The influence of ML Flow test in leprosy classification

A influência do teste sorológico ML Flow na classificação da hanseníase

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ABSTRACT

This is a descriptive, exploratory study correlating ML Flow, bacilloscopy and classification of paucibacillary (PB) and multibacillary (MB), involving 1,041 new leprosy cases in 13 municipalities of Minas Gerais State, from October 2002 to March 2004. Agreement between ML Flow and the classification of the number of skin lesions and bacilloscopy was moderate (kappa: 0.51 and Kappa: 0.48, respectively); and substantial for final classification (Kappa: 0.77). From January 2000 to March 2004, the proportion of new MB cases in Minas Gerais decreased from 78.1 to 65.8%. The reduction in the percentage of MB cases was higher in health centers that participated in the ML Flow study (73.1% to 53.3%). The difference between PB and MB in the participating and non-participating health centers from January to March 2004 was statistically significant. Implementation of the ML Flow test influenced the classification of patients, suggesting a direct and beneficial impact on patient treatment and the control of the leprosy endemic in Minas Gerais, Brazil.

Key-words: Leprosy. Classification. Serology. Bacilloscopy.

RESUMO

Estudo descritivo e exploratório correlacionando o ML Flow, a baciloscopia e a classificação em paucibacilar (PB) e multibacilar (MB), envolveu 1.041 casos novos com hanseníase em 13 municípios de Minas Gerais, de outubro de 2002 a março de 2004. A concordância entre o ML Flow e a classificação pelo número de lesões cutâneas e a baciloscopia foi moderada (Kappa:0,51 e 0,48, respectivamente) e, substancial (Kappa:0,77) com a classificação final. De janeiro de 2000 a março de 2004, a proporção de casos novos MB no Estado, passou de 78,1 para 65,8%. A queda no percentual de MB foi maior nos serviços participantes da pesquisa ML Flow (73,1 para 53,3%). A diferença de PB e MB nos serviços participantes e não participantes, de janeiro a março de 2004, foi estatisticamente significativa, indicando implicação direta e benéfica no tratamento e no controle da endemia em Minas Gerais.

Palavras-chaves: Hanseníase. Classificação. Sorologia. Baciloscopia.

Leprosy is a chronic, infectious disease that is of considerable importance for public health, given its magnitude and potential for causing disabilities and the fact that it mostly affects those of an economically active age[3].

The interaction between *Mycobacterium leprae* and a human being can result in different clinical manifestations with varying signs and symptoms. This is the result of diverse physiopathological mechanisms, different levels of transmissibility and variations in evolution and prognosis, giving rise to innumerous classification systems throughout history[1].

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The 1953 Madrid classification considered two stable and opposite ends of a spectrum: tuberculoid and Virchowian, and two unstable groups: indeterminate and dimorphous, which, in the natural evolution of the disease, move towards the ends of the spectrum. Indeterminate leprosy is considered the first clinical manifestation of the disease, which may lead to spontaneous cure or to another clinical form after a period that can vary from a few months to several years[1].

The classification system proposed by Ridley and Jopling in 1966 is the one most used in research and takes immunity into consideration within a spectrum of host resistance and histopathology. This, however, makes it more difficult to use in the field in smaller health centers. The forms are described as: tuberculoid, borderline (subdivided as borderline-tuberculoid, borderline-borderline and borderline-lepromatous), subpolar lepromatous and lepromatous[1 19].

In 2000, the World Health Organization (WHO) recommended a simplified method of leprosy classification for treatment purposes, based on the counting of skin lesions. This system was adopted by the Brazilian Ministry of Health (MoH) in 2002. Patients

with five cutaneous lesions or less are classified as PB, whereas those with more than five lesions are MB[24,25]. The clinical classification of leprosy cases, as proposed by the WHO, simply for treatment, involves the risk of overestimating the number of MB cases[12].

In Brazil, skin smear bacilloscopy is used as an auxiliary exam, when available, to classify patients as PB or MB. A positive bacilloscopy classifies the patient as MB, regardless of the number of skin lesions[2], but a negative result does not exclude the possibility of leprosy diagnosis[3].

Due to the limited availability of bacilloscopy in many leprosy control programs, other clinical methods were developed for patient classification[10,12,18,20,23]. Sensitivity and specificity were established for clinical criteria, based on bacilloscopic references[20]. The use of the three cardinal signs of leprosy: skin lesions with altered sensation, thickened peripheral nerves and a positive bacilloscopy, resulted in a sensitivity of 97%, with a positive predictive value of 98% to establish leprosy diagnosis[5].

This classification is necessary for the allocation of patients within the two treatment regimens currently in existence and would have no importance if a single course of chemotherapy existed for all patients. However, it is important to remember that PB and MB present very different bacterial loads and distinct risks of developing relapse, disabilities and deformities. Therefore, correct classification is an important tool to ensure quality treatment for patients[20].

The introduction of multi-drug therapy (MDT) for leprosy occurred in Brazil in 1986. The detection rate of MB forms increased from 5.6 to 11.7/10,000 population in the period from 1986 to 1993. That increase took place in nearly all Brazilian states, with a greater tendency to administer 24 doses of MDT/MB, in contrast to the six doses given to PB patients. The percentage of new MB cases in Brazil as whole, increased from 57% to 71.4% over this period and in the State of Minas Gerais, the shift was from 67.7% MB to 94.7%. The introduction of MDT, led to a massive change on a national scale. The alteration in the frequency of MB case treatment with MDT seems to more closely reflect the conservative clinical attitudes regarding the treatment regimen to be administered rather than any real change in epidemiological profile[15].

Beginning in 1980, PGL-I was described as the immunogenic antigen specific to *Mycobacterium leprae*[4] and the first serological tests appeared. The most commonly applied was the enzyme linked immunosorbent assay (ELISA), used to detect anti-PGL-I antibodies, specifically IgM[9].

ML Flow, developed in 2003, is an immunochromatographic test that reveals the presence of IgM antibodies against *Mycobacterium leprae*-specific PGL-I. It is a rapid test that uses either serum or whole blood and can be used directly by health professionals without the need for laboratories or special equipment[6]. The reagents are highly stable and can be stored at room temperature[6].

The test can be used as a tool for the correct classification of new leprosy cases as PB or MB and for use in the identification of contacts with a higher risk of developing the disease in the future[6]. More recent reports indicate that if serology was used to classify leprosy, the number of patients treated as MB would drop.

In a multicentric study of 2,632 new leprosy cases, ML Flow seropositivity was 50.8% in Brazil, 62.9% in Nigeria and 31.9% in Nepal. The proportion of MB cases, according to the counting of anesthetic skin lesions was 39.5% in Brazil, 19.4% in Nigeria and 35.6% in Nepal. Bacilloscopy was positive in 27.1% in Brazil and 11.6% in Nepal[7]. In Nigeria, bacilloscopy is not part of the clinical routine and a strong tendency to classify patients as MB was observed as a result, such that 95.7% of patients received MDT/MB.

The incorrect classification of a patient can lead to insufficient treatment for those MB cases misclassified as PB and excessive treatment for PB cases classified as MB[22]. Correct classification will make leprosy control more effective, avoiding excessive treatment and preventing future relapse due to insufficient treatment[6].

Early diagnosis, correct classification of leprosy patients and adequate treatment are challenges to the elimination of the disease, principally where the management of patients is being integrated into basic healthcare services[22]. This study showed that the implementation of this test influenced the classification of patients in Minas Gerais, Brazil.

PATIENTS AND METHODS

As part of a multicentric study in Brazil, Nepal and Nigeria, a descriptive, exploratory study to compare the results of the ML Flow serological test, bacilloscopy and classification of paucibacillary (PB) and multibacillary (MB) leprosy was conducted by the Minas Gerais State Health Secretariat, coordinated by the Biomedical Research Department, Royal Tropical Institute, Amsterdam and financed by Netherlands Leprosy Relief, Amsterdam, Holland [11]. It was approved by the Research Ethics Committee of the *Santa Casa de Misericórdia* of Belo Horizonte on 22/11/2001, under protocol no. 39/01 and that of the Federal University of Minas Gerais, on 16/02/2004, nº 312/04. All research subjects agreed to participate in the study and signed a free informed consent form.

Study population. Field research was conducted between October 2002 and March 2004, in 14 healthcare institutions in 13 municipalities of the State of Minas Gerais, in 8 local health centers, 4 regional referral centers and 2 state referral centers, involving 1,041 new leprosy cases. Cases with positive bacilloscopy and/or serology were classified as MB for treatment purposes, regardless of the number of cutaneous lesions or affected nerves.

Laboratory exams. The ML Flow test was conducted as described by Bührer-Sékula *et al*[6] and the results recorded qualitatively as either positive or negative.

The collection of skin smears for bacilloscopy was performed at four sites: skin lesion; earlobes; and elbow on the opposite side of the body from the lesion, or in the absence of lesions, from both elbows. The slides were stained using the Ziehl-Neelsen method and the smears examined in immersion at 100x. The bacteriological index (BI) was calculated according to Ridley's logarithmic scale from 0 to 6[19].

Data analysis. The data were collected by health service professionals who agreed to participate in the study and were trained to conduct tests. The results were noted on the patient's medical record and a research form was sent to the State Health Secretariat monthly, together with the dipsticks of the serological tests, duly conserved for a second, confirmatory reading.

The data were entered using Epi-info software, version 6.0, with double entry, corrected, validated, checked for consistency and, when necessary, returned to the health center to clarify doubts. The identity of the patients was kept in strict confidence throughout the study.

The agreement between the first reading of the ML Flow test, as recorded by the local health professionals involved in the study, and the second reading taken by a single, independent examiner in Belo Horizonte was nearly perfect (kappa: 0.81) with 91% agreement in the results.

Agreement between the ML Flow results and a) classification using the number of skin lesions, b) bacilloscopy and c) final classification used by health center to treat the patient was analyzed, taking into consideration clinical signs, number of affected nerves, bacilloscopy and the ML Flow outcome.

Cohen's kappa coefficient was used to evaluate any agreement according to Landis and Koch's criteria for interpretation[13]: no agreement for an index below 0.00; slight agreement between 0.00 and 0.20; fair agreement from 0.21 to 0.40; moderate agreement from 0.41 to 0.60; substantial agreement from 0.61 to 0.80 and almost perfect agreement from 0.81 to 1.00.

RESULTS

Seropositivity in the ML Flow test was verified in 50.8% of new cases, while bacilloscopy was positive in 27.1% and 39.5% of patients tested presented six or more cutaneous lesions (**Table 1**).

Agreement between ML Flow and classification by skin lesion counts and bacilloscopy was moderate (Kappa: 0.51 and Kappa: 0.48, respectively), but substantial for final classification given by the health center for treatment purposes (Kappa: 0.77) (**Table 1**).

Comparing classification by skin lesion counts and the result of the ML Flow test, 188 (29.8%) of patients classified as PB were seropositive, while 70 (17%) of those classified as MB were seronegative (**Table 1**).

When the final classification given by the health centers is analyzed with the ML Flow results, 107 (17.1%) patients treated as MB had a negative ML Flow, while 11 (2.6%) of those treated as PB had a positive ML Flow (**Table 1**).

A reduction in the percentage of MB patients among the total of new cases diagnosed in Minas Gerais was observed, falling from 78.1% in 2000, to 65.8%, in March 2004. The reduction in MB cases was greater in health services that participated in the ML Flow study, decreasing from 73.1 to 53.3%, than in the non-participating services, which showed a decrease from 80.6 to 72.2% (**Figure 1**). The reduction between the percentages of MB cases was greater in the participating institutions and the difference among PB and MB patients diagnosed from January to March 2004 in participating and non-participating centers was statistically significant, with a $\chi^2 = 23.8$ and P-value < 0.001 (**Figure 1**).

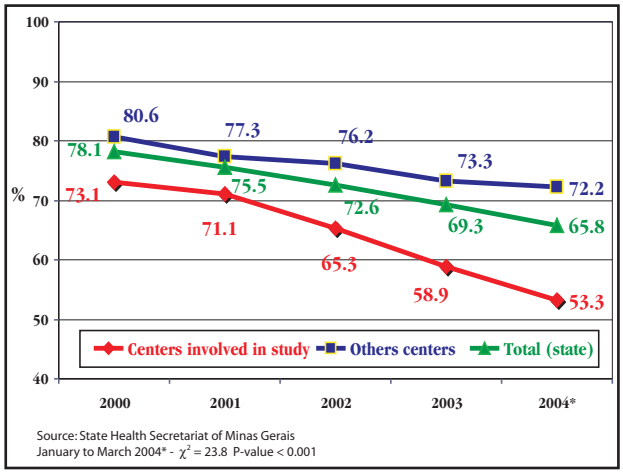

FIGURE 1

Graph with the percentage of new MB leprosy cases in the health centers that participated in the ML Flow study, in other health centers and in Minas Gerais as a whole from 2000 to 2004.

TABLE 1

Distribution of 1.041 new leprosy cases, according to the results of ML Flow, bacilloscopy, skin lesion count and final classification in Minas Gerais from October 2002 to March 2004.

		Number of cutaneous lesions				Baciloscopy				Final classification					
		≥6 lesions (MB)		< 6 lesions (PB)		positive		negative		MB		PB		Total	
		nº	%	nº	%	nº	%	nº	%	nº	%	nº	%	nº	%
ML Flow	Positive	341	83.0	188	29.8	270	95.7	259	34.1	518	82.9	11	2.6	529	50.8
	Negative	70	17.0	442	70.2	12	4.3	500	65.9	107	17.1	405	97.4	512	49.2
	Total	411	39.5	630	60.5	282	27.1	759	72.9	625	60.0	416	40.0	1.041	100.0
Kappa index		0.51 - 95% CI: 0.45; 0.57				0.48 - 95% CI: 0.43; 0.54				0.77 - 95% CI: 0.71; 0.83					

PB: paucibacillary, MB: multibacillary, CI: confidence intervals.

DISCUSSION

The difference in seropositivity of the ML Flow test in this study (50.8%), compared to that obtained by other authors in the same State, Lyon *et al* (57%)[14] and Castorina-Silva[8] (70%) and in other countries, such as Nigeria (62.9%) and Nepal (35.6%)[7], may be explained by the epidemiological and clinical characteristics of the patients, as well as the type of healthcare institution where treatment was administered.

Bacilloscopy positivity varied from 10.6 to 46.2% in the 14 centers involved in the study (data not presented) and the average of 27.1% was shown to be lower than that described in articles from referral centers in Minas Gerais, Lyon *et al* (35.9%)[14] and Castorina-Silva (40%)[8]; although it was much higher that results from Nepal (11.6%)[7] and others frequently cited in the literature[1 5 12 16 20 21]. The high percentage found in the study of Gallo *et al* (77.9%)[10], can probably be explained by the fact that it was conducted within a national leprosy reference center. Smear bacilloscopy, while a relatively simple and easy test to perform, has a number of operational limitations and the results are often not reliable[5 20], in contrast with the high reproducibility and reliability observed with ML Flow; not to mention the fact that the serological test reflects the body's general immunological response to the presence of bacilli. This fact is confirmed by the variation in the ML Flow positivity that accompanied the variation in bacilloscopy. It was positive in 95.7% of patients with positive bacilloscopy, detecting *Mycobacterium Leprae*-specific antibodies in more than a third of new leprosy cases with negative bacilloscopy.

In the cases studied, the percentage of seropositivity was almost twice that of bacilloscopic positivity, corroborating the lower sensitivity of bacilloscopy that has been reported by several authors[1 5 12 16 20 21]. The use of serology by health centers may assist in the correct classification and appropriate treatment of true multibacillary patients, thus eliminating many sources of transmission and possibly preventing relapse in those cases where a negative skin smear result leads to the administration of insufficient treatment.

Agreement between ML Flow and the final classification made by the health Centers was substantial, witnessed by the fact that the ML Flow results altered the final classification. Agreement was only moderate compared with the clinical classification standardized by the WHO.

The proportion of MB cases (39.5%) using the criteria of skin lesion counts, as proposed by the WHO and adopted in Brazil, was higher than that observed in Nepal (35.6%)[7], and cited by Castorina-Silva (35%)[8]. It was considerably higher than the percentage observed in Nigeria (19.4%)[7] and in Ethiopia (20%)[16], yet lower than in studies conducted in state and national reference centers by Lyon *et al* (43.7%)[14] and Gallo *et al* (73.4%)[10], reinforcing the probable differences in the clinical characteristics of patients attended in referral centers.

Comparing the classification by skin lesions and the result of the ML Flow test, 188 (29.8%) of patients classified as PB were seropositive, indicating the treatment received could be insufficient. Meanwhile, 70 (17%) of those classified as MB were seronegative and may have been administered excessive treatment if this was the only criterion used for classification (**Table 1**).

In relation to the final classification given by the health centers, observation showed that 60% of patients were treated as MB. When the final classification is analyzed in conjunction with the ML Flow results, 107 (17.1%) patients treated as MB presented a negative ML Flow, while 11 (2.6%) of those treated as PB presented a positive ML Flow (**Table 1**). These data seem to suggest that health professionals place greater value on clinical observations.

A strong tendency exists among health professionals to classify leprosy patients as MB, as per the example from Nigeria, where 95.7% were treated as MB, even though only 19.4% had 6 or more cutaneous lesions[7]. This appears to be related to the absence of laboratory exams, such as bacilloscopy or biopsy (histopathology), which provide security to the professional when making the decision. All this data indicates the likely benefit of including the ML Flow test as an auxiliary tool in the classification of leprosy cases, above all for Family Health Strategy teams, which often have less experience with the disease. This fact suggests that the use of ML Flow could have a significant impact on the prevention of excessive or insufficient treatment regimens and promote a reduction in MB diagnosed cases.

A reduction was seen in the MB proportion of all new leprosy cases detected in the health centers that participated in the study, which subsequently had a strong repercussion on the MB percentage in the State of Minas Gerais as a whole (**Figure 1**). The use of the ML Flow test to clarify leprosy classification effectively modified the classification of new cases in the State by reducing the number of patients treated as MB during the research period, thereby reducing the use of MDT/MB blisters and the number of consultations, a direct and beneficial impact on treatment and the control of the leprosy endemic.

The results of this study indicate that the use of ML Flow as an auxiliary tool in the classification of leprosy patients and the definition of the most appropriate course of treatment may facilitate the work of basic healthcare professionals, especially those who have minimal experience with the disease and who do not have laboratory access, thereby helping to increase the coverage and impact of leprosy control measures.

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