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Classification of leprosy cases under field conditions in Bangladesh. I. Usefulness of skin-smear examinations

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Summary In 2 non-governmental organization projects in Bangladesh 244 new leprosy patients were classified in the field according to clinical criteria. Skin smears were taken at 4 standardized sites and at the most active peripheral lesion, where a biopsy was also taken.

Comparison of the clinical field classification with the results of the skin smears and biopsies gives a sensitivity of 92·1% for the clinical criteria, but a specificity of only 41·3%. The skin-smear results, on the other hand, have a sensitivity of 88·4% and a specificity of 98·1%.

Thus, skin smears may contribute considerably to the operational classification of leprosy patients under field conditions.

Quality control of the peripheral laboratory is essential. Appropriate site selection for the smear taking will also contribute to increased performance. Analysis of the skin-smear results suggests that the policy of taking smears at standardized sites should be abandoned in favour of the earlobes and active peripheral lesions.

Introduction

According to WHO recommendations,[1] the operational classification of leprosy cases in paucibacillary (PB) and multibacillary (MB) patients should be based on the bacteriological index, whereby the presence of acid-fast bacilli (AFB) at any single site is the criterion used for classification as MB. However, the validity of skin smear results has often been questioned,[2–4] and many leprosy control programmes base their operational

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classification on clinical criteria.[3] These criteria differ from programme to programme, and their rationale is not always clear. The validity of the clinical criteria used in leprosy control programmes in Ethiopia[5] and Nepal[6] has been studied. The present study looks at the results of 2 programmes in Bangladesh: the Danish–Bangladesh Leprosy Mission project in Nilphamari and the Damien Foundation project in Jalchatra.

In the first part of the analysis, the usefulness of skin smears and their potential contribution to the classification of patients under field conditions is examined.

Materials and methods

From 1 March to 31 July 1993 all new cases diagnosed in Nilphamari (NIL) and Jalchatra (JAL) leprosy control projects were enrolled in the study. After a detailed clinical examination, the leprosy control assistant (LCA) drew a body chart, indicating skin lesions, skin infiltration, enlarged nerves, anesthesia and deformities. The results of sensory testing, voluntary muscle testing (VMT) and WHO disability grading were entered on the patient record by a physiotechnician, who also verified the nerve palpation.

Skin smears were taken at 4 standard sites (JAL: left earlobe, right forehead, left cheek, right elbow; NIL: right earlobe, left forehead, chin, left buttock (in males) or left thigh (in females), and at the biopsy site. In JAL, smears were taken by the LCA, in NIL by the laboratory technician. Smears were stained according to the Ziehl–Neelsen hot method, using sulphuric acid (20%) for the decoloration. The skin smears were read by the laboratory technician, and the bacteriological index (BI) according to the Ridley–Jopling scale was determined.

A punch biopsy of 5-mm diameter was taken by the physician at the most active area of the most peripheral active lesion, excluding the face. The biopsy was fixed in formalin 10% and sent to the leprosy laboratory of the Institute of Tropical Medicine, Antwerp, Belgium, where sections were stained using the Trichrome–Fite–Faracco technique.

The physician classified the patient clinically according to the Ridley–Jopling classification, and prescribed the MDT treatment regimen in function of the operational classification. The following criteria were used in the projects in order to arrive at the operational classification:

if > = 10 lesions: MB;
if < = 3 lesions: PB;
if 4 to 9 lesions:
  if > = 2 enlarged nerves: MB;
  if < = 1 enlarged nerve: PB;
whereby lesion = either skin lesion or enlarged nerve.

(If, afterwards, the skin-smear result of a patient classified as PB turns out to be positive, this patient is changed to MB.)

For the purpose of the present study, the operational classification was compared to the bacteriological classification based on biopsy and skin-smear results.

A patient is bacteriologically classified as MB if the biopsy result is either LL or BL or BB *or* if the BI of the biopsy is positive *or* if the BI of the skin smear is positive at any

single site. A patient is bacteriologically classified as PB if the biopsy result is either BT or TT or I *and* if the BI of the biopsy is negative *and* if the BI of the skin smears is negative.

Results

Between 1 March, and 31 July, 1993, a total of 244 leprosy patients were diagnosed in NIL and JAL. They were classified in 6 groups (Table 1). In all, 1 patient refused the biopsy, and the biopsies of 3 patients were impossible to cut or stain properly; 2 patients, treated as PB, had negative skin smears, and are included in group 6; 1 patient, treated as MB, also had negative skin smears, and is included in group 5; 1 patient, treated as MB, had positive skin smears and is included in group 1. Positive skin smears were found in 79 patients.

Standard skin-smear sites in NIL and JAL are not identical, but are sufficiently similar to allow the combination of the results of both projects. The BI values obtained at the 4 standard sites and at the biopsy site could vary considerably in the same patient. The difference (in BI units) between the highest and the lowest value was 0 in 7 patients and 1 in 29 patients (together constituting 45·6% of the smear positive patients), but it was 2 in 16 patients, 3 in 18 patients, 4 in 8 patients, and 5 in 1 patient, giving a mean variation (in BI units) between the highest and the lowest value of 1·92.

The bacterial yield of the various skin-smear sites is given in Table 2. Results for the

Table 1. Operational and bacteriological classification of the leprosy patients

		Operational classification[1]	
		MB	PB
Group	Bacteriological classification[2]		
1. Confirmed MB	MB	82	
2. Confirmed PB	PB		50
3. False PB	MB		7
4. Unconfirmed MB	PB	73	
5. Unconfirmed leprosy treated as MB	Unconfirmed	18	
6. Unconfirmed leprosy treated as PB	Unconfirmed		14
Total		173	71
		244	

[1] If > = 10 lesions: MB. If < = 3 lesions: PB. If 4 to 9 lesions: MB if > = 2 enlarged nerves; PB if < = 1 enlarged nerve (whereby lesion = either skin lesion or enlarged nerve).

[2] MB: either the biopsy result is LL or BL or BB, or the BI of the biopsy is positive, or the BI of the skin smear is positive at any 1 site.

PB: the biopsy result is BT or TT or I, and the BI of the biopsy is negative, and the BI of the skin smear is negative.

Unconfirmed: no evidence of leprosy found in the biopsy and all skin smears negative.

Table 2. Bacteriological yield of the various skin smear-sites in the skin-smear positive leprosy patients (total of 79 patients)

Skin-smear site	Earlobe (right or left	Forehead	Face (cheek or chin)	Limb (elbow, buttock or thigh)	Biopsy site	Earlobe + biopsy site taken together
Sum of the BI* values of all patients, at each of the sites	239	208	207	211	251	Not applicable
Mean BI, at each of the sites	3·0	2·6	2·6	2·7	3·2	3·1
frequency of highest scores:						
number of patients[1]	47	27	24	25	42	66
% of patients[2]	60%	35%	31%	32%	54%	84%

*BI, bacteriological index.
[1] Number of patients in whom a particular site presented the highest BI value (either as sole highest site, or as 1 of several sites with the highest BI value).
[2] Percentage of patients in whom a particular site presented the highest BI value.

earlobe plus the biopsy site taken together are also given. The BI found in the biopsy is compared to the BI found in the skin smear at the biopsy site in Table 3. A patient without a biopsy and a patient who was not smeared at this site are not included in this

Table 3. Difference in bacterial yield between biopsy and skin smear at the biopsy site. (Total of 87 patients: 1 patient without a biopsy result and 1 patient without a skin-smear result are not included)

Group	Difference (in BI units) between the BI in the biopsy (reference BI) and the BI in the skin smear taken at the biopsy site	Number of patients
Biopsy: BI positive	0	31
Skin smear: BI positive	−1	20
	+1	6
	−2	4
	+2	2
	−3	1
		Total: 64
Biopsy: BI positive	0	0
Skin smear: BI negative	−1	6
	−2	3
	−3	1
		Total: 10
Biopsy: BI negative	0	2
Skin smear: BI positive	+1	7
	+2	1
	+3	1
	+4	1
	+5	1
		Total: 13

Table 4. Bacteriological characteristics of MB patients with AFB in the biopsy present solely in the dermal nerves

Operational classification[1]	BI* in nerves	BI in skin smear (highest score)	Histopathological image	Number
MB	1+	0	I	1
		0	BT	4
	2+	0	BT	3
		1+	BT	3
		2+	BL	1
		4+	BT	1
	3+	0	BT	1
		3+	BT	3
PB	1+	0	BT	1
		1+	I	1
		2+	I	1

[1] See legend of Table 1 for definition of the operational classification categories.
* BI, bacteriological index.

comparison. Twenty patients presented a positive BI in the biopsy, but with the AFB present solely in the dermal nerve sections—10 of these patients had negative skin smears. The characteristics of these patients are summarized in Table 4.

There were 13 patients who had a positive skin-smear result while presenting a BT anatomopathological image with a negative BI in the biopsy.

Discussion

From a strictly bacteriological point of view, only those cases with an anatomopathological image of LL, BL or BB, or with a positive BI in the biopsy, or with a positive BI at any 1 skin-smear site, should be classified as MB.

Applying these criteria to the patients in Table 1, there are 89 MB cases in the study group. Of these, 82 were correctly identified in the field (a sensitivity of 92·1%). However, an additional 91 cases were also diagnosed as MB leprosy, thus bringing down the specificity to 41·3%. If only the cases confirmed as leprosy by the biopsy (groups 1–4) are taken into consideration, the sensitivity remains the same (92·1%) and the specificity is almost identical (50/123 = 40·7%).

If classification were based on the skin-smear results, 79 MB cases out of 89 would have been correctly identified (a sensitivity of 88·8%). Since all other cases were skin-smear negative, the specificity is 100%. The 10 MB cases which were missed all belong to the BT group with bacilli present solely in the dermal nerves—6 of these (see Table 4) presented a BI of 1+. Since the original cut-off point for MB classification[7] was a BI of 2+, it can be argued that these 6 cases would have been correctly treated if they had received a PB regimen. Of the remaining 4, 3 had a BI of 2+ in the nerves, and 1 of 3+. As *Mycobacterium leprae* is a nerve pathogen, the nerves could well be the primary site of multiplication. Thus, patients with a negative skin-smear result but many bacilli in the nerve should be considered as true MB which cannot be detected by smear

taking only. The incidence of such cases in the present study is 4/244, or 1·6%. Treatment of these patients with a PB regimen may result in an MB relapse. However, the chance of selecting drug-resistant bacilli after regular PB treatment would be exceedingly small.

The question that should be asked is whether the skin-smear negative patients were truly negative. Quality control of the performance of the laboratory technicians, done independently from the present study, had revealed that no positive slides (BI > =2) were missed, and that BI readings did not differ more than 1 unit from the control reading in 95% of the cases. This suggests that the smear reading results obtained in a well-functioning peripheral laboratory where regular quality control is performed, can be reliable. This has also been observed in other studies:[8] the peripheral laboratory results had a positive predictive value of 96·6% (253/262) and a negative predictive value of 82·2% (106/128), with 10 out of the 22 false negatives having a BI of 1+ at the reference laboratory.

Thus, it is safe to assume that all positive readings are truly positive, and if any errors are made, they will be made to the advantage of the patient. Nevertheless, it could be argued that the 13 cases with a positive skin smear but a negative BI in the BT biopsy constitute false positives. But closer analysis reveals that in 10 out of those 13 patients, a positive BI was found in at least 4 out of 5 skin-smear sites, making a reading error unlikely. Of the 3 remaining patients, 2 had a BI = 1+ at 2 sites, and 1 had a BI = 1+ at 1 site. If these 3 patients would be considered false positive, the sensitivity of the skin-smear examination would be 88·4% (76/86) and the specificity 98·1% (155/158).

These results are remarkably similar to a study of 204 patients in Senegal[9] who were classified according to BI and histopathological result. Out of 85 patients histopathologically classified as MB, 80 were correctly identified by the skin-smear examination (a sensitivity of 94%), while 6 smear positive patients were not confirmed by the biopsy (a specificity of 113/119 = 95%).

In a study in Nepal,[6] a computer simulation based on observations in 54 patients showed that 24 out of 30 patients classified as MB according to biopsy result were correctly identified by the skin-smear examination (sensitivity 80%) while 4 smear-positive patients had a PB biopsy result (specificity 19/23 = 83%). The lower performance in Nepal, as suggested by the authors, may be due to problems with the quality of smear examination.

It is always possible that bacilli in the skin smear are missed due to uneven distribution, especially in the case of a BI of 1+: when examining 100 microscopic fields, only 2·5% of the smear area is covered.[8] Bacilli in the biopsy may be missed because the biopsy is taken at the wrong spot or because the bacilli are concentrated in 1 specific area but the biopsy slices do not include that area, especially in the case of low density bacillary load.[10] In this connection, it is particularly interesting to compare the BI found in the biopsy with the BI found in the skin smear at the biopsy site (see Table 3). In 72 out of 87 patients (82·7%), the difference does not exceed 1 unit. In 9 patients (10·3%), the BI in the biopsy is 2 or 3 units higher than the BI in the skin smear at the biopsy site, but in 5 of these patients the bacilli in the biopsy were present only in the nerves. In 6 patients (6·9%), the BI is 2 or more units higher in the skin smear at the biopsy site than in the biopsy, while in 4 of these patients the biopsy actually has a negative BI. It has to be pointed out that in each of these 4 cases the BI is at least 2+ at each of the 5 smear sites. If we exclude clerical errors, of which we find no evidence, we

can only conclude that the discrepancy is due to an unfortunate selection of the biopsy site.

In all, 73 (80·2%) of the 91 patients with a negative skin smear classified in the field as MB presented a PB anatomopathological image. The remaining 18 showed no histological evidence of leprosy. Among the 64 smear-negative patients operationally classified as PB, 14 showed no signs of leprosy in the biopsy. All of these 32 unconfirmed cases presented clinical signs suggesting leprosy: anaesthetic skin lesions (24 patients), enlarged nerves (7 patients), grade 2 disability of both feet (1 patient). Again, the lack of histological evidence of leprosy may be due to an inappropriate selection of the biopsy site. It raises the question of reliability of diagnosis, but this falls outside the scope of the present article.[11] Whether the smear-negative cases without histological leprosy evidence are true leprosy cases has no direct bearing on the main issues under discussion: the false-negative cases and the MB overclassification. The 32 unconfirmed leprosy cases are discussed in more detail in the accompanying article.[12]

Both in NIL and JAL, smears are taken at standard sites, and the average BI is entered on the patient record. However, it was found that there can be considerable variation between the BIs at various sites. As a result, the average BI will often not reflect the bacillary status of the patient accurately.

This is mainly due to the policy of taking skin smears at standardized sites. Table 2 clearly shows that 3 out of 4 standard sites yield considerably less bacilli than the earlobe or the biopsy site. The earlobe and the biopsy site present the highest mean BI, and most often yield the highest BI in a patient. In fact, in 66 of the 79 smear-positive patients (84%), either the earlobe or the biopsy site, or both, yielded the highest BI. Indeed, many leprosy researchers recommend a minimum of either 2 earlobes and 1 active site or 1 earlobe and 2 active sites for smear taking.

If, for the group of patients in the present study, skin smears had only been taken at the earlobe and the biopsy site, 3 out of the 79 smear positive cases (3·8%) would have been missed: 1 patient with a BI = 1+ in the cheek only, and 2 patients with a BI = 1+ in the forehead only. On the other hand, if the smears had been limited to the standard sites, excluding the biopsy site, this would also have resulted in 3 missed cases: 2 with BI = 1+ at the biopsy site, and 1 with BI = 5+ at the biopsy site. This last patient had a biopsy result BL, BI = 4+, illustrating the importance of good site selection.

Conclusion

In spite of the low esteem in which skin-smear results are held in many programmes, the bacteriological performance in NIL and JAL is quite acceptable. In this context, the importance of quality control of the laboratory performance cannot be overstressed.

Relying solely on skin-smear results, 11·2% of MB cases (10 of 89) would have been missed. But more than 50% of the cases missed have a BI of 1+ in the dermal nerves only, and would probably respond satisfactorily to PB treatment. The operational classification strategy in the field missed 7·9% of the MB cases. But the price for this increased sensitivity is a specificity of only 41·3%. In order to treat 3 additional MB cases, 91 'false' MB's have to be treated as well. This MB overclassification puts a serious managerial and financial burden on the leprosy programme.

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