Risk factors for ML Flow seropositivity in leprosy patients

Fatores de risco para a soropositividade do ML Flow em pacientes com hanseníase

Maria Aparecida de Faria Grossi[1, 2], Maria Ana Araújo Leboeuf[2], Ana Regina Coelho de Andrade[1, 2], Samira Bührer-Sékula[3] and Carlos Maurício de Figueiredo Antunes[1]

ABSTRACT

The early diagnosis of leprosy, its correct classification and the risk factors related to seropositivity have become important for patient treatment and disease control, especially where the responsibility for treatment has been transferred to basic health care centers. This descriptive, exploratory study using logistic regression was undertaken to evaluate the association between the variables of sex, age, mode of detection, number of skin lesions and affected nerves, disability grade and bacilloscopy with the results of the ML Flow serological test in 1,072 new leprosy cases in 13 municipalities in Minas Gerais State. Seropositivity (50.7%) was statistically associated with patients 15 years-old or over (OR:2.6) and those with more than five skin lesions (OR:7.5), more than one affected nerve (OR:2.4) and a positive bacilloscopic index (OR:5.5 for 0<BI<2 and OR:191.2 for BI$\geq$2), thus contributing to the appropriate classification and treatment of patients.

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

RESUMO

O diagnóstico precoce da hanseníase, a correta classificação e o estudo dos fatores de risco relacionados à soropositividade, tornam-se importantes para o tratamento do doente e controle da endemia, especialmente, quando a responsabilidade pelo atendimento desses pacientes está sendo absorvida pelos serviços de atenção básica. Estudo descritivo e exploratório utilizando regressão logística avaliou a associação das variáveis: sexo, idade, modo de detecção, número de lesões cutâneas e de nervos acometidos, grau de incapacidade, baciloscopia, com o resultado do teste sorológico ML Flow, em 1.072 casos novos com hanseníase em 13 municípios de Minas Gerais. A soropositividade (50,7%) estava estatisticamente associada aos pacientes com 15 anos ou mais de idade (OR:2,6), mais de cinco lesões cutâneas (OR:7,5), mais de um nervo acometido (OR:2,4) e com baciloscopia positiva (OR:5,5 para IB<2 e OR:191,2 para IB$\geq$2), colaborando, assim, com a classificação e o tratamento adequados dos doentes.

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

Leprosy is an infectious chronic disease caused by *Mycobacterium leprae*, a bacillus with a predisposition for peripheral nerves and skin, and although it does not represent a primary cause of death, it stands out among diseases that lead to disabilities[1 15 19 33]. Its clinical signs are typical, thus making its diagnosis simple in the majority of cases, but in others it can be confused with other neuropathies and dermatoses[1].

1. Post-graduate Program in Health Sciences: Infectious Diseases and Tropical Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil. 2. State Health Secretariat of Minas Gerais, Belo Horizonte, Brazil. 3. KIT-Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands and Tropical Pathology and Public Health Institute, Federal University of Goiás, Goiânia, Goiás, Brazil.
Address to: Dra. Maria Aparecida de Faria Grossi. Rua Juiz da Costa Val, 65/602, São Lucas, 30240-350 Belo Horizonte, MG, Brazil.
Tel: 55 31 3221-0069
e-mail: cida@grossi.com.br

The different clinical manifestations of leprosy are related to the immune response of the host organism[17]. Genetic factors and contact with other microorganisms in the surrounding environment can modify this response[22].

Significant differences have been noted in leprosy detection among men and women. It is customary for men to present with more severe forms of the disease and suffer more disabilities, while women tend to have a more intense immunological response to *Mycobacterium leprae* and are less often diagnosed with multibacillary (MB) forms or with severe disabilities[21 24]. Another factor may be related to the fact that health professionals do not examine women as thoroughly as they do men, due to cultural difficulties[21].

The progressive reduction in the difference between the sexes in the detection of leprosy cases has been explained by the increased presence of women in the workforce after 1960, or by increased access for women to basic health services[21 24].

Leprosy is a disease that occurs in young and middle-aged adults, with a higher number of cases registered in the age group from 20 to 50 years[24]. The appearance of leprosy in those under the age of 15 indicates early exposure to the etiological agent, determined by a higher level of endemicity[24].

Evaluation of the disability grade must be conducted for the eyes, hands and feet at diagnosis and at the time of release from treatment and is an essential step in the planning of prevention of disabilities (PoD) interventions[5]. The percentage of grade 2 disabilities or deformities among newly diagnosed and evaluated cases is defined as an important epidemiological indicator for judging the effectiveness of early leprosy detection activities, as well as estimating the existence of an undetected caseload[14].

Bacilloscopy is the most useful auxiliary exam for diagnosis. It is relatively easy and inexpensive to conduct, although it does require trained professionals and a laboratory, not always present in basic health care centers[1]. The slit skin smear is collected from the lesions suggestive of leprosy, as well as the earlobes and elbows. Staining is performed according to the Ziehl-Neelsen method and the results are recorded in the form of the Bacilloscopic Index (BI) on a scale of 0 to 6+, as proposed by Ridley[29]. The bacilloscopic reading is negative for paucibacillary (PB) patients (indeterminate and tuberculoid on the Madrid scale) and strongly positive in lepromatous patients, while variable for borderline cases[1]. Slit skin smears are highly specific, but not very sensitive, given that approximately 70% of all leprosy patients have a negative bacilloscopic outcome[6 31].

Since 2000, the World Health Organization (WHO) has recommended a simplified method of leprosy classification and treatment based on skin lesion counts. This guideline, adopted by the Brazilian Ministry of Health in 2002, states that patients with as many as five skin lesions are classified as PB, and those with six or more lesions are multibacillary (MB)[4 5 18 25]. The simplified clinical classification of leprosy cases, as proposed by the WHO for treatment purposes, involves the risk of over diagnosing the number of MB cases[10].

Considering the integration of leprosy control into the basic health care structure, in particular the Family Health Program, the responsibility for diagnosis, classification and allocation of multi-drug therapy (MDT) has been taken on by general practitioners[10]. Given this fact, the large-scale implementation of a simplified clinical method became inevitable[10].

The use of a combination of clinical and laboratorial criteria, principally serological tests to detect antibodies specific to phenolic glycolipid I antigen of *Mycobacterium leprae*, for the correct classification of leprosy patients and distribution of the appropriate MDT regimen[10], could greatly benefit the control of the endemic.

ML Flow is an immunochromatographic test that detects IgM antibodies, specific to *Mycobacterium leprae* in serum or blood, is related to the bacterial load of leprosy patients. It is easy to conduct and can be used directly by health professionals without the need for laboratory access or other equipment[7]. The reagents are highly stable can be stored at room temperature[7].

The majority of PB patients are seronegative, while the majority of MB patients are seropositive[7 26]. This shows that the presence of antibodies in leprosy patients can be used to classify them as MB or PB for treatment purposes, but not as a diagnostic exam[7].

In a multicentric study involving 2,632 new leprosy cases, seropositivity in the ML Flow test was 50.8% in Brazil, 62.9% in Nigeria and 31.9% in Nepal. The proportion of MB cases in the same group, using the WHO lesion counting method, was 39.5% in Brazil, 19.4% in Nigeria and 35.6% in Nepal. Bacilloscopy results were positive in 27.1% in Brazil and 11.6% in Nepal[8].

Early diagnosis and correct classification of leprosy, as well as the study of risk factors related to seropositivity, have become important for patient treatment and disease control, especially where the responsibility for treatment has been absorbed by basic health care centers[24 32].

This study aimed to contribute to the greater accuracy of classification of new leprosy cases in order to improve treatment and control activities.

PATIENTS AND METHODS

This descriptive, exploratory study compared ML Flow serological test results with the covariables of sex, age, mode of detection, number of skin lesions and affected nerves, disability grade and bacilloscopy with the results of the ML Flow serological test in 1,072 new leprosy cases. The research was undertaken by the State Health Secretariat of Minas Gerais and comprised part of a multicentric study in Brazil, Nepal and Nigeria, as coordinated by the Biomedical Research Department, Royal Tropical Institute, Amsterdam and financed by Netherlands Leprosy Relief, Amsterdam. It was approved by the Research Ethics Committees of the *Santa Casa de Misericórdia* in Belo Horizonte on Nov. 22, 2001, protocol no. 39/01 and of the Federal University of Minas Gerais, on Feb. 16, 2004, protocol no. 312/04. All research subjects agreed to participate and signed free informed consent forms.

Research was conducted between October 2002 and March 2004 in 14 health centers in 13 priority municipalities in Minas Gerais. The breakdown of these health centers was as follows: eight municipal health centers, four regional referral centers and two state referral centers.

The ML Flow serological test was performed as described by Bührer-Sékula *et al* (2003)[7] and the results were recorded as positive or negative. The other variables studied were: sex; age in years, categorized as under 15 or 15 years-old and over; means of detection, contact examination, self-presentation or others; number of skin lesions, five lesions or less or six and above; number of thickened nerves, zero, one or more than one; disability grade at diagnosis, zero, 1 or 2 or not evaluated; bacilloscopy, positive, negative, or not tested; and bacilloscopic index, BI from zero to 6+ and categorized as negative, positive less than 2, positive two or higher, or not tested. The variable of disability

grade was used according to WHO criteria, as recommended by the Ministry of Health[2 4 5].

Final classification was defined as that used by the health center to determine patient treatment with MDT, taking into consideration the number of skin lesions and affected nerves in combination with bacilloscopy and ML Flow results. All patients with a positive BI were classified as MB, regardless of the number of skin lesions and thickened nerves.

The bacilloscopy was conducted on skin smears taken from four sites (skin lesion, both earlobes and the elbow on the other side of the body relative to the lesion; or in the absence of skin lesions, in both elbows). The slides were stained according to the Ziehl-Neelsen method, and the smears examined with an oil immersion lens (100x). The BI was calculated according to Ridley's logarithmic scale from 0 to 6[29].

The association between ML Flow positivity and the variables under study was defined using odds ratio (OR) and a 95% confidence interval (95% CI), and univariate/multivariate analyses. For the multivariate analysis, the logistic regression method was used with the Hosmer & Lemeshow test to verify model adjustment[11]. All variables were included that had a significance probability lower than 0.25 in the univariate analysis.

RESULTS

A descriptive analysis of the variables studied is presented in **Table 1**. Seropositivity in the ML Flow test occurred in 50.7% of the study participants. No predominance in seropositivity occurred between the sexes. Age varied from two to 98 years-old, with an average of 42, and 6.9% under the age of 15. In relation to the means of detection, 8.1% were diagnosed by way of household contact examinations, 29.5% via self-presentation and 62.4% through other means, mostly referral from other health centers. In this study, 38.9% of patients were attended at eight municipal health centers, 45.8% at four regional referral centers and 15.3% at two state referral centers.

Of the total cases researched, 59% were classified and treated as MB, 60.4% had five or fewer skin lesions and 7% had grade 2 disabilities. Among the 1,041 patients for whom data were available on the number of nerves affected, 38.7% had more than one thickened nerve. Bacilloscopy results were negative in 73% of cases, 7.8% had a positive BI under 2 and 19.2% had a BI of 2 or higher.

Univariate analysis between ML Flow and the other covariables showed that seropositivity was higher among men (58.6%); patients over 15 years of age (97.6%); those who had six or more skin lesions (64.7%); more than one affected nerve (52.4%); grade 1 disability (31%); grade 2 disability (10.4%); positive bacilloscopy (50.9%); positive BI under 2 (13.2%) and BI of 2 or higher (37.7%), **Table 2**.

TABLE 1

Descriptive statistics of study variables.

Variable	Number	Percentage
ML serologic test		
positive	544	50.7
negative	528	49.3
Sex		
male	531	49.5
female	541	50.5
Age		
Average (years)	42	(2-98)
< 15	74	6.9
≥ 15	998	93.1
Mode of detection		
contact examination	87	8.1
self-presentation	316	29.5
others	669	62.4
Final classification		
paucibacillary	439	41.0
multibacillary	633	59.0
Number of affected nerves*		
≤ 1 nerve	657	63.1
> 1 nerve	384	36.9
Number of skin lesions		
< 6 lesions	648	60.4
≥ 6 lesions	424	39.6
Disability*		
grade 0 (no disability)	760	71.3
grade 1 (loss of sensitivity)	231	21.7
grade 2 (deformity)	75	7.0
Bacilloscopic index		
negative	782	73.0
positive < 2	84	7.8
positive ≥ 2	206	19.2
Health center		
state reference center	164	15.3
regional reference center	491	45.8
basic health center	417	38.9

*patients not examined were excluded from totals.

In the multivariate analysis, seropositivity was statistically associated with patients 15 years of age or older (OR: 2.6); six or more skin lesions (OR: 7.5); more than one thickened nerve (OR: 2.4); positive BI under 2 (OR: 5.3); while the patient with a positive BI of 2 or higher had roughly a 191-fold chance of a positive serology compared to those with a negative BI (**Table 3**).

The probability of seropositivity increased as the number of affected nerves or skin lesions in the patient increased, although the rise was higher when the number of skin lesions increased (**Table 4**)

TABLE 2

Univariate analysis – ML flow versus covariables.

Variables	ML Flow (+)		Odds	CI (95%)
	nº	%	ratio	p/ odds ratio
Sex				
female	225	41.4	1.0	-
male	319	58.6	2.1	(1.7; 2.7)
Age (years)				
< 15	13	2.4	1.0	-
≥ 15	531	97.6	5.3	(2.9; 9.8)
Mode of detection				
contact examination	38	7.0	1.0	-
self-presentation	156	28.7	1.3	(0.8; 2.0)
others	350	64.3	1.4	(0.9; 2.2)
Number of skin lesions				
< 6 lesions	192	35.3	1.0	-
≥ 6 lesions	352	64.7	11.6	(8.6; 15.7)
Number of affected nerves*				
≤ 1 nerve	252	47.6	1.0	-
> 1 nerve	277	52.4	4.2	(3.2; 5.5)
Disability*				
grade 0 (No disability)	316	58.6	1.0	-
grade 1 (Loss of sensitivity)	167	31.0	3.7	(2.7; 5.1)
grade 2 (Deformity)	56	10.4	4.1	(2.4; 7.1)
Bacilloscopy				
negative	267	49.1	1.0	-
positive	277	50.9	41.1	(23.1; 73.1)
Bacilloscopic index				
negative	267	49.1	1.0	-
positive < 2	72	13.2	11.6	(6.2; 21.7)
positive ≥ 2	205	37.7	395.4	(55.1; 2836.1)

*Patients not examined were excluded from totals, CI: confidence intervals.

TABLE 3

Multivariate analysis of the factors Associated to ML flow Seropositivity.

Variables	ML Flow (+)		Odds	CI (95%)
	nº	%	ratio	p/ odds ratio
Age (years)				
< 15	13	2.4	1.0	
≥ 15	531	97.6	2.6	(1.2; 5.3)
Number of skin lesions				
< 6 lesions	192	35.3	1.0	
≥ 6 lesions	352	64.7	7.5	(4.6; 12.4)
Number of affected nerves				
≤ 1 nerve	252	47.6	1.0	
> 1 nerve	277	52.4	2.4	(1.3; 4.4)
Bacilloscopic index				
negative	267	49.1	1.0	
positive < 2	72	13.2	5.3	(2.6; 10.8)
positive ≥ 2	205	37.7	191.2	(26.1; 1.399.0)

CI: confidence intervals.

TABLE 4

Evaluation of the odds of testing ML Flow positive. according to the number of affected nerves and skin lesions.

Nº of Nerves	Nº of Lesions	ML FLOW		Odds ratio	CI (95%) p/ odds ratio
		Positive	Negative		
≤ 1	< 6	107	373	1.0	-
> 1	< 6	81	69	4.1	(2.7; 6.1)
≤ 1	≥ 6	145	32	15.8	(10.0; 25.1)
> 1	≥ 6	196	38	18.0	(11.7; 27.7)

CI: confidence intervals.

DISCUSSION

Early diagnosis of leprosy and its correct classification are important tools to ensure the quality of patient treatment and disease control.

There were a similar number of men and women among the cases studied, although it is known that women are predominant among the general population of Minas Gerais (**Table 1**). Differences in the detection of leprosy among men and women have been previously cited[12 20 21 24 27 28]. Among new cases diagnosed in Minas Gerais in 2003, 51.1% were men, while the state population was 52% female[20]. In Nigeria more women (56.7%) were attended in out-patient clinics, while men were the majority (75.3%) in hospitals[28]. Although biological factors appear to play an important role in protecting women from leprosy and other infections, these cannot satisfactorily explain the differences cited above. It is probable that, besides biological factors, economic, sociocultural and operational aspects related to health services are equally important. Even though there were more male patients who presented seropositivity in this study, when the statistical models were adjusted for the other variables (multivariate analysis), no statistically significant association was observed between the variables of sex and seropositivity (**Tables 2 and 3**).

In reference to age, leprosy is a disease that occurs in young adults and the middle-aged, with a higher number of cases registered in the age group from 20 to 50 years[21 24]; a fact confirmed in this study in which the average age was 42 years-old (**Table 1**), thus showing that leprosy affects the individual in the most productive years. The percentage of children under 15 years-old among the cases studied (6.9%) was higher than the average of all new cases diagnosed in Minas Gerais in 2003 (5.8%)[20], yet lower than Brazil as a whole in that year (8%)[3] (**Table 1**). Although the under-15 age group represented a small percentage of all seropositive cases (2.4%), nearly a fifth of the children studied (17.6%) were seropositive. This fact may suggest that the number of MB cases in this age group is greater than expected, thereby further indicating the importance of ML Flow in the classification of leprosy in children.

Regarding the mode of detection, observation showed that referrals were the predominant source, something that was true for all new cases in the State in 2003[20]. This may be related to leprosy control training provided to Family Health Program teams, which in many cases still do not have the skills to diagnose cases, but can refer suspected cases from their areas of coverage for

confirmation of diagnosis in centers with greater experience. This procedure alters the means of detection, in that cases that would otherwise be listed as having been diagnosed by means of contact examination or self-presentation would be notified as referrals after confirmation of diagnosis in other referral centers.

In this study, the vast majority of patients presented no neural thickening or only one affected nerve (**Table 1**); less involvement of peripheral nerves was observed in relation to cases studied in Africa[13] or India[12].

The majority of subjects studied (60.4%) presented five skin lesions or less (**Table 1**), a fact also observed in Brazil by Lyon *et al*[16] (56.3%), Castorina-Silva[9] (65%) and in Ethiopia (80%)[23], though in disagreement with a study conducted in Rio de Janeiro, in which 73.4% of patients presented six or more skin lesions[10].

The percentage of grade 2 disability among the new cases analyzed (7%) is considered average by Ministry of Health parameters[24] (**Table 1**). The risk of seropositivity increased with higher disability grade, demonstrating the importance of early diagnosis and the use of ML Flow in the correct classification of these cases (**Table 2**).

A majority of the new cases under study were treated at referral centers (**Table 1**), despite the fact that the Single Health System (SUS) introduced decentralized leprosy care in 1998, integrating the diagnosis and treatment of leprosy into the larger scope of services provided at the primary care level[32].

The positivity of bacilloscopic results in this study (27%), while similar to other studies in the literature[6 30] (**Table 1**), was lower than that observed by Lyon *et al*[16] (35.9%) and by Castorina-Silva[9] (40%) in a referral center in Minas Gerais, yet much higher than that obtained in Nepal (11.6%)[8]. A higher percentage was reported by Gallo *et al* (77.9%)[10], but this may be explained by the fact that their cases were treated at a national reference center, where more complex cases are attended and a higher quality of bacilloscopic exam is maintained.

In agreement with the existing correlation between bacilloscopy and serology, observation revealed that seropositivity (50.7%) was lower than that described by other authors in the same state, Lyon *et al* (57%)[16], Castorina-Silva (70%)[9] and in Nigeria (62.9%)[8], yet higher than that obtained in Nepal (35.6%)[8]. These differences can probably be explained by the clinical and epidemiological characteristics of the patients and by the type of health center involved.

Seropositivity (50.7%) was almost twice as high as bacilloscopic positivity (27%), suggesting that the sensitivity of the latter exam is low, as discussed by several authors[1 6 13 23 30 31] (**Table 1**). This suggests that the use of serology by health services may aide in the correct classification and treatment of true multibacillary cases, thereby collaborating in the elimination of potential sources of infection, the prevention of relapse and increased coverage of leprosy control at the basic health care level.

ML Flow showed a high correlation with bacterial load, with positive results in all patients with a BI $\geq$ 2 and nearly all patients with 0 < BI < 2. Additionally, it was able to detect *M.Leprae*-specific antibodies in more than a third of individuals with a negative bacilloscopy (**Table 3**). Bacilloscopy is known to have numerous technical difficulties and low reliability[6 30], in contrast with the high replicability and reliability observed with ML Flow.

The probability of seropositivity increased according to the number of affected nerves or skin lesions, although the increase was higher with skin lesions. A patient with up to five lesions and more than one thickened nerve had a four-fold higher chance of being seropositive than a patient with zero or one nerve affected. A patient with six or more skin lesions and up to one thickened nerve had nearly a 16-fold probability of being seropositive than a patient with up to five lesions and zero or one nerve involved. A patient with six or more skin lesions and more than one thickened nerve had nearly a 18-fold probability of being seropositive than a patient with up to five lesions and zero or one nerve involved. Therefore, the increased chance of seropositivity was greater with a higher number of skin lesions than with a higher number of affected nerves, while no multiplier effect was observed that might be expected from an increase in both variables (**Table 4**). This fact is in agreement with the operational classification that uses the number of skin lesions, but no longer includes the number of thickened nerves[4 5 25].

Seropositivity was statistically associated with patients over the age of 15 years-old, more than five skin lesions, more than one affected nerve and positive bacilloscopy (**Table 3**), suggesting that ML Flow could be used as a reliable means of correctly classifying leprosy patients, ensuring the quality of patient treatment and disease control.

ACKNOWLEDGEMENTS

REFERENCES

1. Araújo MG. Hanseníase no Brasil. Artigo de atualização. Revista da Sociedade Brasileira de Medicina Tropical 36: 373-382, 2003.

2. Brasil. Ministério da Saúde. Área Técnica de Dermatologia Sanitária. *Hanseníase* - Atividades de Controle e Manual de Procedimentos. Brasília, 2001.

3. Brasil. Ministério da Saúde. DATASUS/SINAN/MS/IBGE. Disponível em: <www.saude.gov.br>. Acesso em: 15 out. 2004.

4. Brasil. Ministério da Saúde. *Guia para o controle da hanseníase*. (Cadernos de Atenção Básica, n. 10) Brasília, 2002.

5. Brasil. Ministério da Saúde. Hanseníase *in* Vigilância em Saúde. (Cadernos de Atenção Básica 21: 70-104), Brasília, 2007.

6. Britton WJ, Lockwood DNJ. Seminar leprosy. The Lancet 363: 1209-1219, 2004.

7. Bührer-Sékula S, Smits HL, Gussenhoven GC, 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.

8. Bührer-Sékula S, Visschedijk J, Grossi MAF, 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.

9. Castorina-Silva R. Estudo do comportamento dos testes sorológicos ML Flow e ELISA (PGL-I) em áreas endêmica e não endêmica de hanseníase. Tese (Doutorado em Ciências da Saúde). Universidade Federal de Minas Gerais, Belo Horizonte, 2008.

10. Gallo EN, Nery AC, Junior Lanr, Sales AM, Albuquerque ECA. Alocação do paciente hanseniano na poliquimioterapia: correlação da classificação baseada no número de lesões cutâneas com os exames baciloscópicos. Anais Brasileiros de Dermatologia 78: 415-424, 2003.

11. Hosmer DW, Lemeshow S. *Applied logistic regression*. John Wiley, New York, 1989.

12. Jain S, Reddy OSN, Lockwood DNJ, Suneetha S. Childhood leprosy in an urban clinic, Hyderabad, India: clinical presentation and the role of household contacts. Leprosy Review 73: 248-253, 2002.

13. Keita S, Faye O, Konare HD, Sow SO, Ndiaye HT, Traore I. Evaluation de la classification clinique des nouveaux cas de lèpre. Etude réalisée à l'Institut Marchoux (Bamako, Mali). Annales de Dermatologie et de Venerologie 130: 184-186, 2003.

14. Leboeuf MAA, Grossi MAF. Prevalência oculta de hanseníase nas áreas de abrangência das diretorias regionais de saúde. Belo Horizonte: Secretaria de Estado da Saúde de Minas Gerais, 2000.

15. Lehman LF, Orsini MBP, Grossi MAF, Villarroel MF. A mão na hanseníase. *In:* Freitas PP (ed) Reabilitação da mão. São Paulo: Atheneu, 301-318, 2005.

16. Lyon S, Lyon AC, Silva RC, Grossi MAF, Lyon SH, Bührer-Sékula S, Rocha MOC. A comparison of ML Flow serology and slit skin smears to assess the bacterial load in newly diagnosed leprosy patients in Brazil. Leprosy Review 79: 1-9, 2008.

17. Maeda SM, Rotta O, Michalany NS, Camargo ZP, Sunderkötter C, Tomimori-Yamashita J. Comparison between anti- pgl-1 serology and mitsuda reation: clinical reading, microscopic findings and immunohistochemical analysis. Leprosy Review 74: 263-274, 2002.

18. Martelli CMT, Andrade ALSS, Grossi MAF, Leboeuf MAA, Lombardi C, Zicker F. Clanges in leprosy pattern after multidrug therapy implementation. International Journal of Leprosy 63: 95-97, 1995.

19. Meima A. The impact of leprosy control. Epidemiological and modelling studies. Thesis Erasmus MC, University Medical Center Rotterdam, 2004.

20. Minas Gerais. Secretaria de Estado da Saúde. Coordenadoria Estadual de Dermatologia Sanitária. Hanseníase: procurar para curar e eliminar. Belo Horizonte, out. (Cadernos do Seminário Estadual de Hanseníase), 2004.

21. Moreira TMA, Varkevisser MC. Gender leprosy and leprosy control: a case study in Rio de Janeiro State, Brasil. Amsterdã: Royal Tropical Institute Publishers, 2002.

22. Naafs B, Silva E, Vilani-Moreno F, Marcos EC, Nogueira ME, Opromolla DV. Factors influencing the development of leprosy: An overview. International Journal of Leprosy and Other Mycobacterial Diseases 69: 26-33, 2001.

23. Norman G, Joseph G, Richard J. Validity of the WHO Operational classification and value of other clinical signs in the classification of leprosy. International Journal of Leprosy and Other Mycobacterial Diseases 72: 278-83, 2004.

24. Opromolla DVA. Noções de hansenologia. Bauru: Centro de Estudos Dr. Reynaldo Quagliato, 2000.

25. Organização Mundial da Saúde. Disponível em: <www.who.int>. Acesso em: 15 out. 2004.

26. Oskam L, Slim E, Bührer-Sékula S. Serology: recent developments, strengths, limitations and prospects: A state of the art overview. Leprosy Review 74: 196-205, 2003.

27. Peters ES, Eshiet AL. Male-female (sex) differences in leprosy patients in South Eastern Nigeria: females present late for diagnosis and treatment and have higher rates of deformity. Leprosy Review 73: 262-67, 2002.

28. Pfaltzgraff RE. What is the actual male/female sex ratio in leprosy pacients? Leprosy Review 74: 180-181, 2003.

29. Ridley DS, Jopling WH. A Classification of leprosy for research purpores. Leprosy Review 33: 119-128, 1962.

30. Summary of the Report of The International Leprosy Association Technical Forum. International Journal of Leprosy and Other Mycobacterial Diseases 70: (suppl 1), 2002.

31. Ustianowski AP, Lockwood DNJ. Leprosy: current diagnostic and treatment approaches. Current Opinion Infectious Diseases 16: 421-427, 2003.

32. Visschedijk J, Engelhard A, Lever P, Grossi MAF, Feenstra P. Leprosy control strategies and the integration of health services: an international perspective. Cadernos de Saúde Pública, 19: 1567-1581, 2003.

33. Visschedijk J, Van de Broek J, Eggens H, Lever P, Van Beers S, Klatser P. Review: Mycobacterium leprae - millennium resistant! Leprosy control on the threshold of a new era. Tropical Medicine and International Health 5: 388-399, 2000.