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Use of the ML-Flow test as a tool in classifying and treating leprosy *

Uso do teste ML-Flow como auxiliar na classificação e tratamento da hanseníase

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Abstract: BACKGROUND: The treatment of leprosy is defined by the classification of patients as paucibacillary (PB) or multibacillary (MB). The WHO (World Health Organization) classifies patients according to the number of lesions, but Ridley-Jopling (R & J) also uses complementary exams, which are difficult to use outside reference services. In 2003, a test called ML-Flow, an alternative to Elisa serology, was developed to help classify patients as PB or MB and decide about their treatment.

OBJECTIVES: To assess the agreement between the ML-Flow test and slit skin smears, already largely used for MB detection, and to observe the efficacy of the ML-Flow test in the field.

MATERIAL AND METHODS: A retrospective study evaluating the medical records of 55 patients who had not undergone previous treatment, diagnosed as PB or MB according to R & J and subjected to slit skin smears and the ML-Flow test.

RESULTS: In MB patients, slit skin smears were positive in 80% of the cases, the ML-flow was positive in 82.5%. Among PB patients, the ML-Flow was positive in 37.5% and slit skin smears were negative in 100% of the cases. The agreement between skin smear and ML-Flow results was 87.5%, with a kappa value of 0.59, p <0.001.

CONCLUSION: No laboratory test is 100% sensitive and specific for the correct classification of all forms of leprosy. The ML-Flow test is faster, easier to use, and less invasive than slit skin smears and therefore may be useful when making therapeutic decisions in areas of difficult access to reference services.

Keywords: Classification; Leprosy; World Health Organization

Resumo: FUNDAMENTOS: O tratamento da hanseníase é definido pela classificação de pacientes em paucibacilares (PB) e multibacilares (MB). A OMS (Organização Mundial de Saúde) classifica os doentes de acordo com o número de lesões, mas Ridley-Jopling (R&J) utiliza também exames complementares, porém é de difícil utilização fora dos serviços de referência. Em 2003 foi desenvolvido um teste denominado ML-Flow, uma alternativa à sorologia por ELISA para auxiliar na classificação de pacientes em PB e MB e auxiliar na decisão terapêutica.

OBJETIVOS: Observar a concordância entre o teste de ML-Flow e baciloscopia de linfa, exame já consagrado para detecção de MB. Analisar a utilidade do teste de ML-Flow em campo.

MATERIAL E MÉTODOS: Estudo retrospectivo avaliando prontuário de 55 pacientes virgens de tratamento, diagnosticados como PB ou MB por R&J. Submetidos à baciloscopia e ao teste de ML-Flow.

RESULTADOS: Nos MB, a baciloscopia foi positiva em 80% dos casos, o ML-flow foi positivo em 82,5%. Entre os PB, o ML-Flow foi positivo em 37,5% e a baciloscopia do esfregaço foi negativa em 100% dos casos. A concordância entre os resultados da baciloscopia do esfregaço e ML-Flow foi de 87,5%, kappa=0,59, p<0,001.

CONCLUSÃO: Nenhum teste laboratorial é 100% sensível e específico para a correta classificação de todas as formas de hanseníase. O ML-Flow é um teste rápido, de fácil manuseio em campo, menos invasivo que a baciloscopia podendo ser útil para auxiliar na decisão terapêutica em locais de difícil acesso a serviços de referência.

Palavras-chave: Classificação; Hanseníase; Organização Mundial da Saúde

INTRODUCTION

Leprosy is a chronic infection caused by *M. leprae* that primarily affects the skin and peripheral nerves; its clinical aspects are varied and often difficult to recognize[1]. It particularly affects the less-favored population in countries where the disease is endemic[2]. Its diagnosis is primarily clinical, based on the presence of a painful or possibly tactile hypopigmented or reddish lesion with loss of thermal sensation, with or without thickening of peripheral nerves with loss of sensation at the site of innervation[3].

Most patients can be diagnosed based on the presence of an anesthetic lesion, but 30%, including several multibacillary patients, do not present this clinical manifestation[4], which complicates the diagnosis and makes breaking the transmission chain difficult.

It is very important to classify patients into paucibacillary (PB) and multibacillary (MB), since treatment differs for each of the two groups.

Methods of classification of the clinical forms of leprosy have changed significantly over the years. In 1953, the Madrid classification was developed, which was used in field evaluation until recently. It divided PB patients into Indeterminate – MHI (early form of the disease) and Tuberculoid – MHT (pole resistant), and MB patients into Borderline or Dimorphous – MHD (partial immunity) and Lepromatous or Virchowian – MHV (virtually anergic).

In 1962 and 1966, Ridley and Jopling (R & J) proposed a classification of leprosy into five groups based on clinical, histopathological and immunologic criteria, which is still used in many reference centers and especially by researchers[5].

In 1971, Ridley, after reviewing the 5-group classification, formulated the classification "5 of 7 groups," where he recognizes two polar and immunologically stable types, known as polar Tuberculoid (TTp) and polar Lepromatous (LLp), and the interpolar immunologically unstable types, called secondary Tuberculoid (TI or Ts), Borderline-tuberculoid (BT), Borderline-borderline (BB), Borderline-lepromatous (BL) and subpolar Lepromatous (LI or LLs).[6,7]

Since field use of this classification is impracticable, in 1982, the WHO (World Health Organization) defined Indeterminate, TT and BT with bacteriological index below 2 as PB patients, and the remaining ones[8] as MB, despite knowing that often direct smears for bacilli performed in the field were of low quality or not available[9].

For this reason, in 1995, the WHO proposed separating PB from MB according to the number of lesions, and those who had up to 5 lesions were classified as PB, while those with 6 or more lesions were classified as MB.[10]

Studies show that the operational classification proposed by the WHO leads to some patients being inadequately treated and predisposed to having reactions for an indefinite period of time, which can lead to reactivation of the disease or even secondary resistance.[11,12]

Laboratory tests (histopathology, Mitsuda reaction, slit skin smear and serology) may be used to help in the correct classification of these patients, when available, especially in reference centers. However, they are not available in most health centers.

In 2003, a method called ML Flow, an alternative to ELISA (serology) for detection of anti-PGL-1 IgM antibodies, was described by Bührer Sékula *et al.* It is not a method for diagnosis, but an examination to classify patients as PB or MB and to help decide on their therapy. It does not appear to cross-react with other mycobacterioses.[13]

TABLE 1: Comparison between the classification based on number of skin lesions, results of the ML Flow test, and slit skin smears

Classification based on number of lesions (WHO)	Confirmatory tests				
	ML Flow	Slit skin smear	N	%	
PB	POS	NEG	3	37,5	
	NEG	NEG	5	62,5	PB Confirmado*
Total			8	100	
MB	POS	POS	30	75,0	MB confirmado*
	NEG	POS	3	7,5	MB confirmado*
	POS	NEG	2	5,0	
	NEG	NEG	5	12,5	
Total			40	100	

* = Based on the positivity of slit skin smears. PB = paucibacillary, MB = multibacillary.

OBJECTIVES

Assess the agreement between the ML-Flow test and slit skin smears.

Observe the field efficacy of the ML-Flow test to help decide on the therapy for patients with leprosy.

MATERIAL AND METHODS

In a retrospective study, we evaluated the medical records of 55 patients with leprosy diagnosed between January 2004 and December 2007 - who had not undergone any previous treatment - at the Lauro de Souza Lima Institute. The patients' age ranged from 5 to 83 years, 36 were male and 19 were female, all of them from the State of Sao Paulo. We excluded cases from other states due to difficulty concerning booking appointments for evaluation of examinations and clinical follow-up.

The cases were included when analyzed according to initial clinical criteria and after completion of the following examinations: histopathology analyzing histological aspect and bacterial index (BI), Mitsuda reaction, occurrence of borderline reactions or erythema nodosum during evolution (for a follow-up period of five years on average), and slit skin smear (in lesions, when present, and in index-points otherwise), all of which conducted at the institution. (Ridley & Jopling and other complementary examinations). We considered MHT and MHI as PB and MHD and MHV as MB[14].

Next, they were all submitted to the ML-Flow test, which consists of an immunological test based on a nitrocellulose strip that features on one side a surface containing IgM antibodies labeled with gold and on the other side an absorbing surface. A semi-synthetic trisaccharide similar to PGL-1, bound to human albumin, is used as an antigen in a 1 mm line on the strip surface. Parallel to this line, a human IgM conjugate is used as control. A sample of blood or serum is placed in the sample well and carried with the fluid of the sample. The reagent binds to the IgM in the sample. When the antibody is specific, it binds to the antigen and a red line appears in the test area. Otherwise, only the control line shows positive. The test is considered negative when a line is not formed or when it is pale. The test is considered positive and graded from 1 to 4 + according to the stain intensity in the test line[13].

RESULTS

Table 1 shows the clinical classification, results of slit skin smears and the ML-Flow test of patients who met the criteria for inclusion in this study. The ML-Flow was positive in 82.5% of the MB patients and the slit skin smears, in 80%. Among the PB, the ML-Flow was positive in 37.5% of the patients, while slit skin smears were negative in 100% of the cases.

Table 2 shows a concordance between slit skin smears and the ML-Flow in MB patients of 87.5%, a kappa value of 0.59, p <0.001.

DISCUSSION

Slit skin smear is the test usually performed to confirm the operational diagnosis of leprosy. It helps to distinguish between PB and MB. The test requires, however, trained personnel for sample collection and reading and it is relatively invasive[15].

It has high specificity but low sensitivity due to errors in collection, fixation and reading, usually being negative in many BT patients and always negative in the pure neural cases - borderline. In these two latter situations, slit skin smears are negative, that is, an operational classification based solely on this test would be PB, but histopathology shows rich bacterial load within the dermal nerve network or peripheral sensory nerves[12]. In 2003, Gallo et al. [16] observed a concordance between the clinical method of number of lesions (WHO) and the results of slit skin smears of 83.8%, with a relative test sensitivity of 89.6%, relative specificity of 89.6%, positive predictive value of 95.1% and negative predictive value of 69.5%. However, in 2004, Crippa et al. [17] found a sensitivity of 73.6% for slit skin smears compared to the physical examination, specificity of 85.6%, positive predictive value of 66.8% and negative predictive value of 89.1%.

Slit skin smears were positive in 33 of the MB cases subjected to the test in this series (82.5%) and negative in all PB cases (100%) (Table 1).

Antibodies to PGL-I, mainly IgM, are considered specific for *M. leprae* and are found in patients with leprosy, but may also occur at low levels in exposed people. The antibodies do not offer protection and indicate infection with *M. leprae*[1]. The vast majority of PB patients are seronegative, and the majority of MB patients are seropositive[13].

Anti-PGL-I ELISA is a method for detection of these antibodies and is used to help in the operational classification, detection of recurrence and of individuals with a higher risk of developing leprosy, and patient follow-up. However, it requires trained personnel, specific material and at least one day for obtaining the results, and the material used needs to be stored in a refrigerated place.[1,4]

The ML-Flow test, another method for detecting antibodies, is easy and simple to perform. It does not require special equipment or refrigeration, and the result can be obtained in 5 to 10 minutes.[15]

In 2003, Bührer-Sékula et al.[4,18] observed a sensitivity of 97.4% for multibacillary and 40% for paucibacillary; the test specificity was 90.2%. The pres-

TABLE 2: Concordance between slit skin smear and the ML-Flow test

ML-Flow	Slit Skin Smear Positive Negative	Total
Positive	30 (75%) 2 (5%)	32
Negative	3 (7,5%) 5 (12,5%)	8
Total	33 7	40

ence of these antibodies suggests a current MB infection and can be used to distinguish between PB and MB patients[15], to recognize potential MB contacts and predict which individuals may present recurrence of the disease.

Elisa and ML-Flow sensitivity and specificity were similar, with a concordance of 91% between them.[4,13,18] Like other serologic tests, the ML-Flow is not diagnostic, because most PB patients do not develop detectable antibodies, but it can be used as a tool for classifying patients as PB or MB after initial diagnosis[13].

Among the PB patients, three (37.5%) presented slightly positive ML-Flow, which corroborates previous studies that cite a sensitivity of 40% for the test in these cases. [15]

This can be explained by the fact that the test is reader-dependent and by a not so significant difference between pale line and 1+, even though the literature shows an agreement of 96% in terms of the reproducibility of the test reading.[13]

In this series, the ML-Flow test was positive in 32 MB patients (Table 1).

There was an agreement of 87.5% between the positivity of slit skin smears and the positivity of the ML-Flow test, Kappa = 0.59, p <0.001. (Table 2)

In 2 MB patients (5%), slit skin smears were negative and the ML-Flow was positive. This may be due to a failure in the methodology of collecting smear material, for example.

One of these patients had bilateral resorption of the phalanges and interosseous atrophy on the left and presented a Mitsuda reaction value of 3.5 mm and a BI of 4+, ML-Flow serology was 3+. The patient evolved without any reaction for a period of 5 years.

The second patient had more than five plaques and ulnar thickening in initial clinical examinations, Mitsuda reaction of 5 mm, BI of 1+, ML-Flow serology of 3+, evolution without reactions.

In three MB patients (7.5%), slit skin smears were positive and the ML-Flow was negative.

These patients were shown to be truly multibacillary in the investigation; therefore, serology (ML-Flow) failed to detect them.

The first had a single plaque at initial clinical diagnosis, BI of 2+, Mitsuda reaction of 7 mm, slit skin

smear of 2+. The patient evolved with reversal reaction.

The second patient had more than 5 lesions and reversal reaction initially, BI of 2+, Mitsuda reaction of 11 mm, slit skin smear of 3+.

The third of these patients had an initial macula, bilateral ulnar thickening, BI of 2+, Mitsuda reaction of 5 mm, slit skin smear of 1+. The disease also evolved with reversal reaction.

Additionally, in 5 MB patients (12.5%), the ML-Flow and slit skin smears were negative (Table 1). Possible explanations for this could be fluctuations of immunity or even the presence of bacilli on a site protected from the immune system (nerve network) when the laboratory examination was performed.

It was observed that the ML-Flow could be used as an alternative to slit skin smear due to its practicality, especially in children and in areas where there is no personnel appropriately trained to recognize leprosy and make proper material collection for skin smear. However, like any diagnostic test, it has its limitations.

It can also be used as a differential criterion, since the test showed high sensitivity in the diagnosis of true MB cases (80%) in our series and, therefore, may be a useful field tool to assist in the correct choice of treatment for leprosy patients. However, in specialized health care centers, where professionals are trained in the detection and clinical classification of patients, the gain in sensitivity of the test will probably be less significant.

CONCLUSION

Slit skin smear, Mitsuda reaction and histology are complementary exams in the diagnosis of leprosy, but no method alone is 100% sensitive and specific for the correct clinical classification of all forms of leprosy.

The ML-Flow test is faster and easier to use in areas of difficult access to reference services, it is less invasive than the slit skin smear, and shows good agreement with the latter, which makes it useful to assist in therapeutic decisions in these areas.   ❑

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