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Quantitation of the Granuloma Fraction in Leprosy Skin Biopsies by Planimetry[1]

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Currently available methods for the assessment of disease activity in leprosy are essentially semi-quantitative and subjective. One commonly employed approach is the estimation of the proportion of the dermis occupied by the granuloma from direct microscopy of histological sections of skin biopsies from the edge of the leprosy lesions. This is known as the granuloma fraction (GF) ([5]). It is usually estimated by visual observation under a ×4 objective, and the result is expressed as the decimal fraction (0–1.0) of the area of dermis occupied by inflammatory cells ([4, 6]). The accuracy of GF estimation can be improved by the use of an eyepiece grid or by outlining the projected image of the granulomas ([5]), but such methods are both tedious and time consuming; consequently, they are rarely performed.

While the human eye is good at pattern recognition, the basis of histopathological diagnosis, it is considerably less reliable in determining the proportion of any microscopic field occupied by a particular component ([1, 7]), particularly when the field is broken up into irregular areas. Advances in micro-electronics in recent years have made new techniques of image analysis available to the pathologist ([2, 3]).

We report here an objective method for measuring the GF by using a relatively inexpensive image analyzer, which we have compared with the usual method of visual grading.

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MATERIALS AND METHODS

Skin biopsies. Single biopsies were obtained by using a 4 mm skin biopsy punch or surgical excision from 49 patients in Nepal and Malawi. The biopsies were fixed in Formol-Zenker ([5]) and embedded in paraffin wax. The sections (5 μm) were stained with hematoxylin and eosin (H&E) and by the Fite-Faraco modification of the Ziehl-Neelsen method. Histological diagnosis and classification on the Ridley-Jopling scale ([5]) were performed by one of us (ACMcD). Of the 49 biopsies, 31 were categorized as LL or LLs, 2 as BL, 1 as BB, and 7 as BT. The remaining eight biopsies, obtained from patients who had received treatment for long periods of time, could not be classified histologically.

Visual grading. Semi-quantitative visual grading was carried out independently by an experienced leprosy histologist (Observer 1: ACMcD) and by an experienced general histopathologist with an interest in histometry (Observer 2: JSB). Both observers used a Leitz Orthoplan microscope and graded the sections with the ×4 objective on a scale of tenths from 0 to 1.0, according to the method of Ridley and Hilson ([6]). The first observer graded the biopsies sequentially in random order, and the second observer ranked the biopsies in order of decreasing GF before assigning a numerical index to each individual biopsy.

Image analysis. An Imagan planimeter (Graphic Information Systems Ltd., Blairgowrie, Scotland) consisting of a graphic tablet (Summagraphics, Fairfield, Connecticut, U.S.A.), pointer, HP-85 computer (Hewlett-Packard), and SM-Lux microscope (Leitz) was used to measure the GF (Fig. 1). The image of the pointer on the graphic tablet was superimposed on the eyepiece image by means of a tracing device (Leitz). This allows movement of a projected point of light around the edge of each

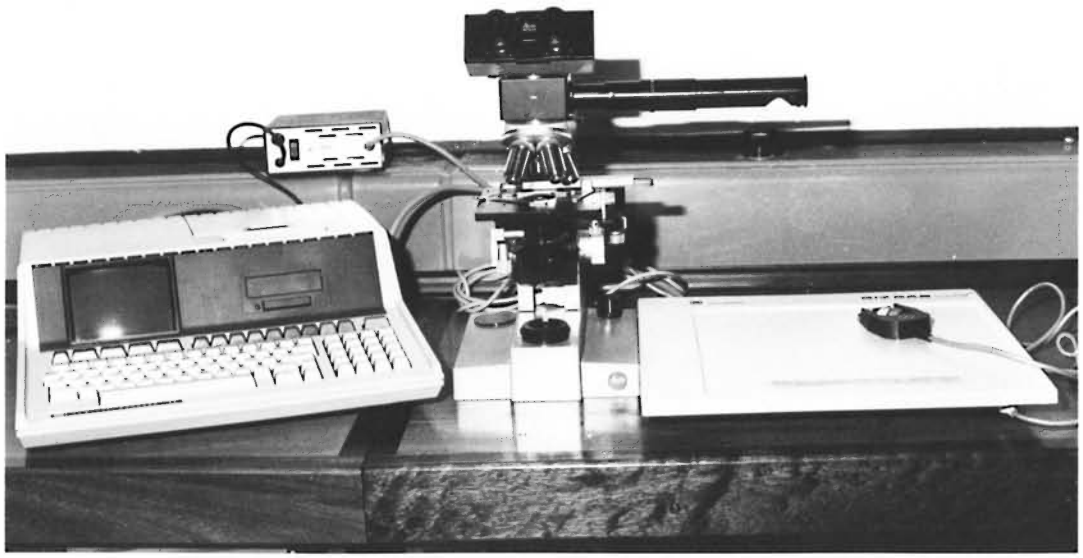

FIG. 1. Imagan planimeter with tracing device side arm attachment to microscope.

granuloma or dermal area. Granulomas were defined as collections of more than ten 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:

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

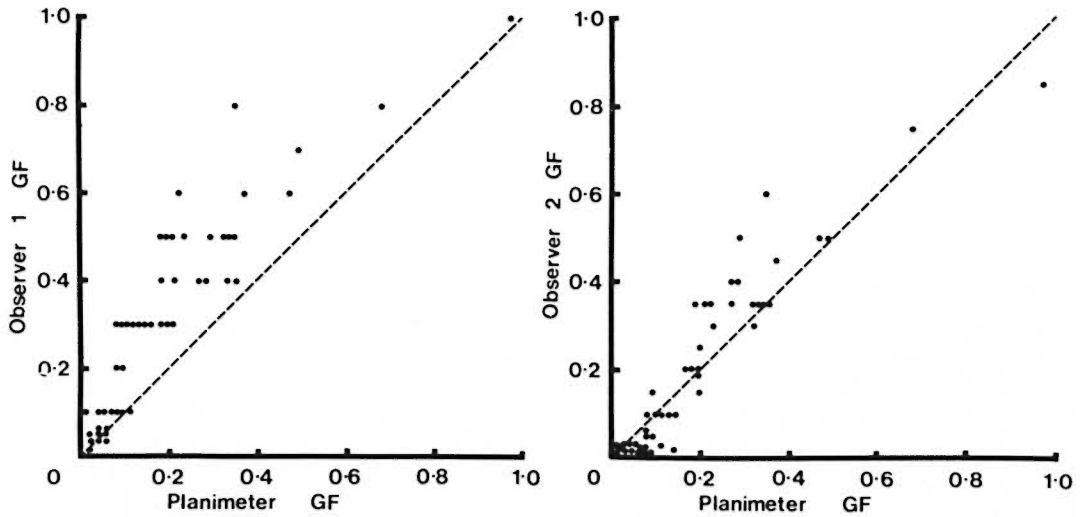

FIG. 2. Comparison of GF measurement by planimetry with direct microscopy by two observers. Dotted lines indicate expected distribution for complete concordance between the two methods of measurement. Correlation coefficient for Observer 1 = 0.89; for Observer 2 = 0.94.

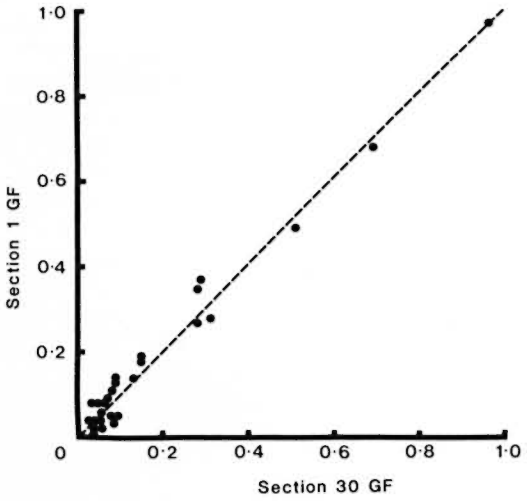

FIG. 3. Comparison of GF measurement by planimetry in the first and 30th serial sections of 4 mm skin punch biopsies.

RESULTS

Good correlation was found between the GF estimated by each of the two observers and the GF obtained by the planimeter (Fig. 2). The GF estimated by Observer 1 disagreed by 0.1 (10% of the dermal area) or more from the planimeter measurement in 33 cases; in 14 cases, the difference was 0.2 (20%) or more. Ranking of the biopsies before assigning the GF values improved the results slightly; Observer 2 disagreed with the planimeter grade in 19 cases by 0.1 (10% of the dermal area) or more and in only 2 of these was the difference 0.2 (20%) or more. All cases of disagreement were apparently caused by overestimation of the GF by the subjective visual observation method. This problem was more marked in sections with more extensive dermal granulomas, i.e., a planimeter GF measurement of 0.3–0.9.

The planimeter gave highly reproducible results; the maximum standard deviation of the GF was 0.016 (1.6%) of the dermal area on triplicate measurements of the GF. This level of error was insignificant unless a very small GF (0.05 or less) was being measured. Use of the planimeter to measure the GF required little practice, and each section could be measured in 10–15 min, depending upon how many individual granulomas were present. The precision of the planimeter GF was assessed by measuring the GF in the first and 30th serial sections from 28 biopsies. The results (Fig. 3) show good correlation between the sections (correlation coefficient = 0.988), and in only two cases was there a difference of 0.05 (5% of the dermal area) or more.

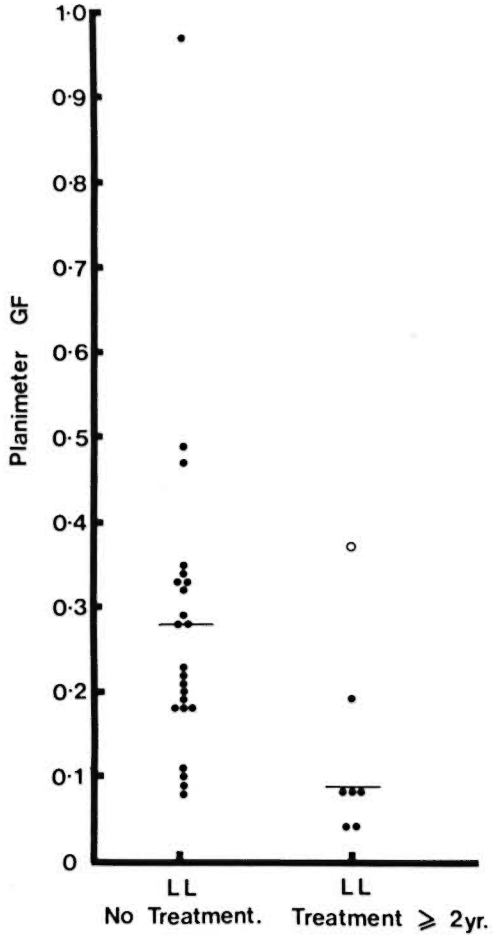

FIG. 4. Granuloma fraction in treated and untreated patients with lepromatous leprosy. Bars = means; o = GF of a dapsone-resistant case. The difference in means is significant ($p < 0.001$, Student's t test).

Although the number of patients included in this initial study is not large, a comparison of the GF in the biopsies from lepromatous patients with length of treatment shows a clear difference between those lepromatous patients treated for two years or more and those who had not received treatment (Fig. 4).

DISCUSSION

The GF values obtained by the usual method of direct microscopy correlated well

with those found using the planimeter, but disagreement of 20% of the dermal area or more occurred in 29% of the cases. This degree of error could be avoided to some extent by ranking the biopsies before assigning each a GF, a simple technique which has been used previously to improve the semi-quantitative grading of iliac crest biopsies ([1]). Perhaps of greater concern is the overestimation of areas occupied by granuloma which both methods of subjective observation have produced. This problem was especially prominent in the group of biopsies from lepromatous patients with larger proportions (GF = 0.3–0.9) of the dermis occupied by granulomas. The lack of precision in the GF estimation in such patients could be of some clinical importance in the assessment of the efficacy of treatment by repeated biopsy since small differences may pass unnoticed by the visual grading method. An assessment of the GF using the planimeter shows a significant difference between treated and untreated lepromatous patients, as expected from the results of previous studies using subjective methods ([6]). The technique described here is undoubtedly able to detect smaller changes in the GF than the methods currently in use, and it is relatively inexpensive in terms of time and financial commitment. Various Hewlett-Packard and IBM computers can be used with the Imagan software. Since real measurements (in millimeters or micrometers) are made, the results using this system are comparable with those of other planimeters giving real measurements.

Whether the greater sensitivity of this technique will ultimately prove of value in leprosy will depend upon the extent of intralesional and interlesional variation in the GF, a problem which we are currently investigating. There appears to be little variation in the GF within individual biopsies, and the error due to this variation is much less than the observer error caused by overestimation of the GF. We believe that planimetry would facilitate the comparison of results of leprosy histopathology for different centers and that it may be valuable in evaluating serial biopsies taken in the course of drug trials. Similar histometric techniques may prove useful in the assessment of other features seen in biopsies from leprosy patients.

SUMMARY

The proportion of the dermis occupied by granuloma in histological sections of skin biopsies from leprosy patients is known as the granuloma fraction. It can be estimated by direct microscopy on a scale of tenths using a low-power objective. We have compared this method with measurement of the granuloma fraction by planimetry, which gives comparable results but is much more accurate and precise. The implications of these findings for the objective assessment of the effects of treatment regimes on leprosy are discussed.

RESUMEN

La porción de la dermis ocupada por el granuloma en los cortes histológicos de las biopsias de piel de pacientes con lepra se conoce con el nombre de fracción granuloma. Esta puede medirse por microscopía directa usando bajos aumentos. En este trabajo, este método se comparó con el de planimetría, encontrándose que el último es mucho más exacto y preciso. Se discute la utilidad de los métodos de medición de la fracción granuloma en la cuantificación del efecto del tratamiento antileproso.

RÉSUMÉ

La fraction granulomateuse (granuloma fraction) est constituée par cette portion du derme qui est occupée par le granulome dans des coupes histologiques de biopsies cutanées chez les malades de la lèpre. On peut estimer l'importance de cette fraction en microscopie directe, en utilisant une échelle graduée en dixièmes, avec un objectif à faible résolution. On a comparé cette méthode avec la mesure de la fraction granulomateuse par planimétrie. Cette dernière méthode fournit des résultats comparables mais elle est plus précise et plus exacte. On discute des conséquences de ces observations dans le cadre d'une évaluation objective des effets de diverses posologies médicamenteuses dans la lèpre.

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REFERENCES

1. Beck, J. S. and Nordin, B. E. C. Histological assessment of osteoporosis by iliac crest biopsy. J. Pathol. Bacteriol. **80** (1960) 391–397.
2. Bradbury, S. Commercial image analyzers and the characterization of microscopical images. J. Microsc. **31** (1983) 203–210.
3. Brugel, G. Image analysis of microscopic prep-

arations. Meth. Achiev. Exp. Pathol. **11** (1984) 1–33.

4. RIDLEY, D. S. A logarithmic index of bacilli in biopsies. 2. Evaluation. Int. J. Lepr. **35** (1967) 187–193.
5. RIDLEY, D. S. *Skin Biopsy in Leprosy. Histological Interpretation and Clinical Applications.* Basle: Documenta Geigy, 1977.
6. RIDLEY, D. S. and HILSON, G. R. F. A logarithmic index of bacilli in biopsies. 1. Method. Int. J. Lepr. **35** (1967) 184–186.
7. ROBERTSON, A. J., BROWN, R. A., CREE, I. A., MACGILLIVRAY, J. B., SLIDDERS, W. and BECK, J. S. Prognostic value of measurement of elastosis in breast carcinoma. J. Clin. Pathol. **34** (1981) 738–743.