Association of the ML Flow serological test with slit skin smear

Associação do teste sorológico ML Flow com a baciloscopia

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ABSTRACT

A descriptive, exploratory study was conducted analyzing the association of covariables in the results of the ML Flow serological test and slit skin smear. A total of 60 leprosy cases diagnosed at the state Sanitary Dermatology Referral Center were investigated. Slit skin smear samples were collected from four sites and the results were expressed by the bacillary index. ML Flow was registered in both qualitative and semi-quantitative terms. Cohen's kappa coefficient was used to study the agreement with Landis and Koch's observer criteria for interpretation. For statistical analysis, the logistic regression model and Kruskal-Wallis test were used. ML Flow showed a strong association with slit skin smear results, since a gradual increase in BI was accompanied by a semi-quantitative rise in antibody levels measured by ML Flow, with 100% positivity in cases presenting a positive slit skin smear. Given its strong correlation to slit skin smear, the results of this study provide evidence that the ML Flow test could be a valuable auxiliary tool in the classification and treatment of leprosy patients.

Key-words: Leprosy. Serological tests. Slit skin smear. ML Flow.

RESUMO

Realizou-se estudo descritivo e exploratório relacionando as covariáveis aos resultados do teste sorológico ML Flow e baciloscopia. Foram estudados 60 casos novos de hanseníase diagnosticados no Centro de Referência em Dermatologia Sanitária. Para a baciloscopia, foi utilizada a coleta de esfregaço dérmico em quatro sítios, sendo o resultado expresso pelo índice bacilocópico. O ML Flow foi registrado de modo qualitativo e semi-quantitativo. Para o estudo da concordância, foi utilizado o índice de Kappa e, para sua interpretação, os critérios de Landis e Koch. Para análise estatística foram realizados a regressão logística e o teste de Kruskal-Wallis. O ML Flow mostrou forte associação com a baciloscopia, observou-se que o aumento gradativo do índice baciloscópico foi acompanhado pelo aumento semi-quantitativo dos níveis de anticorpos medidos pelo ML Flow, tendo sido positivo em 100% dos casos com baciloscopia positiva. Os resultados deste estudo evidenciaram que o ML Flow, por estar fortemente correlacionado à bacilocopia, poderá tornar-se um valioso instrumento auxiliar na classificação e alocação dos pacientes para fins de tratamento.

Palavras-chaves: Hanseníase. Testes sorológicos. Baciloscopia. ML Flow.

Leprosy (Hansen's disease) is an infectious disease caused by *Mycobacterium leprae* and its diagnosis is based on the identification of classic symptoms. It is classified as paucibacillary (PB) or multibacillary (MB) for treatment purposes[2].

The slit skin smear is the most important laboratorial test used to identify the causal agent, although it is negative for the PB forms and in some MB cases. In addition, laboratory infrastructure and trained professionals are required for its proper execution and these conditions are often absent in primary healthcare centers[2].

The phenolic glycolipid-1 (PGL-1) antigen is *Mycobacterium leprae*-specific and leads to the formation of IgG and IgM antibodies. Correlation exists between IgM titers, leprosy clinical forms and disease activity[1]. In the lepromatous leprosy, high levels of anti-PGL-1 have been obtained that tend to decrease with specific treatment[3].

ML Flow is an immunochromatographic test that detects IgM antibodies and can assist in the classification of PB and MB leprosy patients by detecting the patient's bacterial load[3,6]. It is a simple, low-cost and easily-conducted test that does not require a laboratory[3] and can be used routinely in health centers, including primary care units[6].

For treatment purposes, the classification of leprosy patients should be based on the number of bacilli present in the body[5]. The relation between antibody levels and the bacteriological index (BI) indicates that serology can be used as an alternate technique for patient classification[1,7,8].

The combination of clinical and laboratory criteria, more specifically those of serological tests to detect specific antibodies against the phenolic glycolipids of *Mycobacterium leprae,* may be used as an auxiliary test for the correct classification of leprosy patients[6]. Previous studies have shown that the results of ML Flow serological test to detect IgM antibodies correlate with bacterial load and can be used as a tool in the classification of the clinical forms of leprosy[7,8].

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The aim of this work was to study the relation between antibody levels detected by the ML Flow serological test and the bacterial load, as determined by the bacilloscopic index (BI).

CASUISTIC AND METHODS

The study population consisted of 60 new leprosy cases diagnosed at the dermatology clinic of the Eduardo de Menezes Hospital or referred to this clinic during the period of March to December 2006. The research subjects volunteered to take the ML Flow serological test after signing a free informed consent form, as per the document emitted by the Research Ethics Committee of the Eduardo de Menezes Hospital on 12 April 2006.

The 60 patients were classified according to the World Health Organization (WHO) operational guidelines, considering the number of skin lesions. For the slit skin smear, the BI was determined through the collection of samples taken from four sites: a skin lesion, the opposite-side elbow and earlobes and scored according to Ridley's logarithmic scale, ranging from zero to six. The ML Flow test was registered qualitatively (positive or negative) and semi-quantitatively (zero, 1+, 2+, 3+ or 4+) according to Bührer-Sékula *et al*[3]. Data were collected on the patient information form for posterior analysis included identification data, number of skin lesions, number of thickened nerves, slit skin smear results and ML Flow results.

Statistical analysis of the data was performed using the ordinal logistic regression model[9] and the Kruskal-Wallis test[10]. For the agreement analysis, Cohen's kappa coefficient was used, as well as Landis and Koch's interpretation criteria.

RESULTS

Patient age varied from 10 to 78 years with an average of 46.5 years. The number of skin lesions varied from 0 to 20 and thickened nerves from 0 to 8. The average BI was 1.2 with a standard deviation of 1,692 (**Table 1**).

Seropositivity occurred in 70% of patients, while the slit skin smear was positive in 40% (**Table 2**). The agreement observed between the serological results and slit skin smear was 70%, which is considered moderate (Kappa = 0.44), according to the Landis and Koch criteria. Observation revealed that 18 patients, 50% of the cases with a negative slit skin smear, were seropositive. Among patients with a positive slit skin smear, all were ML Flow-positive.

TABLE 1

Descriptive statistics of the 60 new leprosy cases treated at Eduardo de Menezes Hospital. Brazil. 2006.

Variable	Number	Min	Max	Mean	Standart deviation
Age	60	10	78	46.5	14.46
Number of cutaneous lesions	60	0	20	2.7	4.153
Number of nerves affected	60	0	8	0.57	1.661
Bacillary index	60	0	5	1.2	1.692

TABLE 2

Agreement between slit skin smear and the ML Flow serologic test of the 60 new leprosy cases treated at Eduardo de Menezes Hospital, Brazil, 2006.

		Slit skin smear					
		positive		negative		total	
		nº	%	nº	%	nº	%
ML Flow	Positive	24	100.0	18	49.0	42	70.0
	Negative	-	-	18	51.0	18	30.0
Total		24	100.0	36	100.0	60	100.0

kappa coefficient = 0.44, p-value = <0.001

Table 3 presents the multiple analyses of the factors associated with the seropositivity of the ML Flow test, slit skin smear and the number of cutaneous lesions. In the interpretation of this model, patients presenting a positive slit skin smear had approximately a 19-fold greater chance of testing positive on the ML Flow (OR: 19.38) compared to a patient with a negative slit skin smear result. Patient with 6 or more skin lesions had a 6-fold greater chance (OR: 6.04) of a positive ML Flow compared to patients with 5 lesions or less.

The categorized BI was associated with the serological levels of the ML Flow, as shown in **Table 4** and **Figure 1**. Among the patients studied, 60% showed a negative BI and 28.3% presented a BI of 2 or higher, with 18.3% of cases within the BI range of 3.0 to 4.9. Observation also showed that the slit skin smear results were associated with the ML Flow test results.

TABLE 3

Multiple analysis of the factors associated to seropositivity in the ML Flow test of 60 new leprosy cases treated at the Eduardo de Menezes Hospital. Brazil.2006.

Variables	ML Flow (+)		Odds	CI (95%)	P-value
	nº	%	ratio		
Slit skin smear					
negative	18	42.9	1.0	-	0.007
positive	24	57.1	19.38	(2.28; 164.96)	
Number of skin lesions					
≤ 5 lesions	23	54.8	1.0		
≥ 6 lesions	19	45.2	6.04	(1.11; 32.95)	0.020

CI: confidence intervals.

TABLE 4

Distribution of ML Flow results by BI in 60 new leprosy cases treated at Eduardo de Menezes Hospital. 2006.

Bacillary		ML Flow test					Total	
Index		0	+1	+2	+3	+4	nº	%
0.0		18	9	2	5	2	36	60.0
0.1 – 0.9		0	1	1	0	1	3	5.0
1.0 – 1.9		0	1	2	0	1	4	6.7
2.0 – 2.9		0	0	0	1	3	4	6.7
3.0 – 3.9		0	0	0	1	4	5	8.3
4.0 – 4.9		0	1	0	0	5	6	10.0
5.0 – 5.9		0	0	1	0	1	2	3.3
6.0		0	0	0	0	0	0	0
Total	nº	18	12	6	7	17		
	%	30	20	10	11.7	28.3	60	100.0

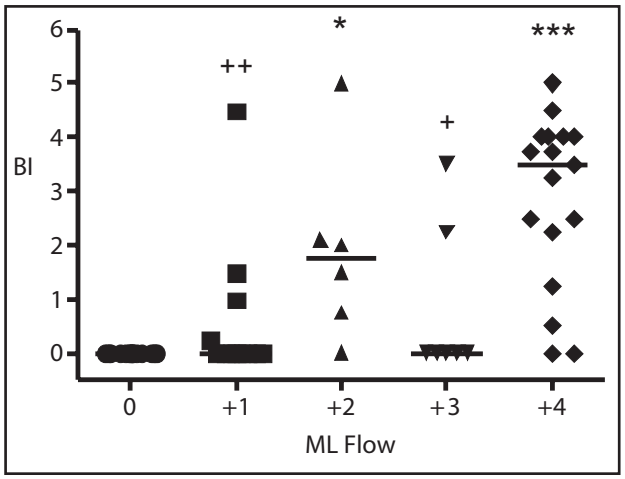

FIGURE 1

Results of the bacillary index according to the semi-quantitative results of the ML Flow test of new leprosy cases treated at Eduardo de Menezes Hospital, Brazil, 2006. 0: N=18; +1: N=12; +2: N=6; +3: N=7; +4: N=17. Statistical analysis was conducted using the Kruskal-Wallis test, followed by Dunn's post-hoc test for multiple comparisons. *p<0.05; ***p<0.0001 (vs. 0); +p<0.05; ++p<0.01 (vs. +4).

Patients that presented a high BI had a 19-fold chance of presenting a ML Flow serological test in the highest category (4+) compared to those patients with lower BI values (Table 3). Even patients with a low BI can present a high response to the ML Flow test (**Table 4** and **Figure 1**).

Figure 1 shows the association of semi-quantitative results with ML Flow and BI, including the Kruskal-Wallis statistical analysis.

DISCUSSION

Seropositivity in this study was higher (70%) than that witnessed by Lyon (57%)[7 8] and Grossi (50.8%)[6], and higher than from Nepal (35.6%)[4] and Nigeria (62.9%)[4]. These studies demonstrate that the level of antibodies specific to the phenolic glycolipid antigens of *Mycobacterium leprae* correlate with the bacterial load of leprosy patients[4 6 7 8]. The majority of patients classified as MB have high levels of anti-PGL-1 IgM antibodies, as opposed to those classified as PB, who are generally seronegative. The level of these antibodies is directly correlated to the quantity of *Mycobacterium leprae* in patients and diminishes throughout the treatment period[5]. In the current study, a moderate agreement (kappa: 0.44) was observed between slit skin smear and ML Flow results (**Table 2**); similar results were obtained by Grossi (Kappa: 0.48)[6] and Lyon (Kappa: 0.49)[7 8]. It should be emphasized that 18 (50%) of the patients who presented a negative slit skin smear were seropositive, meaning that they would be classified as PB if slit skin smear were used as the only criterion for classification. This fact suggests that a serological test may be more sensitive than the slit skin smear for the detection of true MB cases. In contrast, serology was positive in 100% of patients with a positive slit skin smear (**Table 4**). In the study by Bührer-Sékula *et al*[3], ML Flow was positive in 97.4% of MB cases and in 97.8% of patients with a BI ≥ 2.

The association between BI and ML Flow indicated that as the BI increases, the results from the serological test also rise, as previously demonstrated by Lyon (2005)[7 8].

This study provides evidence of a strong association between the ML Flow serological test and slit skin smear. It also showed that the ML Flow test was capable of detecting seropositivity in half of the patients showing a negative BI, which is of great importance, principally for professionals working in primary healthcare units who would be more confident in classifying leprosy patients, given that their results are closely related to field observations.

CONCLUSION

A statistically significant association was established between the results of the ML Flow serological test and slit skin smear. The study showed that the incorporation of the ML Flow test could be an auxiliary tool in the classification of leprosy patients for treatment purposes in an important number of cases. This may prevent the possibility of insufficient treatment in the case of clinically-classified PB cases with a positive serology and excessive treatment in the case of patients diagnosed as MB who are seronegative.

ACKNOWLEDGEMENTS

The authors would like to thank the State Health Secretariat of Minas Gerais (Belo Horizonte, Brazil) and KIT Biomedical Research Department (Amsterdam, the Netherlands) for their technical support and Netherlands Leprosy Relief (Amsterdam, Netherlands) for financial backing. The NT-P-BSA used in the production of the ML Flow serological test was kindly provided by Dr. Fujiwara of the Institute for Natural Science, Nara University, Nara, Japan.

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