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A comparison of ML Flow serology and slit skin smears to assess the bacterial load in newly diagnosed leprosy patients in Brazil

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Summary

Introduction The ML Flow test is an immunochromatographic assay that detects IgM antibodies against *M. leprae*-specific anti-phenolic glycolipid I (PGL-I). In addition to slit skin smears stained by the Ziehl–Neelsen technique, it can be helpful in the operational classification of leprosy patients for treatment purposes.

Objective This work studied the relationship between antibody levels as detected by semi-quantitative ML Flow serologic test and bacterial load as quantified by slit skin smear.

Patients and methods 135 patients with newly detected leprosy at the reference service in Sanitary Dermatology in Brazil had slit skin smears (registered as bacillary index – BI) and an ML Flow test (registered qualitatively and semi-quantitatively) performed at admission. A logistic regression and agreement measures (kappa index) were calculated.

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Results Slit skin smears were positive in 35·9% of patients and 57% of patients were seropositive for PGL-1 antibodies. Among the seropositive patients, 41·6% had five or fewer skin lesions, and 65·8% had more than one peripheral nerve involved. Slit skin smears were positive in only three seronegative patients (5·6%), and negative in 41·9% of seropositive patients. Patients with a BI of 4+ had an OR of 3·3 for being seropositive in comparison to those with a low BI.

Conclusions There is a correlation between serologic test and slit skin smear results. Therefore, an ML Flow test may become a useful tool in the clinical classification of leprosy, besides slit skin smears, which require a proper laboratory infrastructure and experienced personnel.

Introduction

Classification of leprosy patients is important for treatment purposes. The World Health Organization (WHO)'s operational classification is based on the number of skin lesions:[1] paucibacillary (PB) patients have at most five skin lesions, and multibacillary (MB) patients more than five skin lesions. Whatever the clinical presentation, a case with detectable bacteria in a slit skin smear is always classified as MB.

Slit skin smear is currently the most important complementary examination in leprosy patients because, in demonstrating acid-fast bacilli, it can confirm the diagnosis of leprosy and the classification MB. However, a proper laboratory infrastructure and experienced personnel, not always available in primary health care centres, are required for its execution.

The ML Flow test is an immunochromatographic assay that detects *M. leprae*-specific anti-phenolic glycolipid I (PGL-I) IgM antibodies. It is easy to perform and can be used in primary health care centres because its performance does not require a specific infrastructure.[2]

A combination of clinical and laboratory criteria might be helpful in an operational classification of leprosy. In this context, the ML Flow test could be useful, since it is an alternative to a slit skin smear and is simpler to execute.[2]

This work studies the relationship between antibody levels as detected by ML Flow serology and bacterial load as quantified by slit skin smear to determine the bacillary index (BI) of slit skin smears.

Materials and Methods

This work is part of the study performed to evaluate the use of the ML Flow test as an additional serological tool to classify new leprosy patients for treatment purposes.[2] The Sanitary Dermatology Service in Hospital Eduardo de Menezes, Minas Gerais, Brazil, was the only centre that read ML Flow results semi-quantitatively.

PATIENTS

The study group consisted of 135 newly-diagnosed leprosy patients who visited the reference service in Sanitary Dermatology in the city of Belo Horizonte, Minas Gerais, Brazil, from November 2002 to March 2004. All newly-diagnosed patients that agreed to participate and signed the informed consent form were enrolled.

CLASSIFICATION

For field classification patients are classified based on the number of skin lesions (patients with six or more skin lesions are classified as MB) and number of nerves involved (patients with two or more nerves involved as detected by nerve palpation, are classified as MB irrespective of the number of skin lesions), in combination with the bacteriological index. All patients presenting positive slit skin smears are classified as MB, irrespective of the number of skin lesions or nerves involved. Disability level was determined according to neurologic evaluation of eyes, hands and feet: level 0 means no disability, level 1 means decreased sensitivity in one or more of the examined body parts, and level 2 means deformity in one or more of the body parts.

LABORATORY EXAMINATION

Slit skin smear

Skin smears from four sites were used: two from the earlobes, one from an elbow and one from a skin lesion. Whenever it was impossible to take a skin smear from a lesion, a smear from the other elbow was used instead. Smears were stained for AFB by Ziehl–Neelsen's (ZN) method, 100 oil-immersion fields were examined and the bacterial index (BI) was calculated according to logarithmic scale of Ridley (from 0 to 6).[3]

Serology

The ML Flow test was performed as described before[2] and the results recorded both qualitatively (positive or negative) and semi-quantitatively as 0 (seronegative), 1+, 2+, 3+ or 4+ according to colour intensity of the antigen band.

STATISTICAL ANALYSIS

Here we present a descriptive and exploratory study which associates six variables (sex, age, number of skin lesions, number of involved peripheral nerves, disability level at time of diagnosis, and field classification) to slit skin smear and ML Flow test results. In order to investigate the association between the variables mentioned above, a logistic regression was performed (univariate, multivariate and ordinal); significance cut-off level of 0·10 and 0·05 was used, respectively, for univariate and multivariate analysis. Concordance studies between serologic test and slit skin smear results, and between serologic test result and WHO classification of leprosy patients were done using kappa value, which was interpreted according to Landis and Koch criteria. Values of $P < 0{\cdot}05$ were considered statistically significant.

Results

The age of the patients studied ranged from 5 to 78 years (median age 44 years), and most patients were male (60·7%). Slit skin smears were positive in 35·9% of patients and 57% of patients were seropositive for PGL-1 antibodies. While 43·7% had six or more skin lesions (WHO classification), the field classification included 85·2% in the MB group; 49·6% of patients had no disability, 27·5% had level 1, and 22·9% had level 2 disabilities (Table 1).

Table 1. Descriptive statistics of the study population

Variable	*N*	%
Sex		
Female	53	39
Male	82	61
Level of disability		
Level 0	65	50
Level 1	36	27
Level 2	30	23
Slit skin smear		
Negative	82	64
Positive	46	36
ML Flow test (qualitative) results		
Negative	58	43
Positive	77	57
ML Flow test (semi-quantitative) results		
Negative	58	43
Positive 1+	17	13
Positive 2+	14	10
Positive 3+	9	7
Positive 4+	37	27
Number of skin lesions (WHO classification)		
$\leq$ 5 lesions (PB)	76	56
$\geq$ 6 lesions (MB)	59	44
Number of peripheral nerves involved		
$\leq$ 1 nerve	52	39
$\geq$ 1 nerve	81	61
Field classification		
Paucibacillary	20	15
Multibacillary	115	85

Disabilities were detected in 36·7% seronegative patients and 60·5% seropositive patients. Among seropositive patients 41·6% had five or fewer skin lesions and 65·8% had more than one peripheral nerve involved while among seronegative patients 42·1% had six or more lesions and 45·6% had no nerve involvement. Slit skin smear was positive in only three seronegative patients (5·6%), and negative in 41·9% of seropositive patients (Table 2).

The agreement between serology and slit skin smear results was 73·4% with a kappa value of 0·49. Among cases with negative slit skin smear, 37·8% (24·2% of all patients) were seropositive. Three patients (6·5% of cases with positive slit skin smears and 2·3% of the total) were seronegative (Table 3); all of them had BI $<$ 2.

The agreement between serological testing and the WHO classification was 65·9%, with kappa value of 0·33. According to WHO classification, 77 patients (57%) were MB cases, and 41·6% of them were seronegative. Among the PB cases, 24·1% were seropositive (Table 4).

Patients with six or more skin lesions (MB) (OR: 5·0) had five times the chance of being seropositive when compared to the patients with five or less skin lesions (PB). Patients with level 1 of disability had approximately four times (OR: 3·8) the chance of being seropositive in comparison with patients with level 0 of disability. Patients with positive slit skin smear had almost 24 times (OR: 23·6) the chance of being seropositive when compared to patients with negative slit skin smear (Table 5).

Patients with six or more skin lesions (MB) had 5·6 times the chance of being seropositive category 4+ (highest score) when compared to those with five or less skin lesions (PB).

Table 2. Distribution of studied variables according to ML Flow test result

	ML Flow negative		ML Flow positive	
Variable	*N*	%	*N*	%
Sex				
Female	25	43	28	36
Male	33	57	49	64
Level of disability				
Level 0	35	64	30	39
Level 1	8	14	28	37
Level 2	12	22	18	24
Number of skin lesions				
≤ 5 lesions	44	76	32	42
≥ 6 lesions	14	24	45	58
Number of peripheral nerves involved				
≤ 1 nerve	26	46	26	34
> 1 nerve	31	54	50	66
Slit skin smear				
Negative	51	94	31	42
Positive	3	6	43	58
Field classification				
Paucibacillary	20	35	0	0
Multibacillary	38	65	77	100

Patients with level 1 of disability had 3·6 times the chance of being seropositive category (4+) when compared to cases with level 0 of disability (Table 6).

The BI tended to be higher in patients with a higher score in the ML Flow test result, as shown in Figure 1.

In adjustment by ordinal logistic regression model to analyse ML Flow test quantitative results, it was observed that patients with a high BI had more than three times (OR: 3·3) the chance of being seropositive 4+ in comparison to those who had a low BI.

Discussion and Conclusions

As PB and MB patients have different risks for reactions, nerve function impairment, relapse and disability, distinguishing between patients with low and high bacterial loads is important

Table 3. Concordance between ML Flow test and slit skin smear results

	Bacillary index (BI)							Total	
	0	0·1–1·0	1·1–2·0	2·1–3·0	3·1–4·0	4·1–5·0	5·1–6·0	*N*	%
ML Flow									
Negative	52	3	–	–	–	–	–	55	43
POS 1+	15	–	1	–	–	–	–	16	12
POS 2+	8	–	1	–	1	–	–	10	8
POS 3+	4	–	2	–	2	1	1	10	8
POS 4+	4	1	3	5	16	6	2	37	29
Total	83	4	7	5	19	7	3	128	100

Agreement = 73·4; kappa value = 0·49 (moderate concordance).

Table 4. Concordance between ML Flow test and WHO classification

	WHO classification					
	Paucibacillary		Multibacillary		Total	
	N	%	*N*	%	*N*	%
ML Flow						
Negative	23	59	35	36	58	43
POS 1+	10	25	7	7	17	13
POS 2+	1	3	13	14	14	10
POS 3+	4	10	5	5	9	7
POS 4+	1	3	36	38	37	27
Total	39	100	96	100	135	100

Agreement = 65·9%; kappa value = 0·33 (slight concordance).

for appropriate clinical management.[2] Therefore, even if the unified multi drug therapy for all leprosy patients proves to be feasible, health care providers will face the question 'is this patient a PB or an MB case?' Some studies warn that WHO classification, based solely on the number of skin lesions could lead to misclassification, and therefore rigid technical guidelines could make some primary health care professionals feel themselves unable to properly classify the patients.[2,4]

Using slit skin smears as the gold standard, WHO's criterion of five or more skin lesions is 89·6% sensitive and 83·8% specific, respectively.[5] Norman *et al.* when studying 5439 newly detected leprosy cases reported similar results: the field criteria were 88·6% sensitive and 86·7% specific.[4] The WHO classification system is an adequate operational tool, although poorly adhered to in practice as can be seen in our 'field classification' where health workers tend to over-classify patients as MB and probably very difficult to implement appropriately.[2]

Health care providers tend to over-classify patients as MB in order to prevent future problems such as reactions and relapses. Buhrer *et al.* observed that despite the fact that the official Brazilian operational recommendation is based only on the number of skin lesions

Table 5. Multivariate analysis between qualitative ML Flow test result and the studied variables

	(+) ML Flow			
Variables	*N* (%)	OR	95% CI	*P*-value
Number of skin lesions				
≤ 5 lesions	32 (42)	1·0	–	<0·001
> 5 lesions	45 (58)	4·95	2·1–11·4	
Level of disability				
Level 0	30 (40)	1·0	–	0·011
Level 1	28 (37)	3·82	1·4–10·1	0·007
Level 2	18 (23)	1·04	0·4–2·8	0·936
Slit skin smear				
Negative	31 (42)	1·0	–	<0·001
Positive	43 (58)	23·6	6·7–82·5	

OR, odds ratio; CI, confidence interval.

Table 6. Multivariate analysis between semi-quantitative ML Flow test result and the studied variables

Variables	OR	95% CI	*P*-value
Number of skin lesions			
≤ 5 lesions	1·0	–	< 0·001
> 5 lesions	0·18	0·09–0·38	
Level of disability			
Level 0	1·0	–	
Level 1	0·27	0·12–0·63	0·002
Level 2	0·81	0·34–1·94	0·638
Field classification			
Paucibacillary	1·0	–	0·013
Multibacillary	0·08	0·01–0·58	

OR, odds ratio; CI, confidence interval.

(and BI results when available), enlarged nerves were also taken into account in Minas Gerais for classification of patients for treatment purposes.[2] Similarly, in our hospital, while 53·6% of patients had five or less skin lesions and would be classified as PB, a much higher proportion (85·2%) were treated as MB.

High percentages of MB patients were also reported in other studies conducted in Minas Gerais. A study performed in the same hospital in 2003 detected a preponderance of MB cases (99·3%).[6]

The percentage of seropositivity found in our study population (57%) was higher than that found by Bührer-Sékula *et al*.[2] As may be expected in a reference centre, a larger number of leprosy patients with complications, most of them MB, are seen compared to primary health care centres.

Many studies have shown that anti-PGL-I antibody levels correlate with the bacterial load in leprosy patients (reviewed in 7). Most MB cases in our study present high IgM anti-PGL-I levels, in contrast to PB patients, who are usually seronegative; the concentration of this immunoglobulin often decreases with adequate treatment.[5]

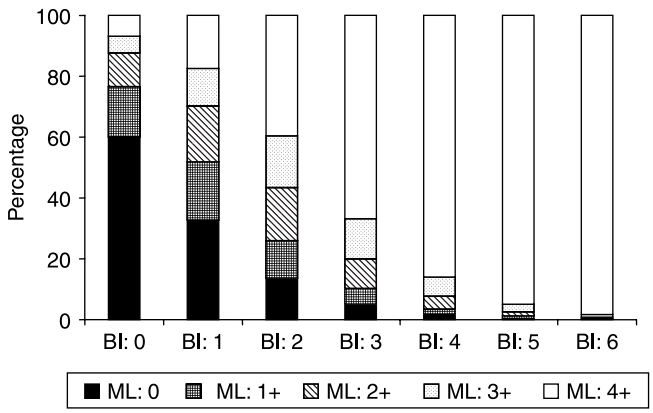

Figure 1. Percentage positivity of each semi-quantitative ML Flow test result level, for each BI value. BI: bacillary index according to logarithmic scale of Ridley (from 0 to 6). ML: ML Flow semi-quantitative results, from 0 (seronegative) through, 1+ , 2+ , 3+ and 4+ according to colour intensity of the antigen band.

In the present work, negative slit skin smears were found in 64·1% of patients. Fifty-seven percent of all patients with positive slit skin smears had BIs ranging from 3·0 to 4·9. When comparing slit skin smears to ML Flow test results we observed a moderate concordance (kappa index = 0·49) (Table 3). Among the cases with a negative slit skin smear (BI = 0), 37·8% were seropositive. These seropositive patients would be treated as PB patients if slit skin smear and lesion counting were the criteria used for classification. By contrast, serology was positive in 93·5% of all patients with positive slit skin smears (Table 4). Similarly, Bührer-Sékula *et al.*[2] reported positive ML Flow results in 97·4% of MB patients and in 97·8% of cases with BI $\geq$ 2. It is known that a slit skin smear is not very sensitive at detecting low bacterial loads (the BI 1+). The result of slit skin smear technique can only be as good as the technique or the skill and patience of the microscopist or of the well-known phenomenon of there being more bacilli in deep punch skin biopsies when compared with slit skin smears. Our results are an indication that the ML Flow might smoothly bridge this gap.

Indeed, the BI tended to be higher in patients with a higher score in the ML Flow test. Patients with BI above 2+ had a 3·3 times higher chance of having a high positive 4+ serologic test than those who had a low BI. In addition, multivariate logistic regression analysis revealed an association between seropositivity of the ML Flow test and the number of skin lesions higher than five, level 1 disability and positive slit skin smears.

There is a correlation between serologic test results and slit skin smear results and therefore the ML Flow test may become a useful tool in the clinical classification of leprosy, particularly in primary health care centres, in which a proper laboratorial infrastructure and experienced personnel required to perform slit skin smears are not usually available. In everyday practice, the ML Flow test will be at least as reliable as counting lesions, because it is a more objective measure and will give the health worker increased confidence in the classification of especially PB leprosy.[2]

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Authors' Contributions

Sandra Lyon wrote the manuscript. Rozana Castorina da Silva and Silvia Helena Lyon supervised the raw data collection. Ana Claudia Lyon analysed the data and helped to prepare the manuscript. Maria Aparecida de Faria Grossi and Manoel O.C. Rocha participated in the design, and supervised the study. Samira Bührer-Sékula participated in the design of the study, trained the participants, supervised data analysis and checked the manuscript. All authors participated in the interpretation of data, read and approved the final manuscript.

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