and others which suggest that there is good concordance in histology between and within lesions, although occasional patients may not conform to this pattern as a result of reversal reactions. In a study of a small group of untreated patients, Gupta, *et al.* (3) suggested that there were significant differences in the histological grade between biopsies taken from different sites. We have failed to substantiate their suggestion that varied histology is common between lesions in leprosy, although there are significant differences in the GF and possibly the BI. The lack of appreciable interlesional variation in the histological classification suggests that the systemic host response to infection by *M. leprae* is not altered by local factors in the untreated patient, although the expression of these in the development of individual lesions may be influenced by local factors, such as the micro-anatomical site of lodgement of leprosy bacilli.

In conclusion, the results of this study suggest that biopsies from the well-defined edge of established lesions are representative of their histology, GF, and BI. It seems likely that planimetry would facilitate comparison of the results of leprosy histopathology from different centers and that it would be of value in evaluating serial biopsies from the edge of the lesion taken during drug trials. It is recommended that if sequential biopsies are used, these should be taken from the same lesion or from a lesion of similar size to avoid errors resulting from interlesional differences in the GF and the BI.

SUMMARY

The variability of three commonly used histological parameters in leprosy histology was examined within and between lesions on individual patients by taking two biopsies, either from opposing edges of the same lesion or from the edge of two separate lesions. There was little variation in granuloma fraction (GF), bacterial index (BI), or histological classification on the Ridley-Jopling scale between biopsies from opposing edges of the same lesion, but there was considerable variation in the GF between biopsies from the edge of different lesions. A lesser degree of variation was seen in the BI between different lesions, and there was little difference in histological classification between established lesions. Thus, it appears that local factors influence the size of the leprosy granuloma, but its histological composition and bacterial load are determined systemically.

RESUMEN

Se examinó la variabilidad de 3 parámetros comunmente usados en la histología de la lepra, dentro y entre las lesiones de pacientes individuales, tomando 2 biopsias de los bordes opuestos de la misma lesión o del borde de dos lesiones separadas. Hubo poca variación en la fracción granuloma (FG), en el índice bacteriano (IB), y en la clasificación histológica según la escala de Ridley-Jopling entre las biopsias tomadas de los bordes opuestos de la misma lesión pero hubo una considerable variación en la FG entre las biopsias del borde

de diferentes lesiones y un menor grado de variación en el IB. Hubieron muy pequeñas diferencias en la clasificación histológica entre las lesiones establecidas. Así, parece que los factores locales influyen en el tamaño del granuloma leproso, mientras que su composición histológica y la carga bacteriana están determinadas sistémicamente.

RÉSUMÉ

On a examiné la variabilité de trois paramètres histologiques communément utilisés dans l'histologie de la lèpre, tant à l'intérieur d'une même lésion, qu'entre lésions différentes chez les mêmes individus. Pour ce faire, on a prélevé deux biopsies, situées soit aux bordures opposées de la même lésion, ou au bord de deux lésions séparées. On a observé peu de variations dans la fraction granulomateuse (GF), l'index bactériologique (BI), ou la classification histologique selon l'échelle de Ridley-Jopling, entre biopsies prises aux bordures opposées de la même lésion. Par contre, la variation de la fraction granulomateuse était considérable lorsqu'on comparait les biopsies prises aux bords de lésions différentes. Dans ce cas, les variations observées pour l'index bactériologique étaient moins prononcées; quant à la classification histologique, on n'a noté qu'une différence très faible entre des lésions bien établies. Il apparaît dès lors que, si des facteurs locaux influencent la dimension du granulome lépreux, sa constitution histologique et sa charge bactérienne obéissent par contre à des facteurs systémiques.

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