Leprosy serology (ML Flow test) in borderline leprosy patients classified as paucibacillary by counting cutaneous lesions: a useful tool

Sorologia rápida para hanseníase (teste ML Flow) em pacientes dimorfos classificados como paucibacilares pelo número de lesões cutâneas: uma ferramenta útil

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ABSTRACT

Leprosy remains an endemic disease in Brazil, with almost 40,000 new cases diagnosed each year. As it is difficult to perform laboratory procedures in the field, operational classification is determined by counting lesions, which can cause underdiagnosis of multibacillary cases and failures in treatment. To evaluate a new tool to diagnose MB cases, the ML Flow test, 21/77 (27.3%) patients with untreated borderline leprosy (6 BL and 15 BT) with 1 to 5 cutaneous lesions were evaluated according to the R&J Classification. The ML Flow test was positive in 14/21 (66.6%) patients; 7/21 (33.3%) cases, 5 BT and 2 BL, showed negative results. Classification of leprosy based only on the number of lesions can fail to diagnose MB leprosy. The ML Flow test is a useful tool to diagnose borderline leprosy in patients with 1 to 5 cutaneous lesions.

Key-words: Borderline leprosy. Classification. Serology. Pathology.

RESUMO

A hanseníase ainda é doença endêmica no Brasil, com cerca de 40.000 novos casos por ano. Devido à dificuldade na realização de exames laboratoriais em campo, classifica-se a forma clínica contando-se lesões, o que pode causar subdiagnóstico de casos multibacilares e falha terapêutica. Para avaliar uma nova ferramenta para diagnóstico de hanseníase multibacilar, o teste ML Flow, foi realizado em 21/77 (27,3%) pacientes com hanseníase dimorfa (6 DV e 15 DT) não tratados, com até cinco lesões de pele, avaliados de acordo com a classificação de Ridley & Jopling (R&J). O teste ML Flow foi positivo em 14/21 (66,6%) pacientes (4 DV e 10 DT); em 7/21 (33,3%) pacientes (5 DT e 2 DV) o resultado foi negativo. A classificação da hanseníase baseada somente na contagem de lesões pode falhar em diagnosticar casos MB. O ML Flow é ferramenta útil no diagnóstico de hanseníase dimorfa com até cinco lesões cutâneas.

Palavras-chaves: Hanseníase dimorfa. Classificação. Sorologia. Patologia.

Leprosy or Hansen's disease (HD) is an infectious disease caused by *Mycobacterium leprae*, an intra-cellular parasite with tropism for the peripheral nervous system and derma. It is endemic to Brazil with close to 40,000 new cases detected each year, despite the reduction in prevalence observed following the introduction of multi-drug therapy by the World Health Organization (MDT-WHO) in 1990[9]. Although its diagnosis is relatively simple, it is not always classified accurately in terms of its clinical form and the proper MDT regimen that should be administered. A precise diagnosis of the clinical form involves five criteria: morphology of the skin lesions, evolutive aspects, smear bacilloscopy, histopathology and the immune response of the patient[9]. Given that only the first two are readily available in the field, the WHO created an operational classification system based on the number of skin lesions in order to facilitate diagnosis. Patients with up to five lesions are classified as paucibacillary (PB) and those with six or more lesions are multibacillary (MB)[1]. This form of classification, however, often causes errors due to the fact that a sizeable number of borderline (dimorphous) patients have a limited number of visible skin lesions; they may in fact be MB if assessed according to all of the standard criteria. This classification error is often only discovered when the patients present leprosy reactions after starting MDT[1]. Therefore, easily-applied laboratory tests are required to assist with the correct classification of patients with few lesions, in order to avoid inadequate treatment.

The ML Flow (lateral flow) test detects IgM antibodies against phenolic glycolipid I (PGL-I) for *M.leprae* with high sensitivity and

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specificity[3]. Studies show that the IgM antibodies against PGL-I are closely linked to the bacterial load of the patient[2] and a proportion of patients classified as PB according to the WHO recommendation on the number of lesions[6] show a positive ML Flow result.

With the objective of evaluating the ML Flow test as an auxiliary tool in the diagnosis and treatment of HD, this test was applied and the Ridley & Jopling (R&J) classification was compared in untreated borderline cases with a number of skin lesions that would lead them to be classified as PB under WHO criteria[5].

MATERIAL AND METHODS

Between July 2004 and July 2008, 182 new HD cases were diagnosed at the Lauro de Souza Lima Institute, Bauru (São Paulo state) with the following classification breakdown according to the Madrid system: 45 lepromatous, 77 borderline, 54 tuberculoid and 6 indeterminate. Among the 77 borderline patients, 21 (27.3%) presented five skin lesions or less, with 6 showing a tendency towards Lepromatous features (BL) and 15 closer to the tuberculoid end of the spectrum (BT). A second counting of the number of skin lesions was conducted, as well as clinical, bacilloscopic and immunological (serology for PGL-I and Mitsuda reaction) evaluations and a review of evolutive characteristics, in order to verify the correct classification of the clinical form based on the R & J criteria of 1971[7].

This study was approved by the Research Ethics Committee of the Lauro de Souza Lima Institute, Bauru-SP, protocol number 0066/2004.

Evaluation of the bacteriological index (BI). The patients with bordeline leprosy who authorized the collection of skin smear, were tested from a representative sample of lesion sites until a maximum of five samples were collected[1], in order to have a total of six collection points. However, some patients did not authorize this exam.

Histopathology. Skin biopsies were obtained using a 4mm punch at the border of the lesion that was most representative for that clinical form. The tissue samples were fixed in formalin 10% buffer solution. Each fragment was processed for inclusion in paraffin and stained for hematoxylin-eosin and Fite-Faraco.

Mitsuda reaction. The Mitsuda reaction was prepared at the ILSL at a concentration of 6.0 x 10^7 *M. leprae*/ml. An injection of 0.1 ml was applied intradermally to the forearm using a tuberculin syringe. After 28 days, a clinical reading of the reaction was realized, following the criteria proposed at the International Leprosy Congress of Madrid in 1953[6]. In the positive cases, a biopsy of the reaction was taken for evaluation of the granulomatous response.

Serology (ML Flow test). A blood sample was taken by means of a finger prick and the test performed according to the protocol described by Bührer-Sékula[3].

RESULTS

Table 1 shows the results from 21 borderline patients that presented as many as five skin lesions (27.3%). Observation revealed that 13% (10/77) of all cases showed a single lesion, among which there were two borderline-lepromatous (BL) cases with a positive ML Flow result. In 66.6% (14/21) of cases with five lesions or less, the ML Flow tested positive. Of the seven cases that were negative for ML Flow, five were borderline-tuberculoid (BT) and two BL.

TABLE 1

Clinical. serologic. bacilloscopic and histopathologic characteristics of the 21 borderline cases studied.

Number of lesions	ML Flow	R&J Classification	BBI (+)	BI (+) (average)	Mitsuda (mm)
1	0	BL	5	0.5	3.5
1	1	BT	2	0,0	0
1	1	BT	3	-	0
1	0	BT	2	0,0	5.0
1	0	BT	2	0.3	7.0
1	1	BL	6	0,0	0
1	4	BL	5	-	3.0
1	1	BT	2	0,3	-
1	1	BT	2	0,0	-
1	2	BT	1	-	-
2	3	BT	2	1,0	0
2	0	BT	2	0.3	5.0
2	3	BT	3	2,0	-
2	1	BT	2	-	-
3	1	BT	3	0.0	0
3	3	BL	6	0.8	0
3	4	BL	6	2.2	0
3	0	BL	5	0.5	0
3	0	BT	2	0,0	4
4	0	BT	2	-	-
5	1	BT	2	0,0	-

BBI: biopsy bacterial index. BI: bacteriological index of skin smears.

The average of the biopsy bacterial index (BBI) from the lesions varied from 0 to 2.2 (1 BL with 3 lesions, ML Flow 4+ and PGL-I positive with OD= 0.805); many patients did not permit the collection of skin smears from the index points for bacilloscopy.

The results of the Mitsuda test varied between negative and 7.0mm (1 BT case with a single lesion, ML Flow negative e PGL-I negative with OD=0.015). Seven patients did not return for the test reading.

DISCUSSION

The classification of HD according to the number of skin lesions can lead to the use of an inadequate therapeutic regimen, resulting in the under-treatment of MB patients who have less than five lesions. This error in treatment is a concern for health professionals who do not have access to laboratory exams, so they tend to treat more patients as MB. In a study conducted in Nigeria, observation showed that 95.7% of patients received MDT/MB, when in reality only 62.9% showed a positive ML Flow. Of these, 55.9% had up to five skin lesions and therefore should have been classified and treated as PB according to WHO guidelines.

Hansen's disease is a systemic disease in which clinical manifestations depend on the patient's immunity level. In those individuals with a high cellular immunity, the tuberculoid form, the disease is limited to the cutaneous nerve branches or to one nerve trunk, but severe nerve damage often occurs[7 9]. On the other hand, in individuals with virtually no cellular response to the bacilli, the lepromatous patients (LL), *Mycobacterium leprae* multiplies in a series of other host cells, such as macrophages, epithelioid cells, endothelia, in addition to viscera, with dissemination of *Mycobacterium leprae* throughout the organism. In these cases, it is possible to find bacilli in almost all the patient's tissues, but particularly in the cooler extremities of the body, such as the ears, elbows and knees. However, in dimorphous or borderline cases, this diffuse dissemination of bacilli in the tissues and viscera does not occur[9] and may not be present at all in these areas, particularly in borderline-tuberculoid patients, where even skin smears taken from lesions may be negative. In these cases, the bacilli remain within the dermal nerve branches and for this reason, they frequently appear in significant numbers only in the bacilloscopy of nerve biopsies[10], but not in skin smears. In addition, skin smear bacilloscopy is rarely performed in the field, even where such services exist, due to the various difficulties inherent in material collection and because it is an exam performed without local anesthesia. All of these facts may lead to classification mistakes of early borderline cases, which can be diagnosed as paucibacillary (PB), but are really MB when analyzed according to all of the standard criteria. In patients with few lesions, the lymph collection taken from the index points may be negative even when the bacilloscopy of the sample taken from a lesion is positive, but in these cases, the serology is often positive and the Mitsuda reaction negative or slightly positive. Individuals with multiple lesions are normally classified as MB, but occasionally someone with only a few visible lesions presents only in areas of previous subclinical infiltration at the onset of reactions[10].

Of the 182 cases evaluated at the Lauro de Souza Lima Institute, 77 were borderline and 21 (27.3%) presented five skin lesions or less. Of these, 10 patients (13%) had only a single visible lesion. This reinforces the fact that a single *visible* lesion may not be the only lesion, but simply the first external manifestation of an infectious granulomatous process. In other words, the bacilli may be present in other sites of the body, protected from the immune system and not have yet been discovered, something that only appears clinically once reactional episodes begin following the initiation of MDT.

The ML Flow test proved to be sensitive in the detection of MB cases, including those that showed few visible skin lesions. In addition, contrary to that which occurred with skin bacilloscopy, none of the patients refused to take the ML Flow test. Although it did not provide a positive reading in all cases, in 66.6% of patients with up to five lesions, it was an effective tool in the correct classification of the clinical borderline form.

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REFERENCES

1. Barreto JA, Belone AFF, and Ghidella CC. Hanseníase multibacilar: estudo de seguimento de 114 pacientes por 6 anos. Anais do 62ª Congresso Brasileiro de Dermatologia, 2007
2. Buhrer-Sekula S, Sarno EN, Oskam L, Koop S, Wichers I, Nery JA, Vieira LM, de Matos HJ, Faber WR, Klatser, P. R. Use of ML dipstick as a tool to classify leprosy patients. International Journal of Leprosy and Other Mycobacterial Diseases 68: 456-463, 2000.
3. Buhrer-Sekula S, Smits HL, Gussenhoven GC, van Leeuwen J, Amador S, Fujiwara T, Klatser PR, Oskam L. Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. Journal of Clinical Microbiology 41: 1991-1995, 2003.
4. Buhrer-Sekula S, Visschedijk J, Grossi MA, Dhakal KP, Namadi AU, Klatser PR, Oskam L. The ML flow test as a point of care test for leprosy control programmes: potential effects on classification of leprosy patients. Leprosy Review 78: 70-79, 2007.
5. Guia de Vigilância Epidemiológica. 6 edição. Secretaria de Vigilância em Saúde, Ministério da Saúde, 2005.
6. Guia de Controle da Hanseníase. Fundação Nacional de Saúde. Centro Nacional de Epidemiologia - CNDS, Ministério da Saúde, 1994.
7. Hastings RC. Leprosy. 2 edition, New York, 1994.
8. International Congress of Leprosy, Madrid 1953. Report of the Committee on Classification. International Journal of Leprosy and Other Mycobacterial Diseases40:102-103, 1972.
9. Opromolla DVA. Noções de Hansenologia. 2000.
10. Opromolla DVA. Ação terapêutica das drogas anti-hansênicas e evidências da persistência microbiana nos casos paucibacilares. Hansen International 29: 1-3, 2004.