The result patterns of ML Flow and ELISA (PGL-I) serological tests in leprosy-endemic and non-endemic areas

Comportamento dos testes sorológicos ML Flow e ELISA (PGL-I) em áreas endêmica e não endêmica de hanseníase

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ABSTRACT

ML Flow and anti-PGL-I ELISA are serological tests that detect IgM antibodies against the phenolic glycolipid I (PGL-I), specific to *Mycobacterium leprae*. To evaluate the outcomes of ML Flow and ELISA (PGL-I) serological tests in leprosy-endemic areas in comparison to non-endemic ones, a total of 351 volunteers from Brazil and Chile were examined, including leprosy patients, healthy controls and others affected by other infectious or non-infectious diseases that are common differential diagnoses for leprosy. The ELISA cut-off point was established using the ROC Curve method (≥ 0.157). In endemic areas, 70% of leprosy patients present positive ML Flow results and 53.3% were ELISA-positive. In non-endemic areas, ML Flow was negative in all the subjects tested and ELISA was positive in 4 volunteers. ML Flow is faster and more easily performed and, therefore, a more adequate test for use in basic, primary-level health care centers. ELISA requires trained personnel, in addition to a more complex laboratory infrastructure.

Key-words: Serologic tests. PGL-I antigen. Leprosy. ML Flow. ELISA.

RESUMO

O ML Flow e o ELISA PGL-I são testes sorológicos que detectam anticorpos IgM contra o glicolipídio fenólico I específico do *Mycobacterium leprae*. Para avaliar o comportamento destes testes em áreas endêmica e não endêmica para hanseníase foram estudados 351 voluntários no Brasil e no Chile, incluindo pacientes com hanseníase, controles sadios, portadores de outras doenças infecciosas, não infecciosas e dermatoses que fazem diagnóstico diferencial com hanseníase. O ponto de corte do ELISA foi estabelecido pelo método da Curva ROC ($\geq 0{,}157$). Em área endêmica, o ML Flow apresentou resultados positivos em 70% dos pacientes com hanseníase; o ELISA foi positivo em 53,3%. Em área não endêmica, o ML Flow foi negativo em todos os voluntários testados; o ELISA foi positivo em 4 voluntários. O ML Flow é um ensaio mais rápido, facilmente aplicável e, portanto, mais adequado para ser utilizado na Atenção Básica; o ELISA necessita, alem de uma infra-estrutura de laboratório adequada, pessoal treinado e especializado em sua execução.

Palavras-chaves: Testes sorológicos. Antígeno PGL-I. Hanseníase. ML Flow. ELISA.

Leprosy is still an endemic disease in a few countries, including Brazil. It was prevalent throughout the world, even in Europe and North America, in different periods of human evolution. The disease is most commonly found in tropical and subtropical regions and is closely tied to areas of poverty[3].

In 1953, Brazil registered 62,010 recognized cases of leprosy among a population of 55,211,268 million[14], while in Chile there was not a single registered case. In 1982, there were still no cases of leprosy in mainland Chile[7], but in 1999, three leprosy cases were registered in that country among Polynesian[1] immigrants who had been working in Peru[11]. Most likely, due to factors such as climate, topography and immunity conferred by the BCG vaccination programs, Continental Chile inhabitants seems to have a greater immunity against leprosy[7]. There is also a theory which considers that the DNA of Chileans protects them against the disease[7].

According to 2006 data from the World Health Organization (WHO), Brazil was considered the only endemic country in the Americas, registering the majority of new cases diagnosed that year: 44,436 cases. Chile did not register a single case that year. In 2006, the prevalence of active leprosy cases in Brazil was 60,567[17].

Assuming that the prevalence of seropositivity in the general population reflects the rate of exposure or infection[2 8], serological tests may be of great advantage in determining the extent of leprosy in a community, as well as serving as an indicator of control measures through repeated application[10 15]. Perhaps it is possible to monitor alterations in exposure intensity to *Mycobacterium leprae* and determine epidemiological tendencies, such as the level of transmission in a given community[15]. Bach *et al* showed that serology can be an useful method in the clinical follow-up of leprosy patients given that the levels of antibodies diminish during the administration of multi-drug therapy (MDT)[1].

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Since the beginning of the 21st century, several methods have been developed to study the antibodies specific to *Mycobacterium leprae* and currently there are several mycobacterial antigens available for research in leprosy patients[12]. The most commonly used method is ELISA, indirect enzyme-linked immunosorbent assay[6,13], to identify the presence of IgM antibodies against the species-specific phenolic glycolipid I of *Mycobacterium leprae* (PGL-I), especially IgM. ML Flow is a fast and simple test that was developed in 2003[5] and it has proven to be an easy exam to conduct that does not require a laboratory infrastructure. Therefore, it is possible to use as an auxiliary tool in the classification of patients for treatment in primary health care units.

This study evaluated the pattern of result patterns of ML Flow and ELISA (PGL-I) serological tests in a leprosy-endemic area and in a non-endemic area. Although previous studies have been conducted in non-endemic areas, there may have been immigrants from endemic countries that could have developed antibodies to *Mycobacterium leprae*, something that has not happened in the population studied in Chile.

MATERIAL AND METHODS

This study was conducted in Brazil and Chile, which are considered leprosy endemic and non-endemic countries, respectively. The Brazilian component was conducted in the out-patient clinic of the Eduardo de Menezes Hospital, which is the reference center for sanitary dermatology in the Minas Gerais State Hospital Foundation. The Chilean component took place in the Internal Medicine sector of the Hospital del Salvador (University of Chile, western campus), in Santiago.

The population studied in the leprosy-endemic area consisted of 60 new leprosy cases, 28 hepatitis patients, 29 HIV-infected patients, 27 tuberculosis (TB) patients, 10 cases of other tropical diseases (pemphigus foliaceus, paracoccidioidomycosis), and six patients with psoriasis. The healthy controls (72 individuals with no symptoms of clinical disease) were blood donors and volunteers from the general population.

The non-endemic study sample consisted of 27 tuberculosis patients, 33 AIDS patients and 28 patients suffering from autoimmune inflammatory illnesses (rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis) and 30 health care professionals with no clinical symptoms, who served as the controls.

The ML Flow and ELISA tests were conducted following the steps previously described in other studies[5]. ML Flow results were registered in qualitative (positive or negative) and semiquantitative (0, 1+, 2+, 3+ and 4+) terms; the cutoff point was established by the ROC (Receiver Operating Characteristic) curve method; positive results were considered when optical density at 450/630nm was equal to or greater than 0.157. For both methods, a semi-synthetic antigen was used: natural trisaccharide linked to bovine serum albumin (NT-P-BSA). The statistical analysis was completed using the ROC curve method, univariate analysis and agreement studies.

This study was approved by the Research Ethics Committee of the Federal University of Minas Gerais in document nº 393/07. The subjects evaluated agreed to participate in the study and signed free informed consent forms.

RESULTS

The ML Flow results in the non-endemic area were all negative; in the leprosy-endemic area, ML Flow was positive in 70% (42/60) of leprosy patients, 6.9% (5/72) of the healthy controls, 7.1% (2/28) of hepatitis patients, 3.4% (1/29) of HIV-positive cases, 11.1% (3/27) of TB patients, 20% (2/10) of patients with other tropical diseases and 33.3% (2/6) of psoriasis patients (**Table 1**). It should be noted that the last two groups were comprised of only 10 and 6 participants, respectively.

The ELISA (PGL-I) test in the non-endemic area was positive results were observed in 53.3% of leprosy patients in 3.4%

TABLE 1

Frequency distribution of semiquantitative results of the ML Flow test in leprosy-endemic and non-endemic areas, 2006.

	Groups	ML Flow										Total
		0		1+		2+		3+		4+		
		nº	%	nº	%	nº	%	nº	%	nº	%	nº
Brazil	Leprosy	18	30.0	12	20.0	6	10.0	7	11.7	17	28.3	60
	Control	67	93.1	5	6.9	0	-	0	-	0	-	72
	Hepatitis	26	92.9	1	3.6	1	3.6	0	-	0	-	28
	AIDS	28	96.6	1	3.4	0	-	0	-	0	-	29
	Tuberculosis	24	88.9	1	3.7	2	7.4	0	-	0	-	27
	Tropical diseases	8	80.0	2	20.0	0	-	0	-	0	-	10
	Psoriasis	4	66.7	1	16.7	1	16.7	0	-	0	-	6
Chile	Control	30	100.0	0	-	0	-	0	-	0	-	30
	Tuberculosis	27	100.0	0	-	0	-	0	-	0	-	27
	AIDS	33	100.0	0	-	0	-	0	-	0	-	33
	Inflamatory diseases	28	100.0	0	-	0	-	0	-	0	-	28

(4/118) of individuals. In the leprosy-endemic area, positive results were observed in 53.3% of leprosy patients, in 10.7% of TB cases and in 6.9% of healthy controls (**Table 2**).

The agreement study between ML Flow and ELISA (PGL-I) showed a kappa coefficient of 0.628, which is considered substantial (**Table 3**).

TABLE 2

Frequency distribution of the ELISA (PGL-I) serologic test in leprosy-endemic and non-endemic areas. 2006.

Group	Number	Frequency of seropositivity 0.157 cut-off point	
		nº	%
Endemic region			
control	72	5	6.9
leprosy	60	32	53.3
hepatitis	28	0	
AIDS	29	0	
tuberculosis	28	3	10.7
tropical diseases	10	0	
psoriasis	6	0	
subtotal	233	40	17.2
Non-endemic region			
control	30	1	3.3
AIDS	33	1	3.0
tuberculosis	27	1	3.7
inflamatory diseases	28	1	3.6
subtotal	118	4	3.4

TABLE 3

Agreement between the ML Flow and anti-PGL-I ELISA serologic tests (*cut-off* ≥0.157) in all groups studied, 2006.

		ML Flow		
		negative	positive	Total
ELISA (PGL-I ≥ 0,157)	negative	285	23	308
	Positive	9	34	43
	Total	294	57	351

kappa coefficient = 0.628 CI 95% (0.510 ; 0.746), p-value = 0.000

DISCUSSION

Seronegativity was observed in the ML Flow tests of all subjects in the non-endemic area. This shows that populations in these areas do not develop antibodies against the *Mycobacterium leprae*-specific PGL-1 antigen. The percentage of seropositivity detected using ML Flow in healthy controls from the endemic area was 6.9%. This proportion was lower than that reported in previous studies, which showed a 12% positivity rate in controls. This may suggest the possibility of previous contact with *Mycobacterium Leprae*, but with no clinical manifestation of the disease[5].

In this study, seropositivity in the ML Flow test among leprosy patients was 70%, while in other studies the percentages were 72.9%[5]; 50.7%[9] and 57%[11]. The high positivity may be explained by the fact that all patients in the present study were identified at the state referral centre, where more complex cases, predominantly multibacillary patients, are referred to for specialized treatment.

The proportion of seropositivity of the ELISA (PGL-I) test in the non-endemic area was low; the majority of patients with a positive result presented titer levels very close to the established cutoff point. These results are compatible with previous studies[4,5].

The study showed that the positivity of the ML Flow and ELISA tests in individuals without leprosy varies according to the level of endemicity in the geographic area where the test is applied. In the non-endemic area, ML Flow seronegativity was witnessed in all participants and only 4 of them were ELISA-positive, regardless of their disease diagnosis. Current etiological models indicate that for infectious diseases, the agent is necessary but not sufficient for the manifestation of disease[6].

CONCLUSION

The ML Flow and ELISA tests show similar outcomes in the detection of anti-PGL-I antibodies. The possible expression of the PGL-I antigen in other microorganisms does not seems to compromise the outcome of these tests in the allocation of treatment for leprosy patients. Positivity of the ML Flow serological test in individuals without leprosy varies significantly according to the level of endemicity in the geographic area where the test is applied.

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