Factors associated with ML Flow test seropositivity in leprosy patients and household contacts under the age of 18

Fatores associados à soropositividade do teste ML Flow em pacientes e contatos de pacientes com hanseníase menores de 18 anos

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ABSTRACT

This article presents the results of a cross-sectional study involving 115 newly-diagnosed leprosy patients and 1,011 household contacts, all under the age of 18. Seropositivity ratios and factors associated with a positive ML Flow were determined. The test was positive in 21.7% of patients and in 19.7% of contacts. Among patients, logistic regression indicated the association of seropositivity with positive slit skin smear and having more than five skin lesions. The tree method analysis indicated associations with skin smear, Madrid classification, number of nerves affected and age. In the contact group, index case classification, age and type of health service were associated with seropositivity in both analyses. The variables that best explained seropositivity are those associated with higher bacterial load. Therefore, the ML Flow test could be used in children to aid correct classification of patients for treatment and to identify contacts at greater risk of developing leprosy.

Key-words: Leprosy. Serologic tests. Leprosy/transmission.

RESUMO

Estudo transversal em menores de 18 anos, sendo 115 casos novos de hanseníase e 1.011 contatos intradomiciliares. Determinaram-se as proporções da soropositividade do ML Flow e fatores associados ao teste positivo. Observou-se soropositividade em 21,7% dos pacientes e 19,7% dos contatos. Nos pacientes, a regressão logística indicou associação com baciloscopia positiva e número de lesões cutâneas maior que cinco. A análise por árvore de decisão mostrou associação com baciloscopia, classificação de Madri, número de nervos acometidos e idade. Nos contatos, as duas análises indicaram as mesmas associações: classificação do caso-índice, idade e tipo de serviço de saúde. As variáveis que explicaram melhor a soropositividade, em menores de 18 anos, são aquelas associadas à maior carga bacilar. Assim, o teste ML Flow poderia ser utilizado também na infância para ajudar na correta classificação dos pacientes para tratamento e na identificação dos contatos com maior risco de desenvolver hanseníase.

Palavras-chaves: Hanseníase. Testes sorológicos. Hanseníase/transmissão.

Leprosy in children is a public health problem which reflects early exposure to the disease, community-level transmission and the limited efficacy of control programs[1 13 17]. Several studies show that greater risk of presenting leprosy exists among household contacts, especially those who live with multibacillary (MB) patients[2 6 16].

The importance of research involving leprosy among children relates to the fact that this disease affects the skin, the largest and most visible organ of the body, and has the potential to cause physical disfiguration. The under-18 age group represents 36% of the Brazilian population[10]. The majority of leprosy cases in this age group are the non-contagious form, with few skin lesions and a negative skin smear. If diagnosis and treatment occur early and

correctly, the tendency in these cases is to progress to a complete cure with no sequelae.

Early detection and correct classification of leprosy cases to define the appropriate treatment regimen are challenges to the control of this disease and justify the search for simpler methods to determine the correct classification of cases[8].

ML Flow is an immunochromatographic serological test that detects IgM antibodies against phenolic glycolipid 1 (PGL-1) of *Mycobacterium leprae*. It was developed by the Royal Tropical Institute in the Netherlands and is a fast test with simple application and reading that does not require a laboratory or any special equipment[5].

Seropositivity is related to the bacterial load, but it is not a test for leprosy diagnosis. It has been proposed as a supplementary tool in classifying patients for treatment with multidrug therapy (MDT) and to identify household contacts of patients at greater risk of developing the disease in the future. However, in studies of ML Flow in the literature, when the age of the study population is mentioned, the majority of individuals are over the age of 15 years-old[3 4 8 14].

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The objective of this study was to determine the seropositivity ratios and the factors associated with ML Flow test seropositivity in leprosy patients and household contacts under the age of 18 years-old.

MATERIAL AND METHODS

This is a cross-sectional study that uses secondary data from the Minas Gerais State Health Secretariat research database concerning the ML Flow test. The study population consisted of 115 new leprosy cases and 1,011 household contacts of leprosy patients, all under the age of 18, who were submitted to the ML Flow test during the period of October 2002 to March 2004.

The ML Flow test was performed using whole blood taken via finger prick. Test results readings were performed after five minutes and the presence of coloration in the nitrocellulose strip indicated a positive result[11]. The definition of a household contact used in the study was any and all persons who reside or resided with an index case of leprosy in the preceding five years[15].

The sample detection power was 99.9%, calculated based on the final logistic regression model in accordance with the method proposed by Hosmer and Lemeshow[9].

Independent statistical analyses were performed for the patient and contact sample groups. The outcome variable was the result of the ML Flow test (negative and positive). The explanatory variables were demographic (i.e. sex, age and type of health service used), clinical (i.e. number of skin lesions, number of nerves affected, disability grade, Madrid classification, treatment classification, index case classification and BCG scar) and laboratory tests (skin smear and bacterial index, BI).

Treatment classification refers to the type of MDT administered to the patient. As per the research protocol for the ML Flow test, a negative skin smear and a negative ML Flow determined the case classification as paucibacillary (PB). A positive skin smear or a positive ML Flow determined a MB classification, regardless of clinical manifestations[11].

In the descriptive analysis, frequency distribution tables and measures of central tendency, position and variability were used. Univariate analysis was conducted using Pearson's chi square test, the Fisher exact test or Student t test. Odds ratios (OR) and their respective 95% confidence intervals (95%CI) were estimated. To construct the logistic regression models for binary observations, all independent variables were considered that had a p-value lower than or equal to 0.25 in the univariate analysis. The logistic models were adjusted, eliminating the variables individually. The criterion for the variables to remain in the final model was a p-value lower than 0.05 using the Wald test. The Hosmer-Lemeshow test was used to assess the model's goodness-of-fit[9]. In addition, the CART (Classification and Regression Trees) algorithm was used for the tree method analysis. The models were adjusted with successive binary division of the data sets. The stop criterion was a minimum improvement value of 0.01. The final fit of the model was evaluated by the estimate of general risk and cross-validation procedure.

The SPSS® 12.0 (2003) and Answer Tree® 3.0 (2001) statistical software packages were used for these analyses. The

Research Ethics Committee of the Federal University of Minas Gerais approved this study on May 18, 2006, under protocol no. ETIC 312/04 (addendum).

RESULTS

Descriptive analysis of the data (**Table 1**) showed that the majority (60.9%) of patients under 18 years of age were female. Regarding the means of detection, 22.6% of the cases were diagnosed through contact examinations. It was also noted that the majority of patients presented five or fewer skin lesions and the same percentage (82.6%) presented zero or one affected nerve. The vast majority (93%) of patients were diagnosed with a zero disability grade. Skin smear was positive in 6.5% of patients. A positive BI of two or more was present in 2.8% of cases. Equal proportions (34.8%) of indeterminate and tuberculoid forms of the disease were observed. A proportion of 4.3% of patients were classified as lepromatous. For treatment purposes, 69.6% of patients were classified as PB. The largest percentage (48.7%) of patients was examined at regional referral services. The average age of the patients was 11.8 years-old. ML Flow test seropositivity in this sample of patients was 21.7%.

Examining the household contacts (**Table 2**), the distribution by sex was almost equal, with 50.2% females. The majority (72.8%) of index cases were MB. Among the contacts, 63.1% had one BCG vaccine scar. The largest percentage of contacts (44.2%) was examined at basic health services. The average age of the contacts was 10.4 years-old. ML Flow seropositivity in this sample of contacts was 19.7%.

According to the results of the univariate analysis of patients (**Table 1**), a statistically significant difference (p < 0.05) in seropositivity occurred for the following variables: number of skin lesions, number of nerves affected, skin smear, BI, Madrid classification, treatment classification and age. Higher seropositivity in the ML Flow test was found among patients with more than five skin lesions (55%), more than one affected nerve (40%), a positive skin smear (85.7%) and a BI equal to or greater than 2 (100%). ML Flow seropositivity was also higher in patients classified as lepromatous and borderline (100% and 66.7% respectively) and in patients classified as MB for treatment purposes (71.4%). The average age of patients with a positive ML Flow test was 13.1 years-old; those with a negative result averaged 11.4 years-old.

For contacts (**Table 2**), a statistically significant difference in seropositivity (p < 0.01) was observed for the following variables: index case classification, type of health service and age. The prevalence of positive ML Flow results was higher among contacts of MB patients (22.6%) and for contacts examined at basic health services (25.3%). Seropositive contacts were older (average age 11.5 years-old) than seronegative contacts (average age 10.1 years-old).

Table 3 shows the final logistic regression models for patients and contacts. The significant variables associated with seropositivity in the ML Flow test in patients were skin smear and number of skin lesions. Patients with a positive skin smear had an 18-fold greater chance of being seropositive than those with

TABLE 1

Descriptive and univariate analyses of the association between ML Flow test result and the characteristics of 115 leprosy patients under the age of 18 years-old, Minas Gerais, Brazil, October 2002 to March 2004.

Patient characteristics	Total (nº =115) %	ML Flow Positive (nº =25) %	Odds ratio	95% CI for odds ratio	P-value
Sex					
female	60.9	20.0	1.00	—	0.573[1]
male	39.1	24.4	1.29	[0.53; 3.17]	
Means of detection					
contact examination	22.6	11.5	1.00	—	0.191[1]
spontaneous demand	32.2	18.9	1.79	[0.42; 7.68]	
others	45.2	28.8	3.11	[0.81; 11.92]	
Number of skin lesions					
≤ 5 lesions	82.6	14.7	1.00	—	**<0.001²**
> 5 lesions	17.4	55.0	7.07	[2.48; 20.16]	
Number of nerves affected					
≤ 1 nerve	82.6	17.9	1.00	—	**0.029[1]**
> 1 nerve	17.4	40.0	3.06	[1.08; 8.63]	
Disability grade					
grade 0	93.0	20.6	1.00	—	0.065²
grade 1	5.2	16.7	0.77	[0.09; 6.96]	
grade 2	1.7	100.0	*		
Slit skin smear					
negative	93.5	18.8	1.00	—	**0.001²**
positive	6.5	85.7	25.89	[2.94; 227.94]	
Bacterial index					
negative	94.4	18.8	1.00		**<0.001²**
positive < 2	2.8	100.0	*	*	
positive ≥ 2	2.8	100.0	*	*	
Madrid classification					
indeterminate	34.8	0.0	1.00		**<0.001²**
tuberculoid	34.8	0.0	*	*	
borderline	26.1	66.7	*	*	
lepromatous	4.3	100.0	*	*	
Treatment classification					
paucibacillary	69.6	0.0	1.00		**<0.001²**
multibacillary	30.4	71.4	*	*	
Type of health service					
basic health service	44.3	21.6	1.00	—	0.519[1]
regional referral service	48.7	19.6	0.89	[0.35; 2.27]	
state referral service	7.0	37.5	2.18	[0.45; 10.58]	
Age					
average	11.8	13.1**	1.16[4]	[1.01; 1.33][4]	**0.035³**
standard deviation	3.6	3.6			
ML Flow test					
negative	78.3				
positive	21.7				

[1]pearson's Chi square test, ²fisher exact test, ³student t test for independent samples, [4]odds ratio and 95% CI calculated based on the logistic regression model for univariate binary observations.

*odds ratio could not be calculated due to the null frequency of the matrix.

**the average age of patients with a negative ML Flow was 11.4 years-old.

CI: confidence intervals.

TABLE 2

Descriptive and univariate analyses of the association between ML Flow test result and the characteristics of the 1,011 under-18 household contacts of leprosy patients, Minas Gerais, Brazil, October 2002 to March 2004.

Patient characteristics	Total (nº = 1,011) %	ML Flow Positive (nº = 199) %	Odds ratio	95% CI for odds ratio	P-value
Sex					
female	50.2	20.5	1.00	—	0.526[1]
male	49.8	18.9	0.90	[0.66; 1.23]	
Index case classification					
paucibacillary	27.2	12.0	1.00	—	**<0.001[1]**
multibacillary	72.8	22.6	2.13	[1.42; 3.19]	
BCG scar					
none	1.2	8.3	0.35	[0.04; 2.79]	0.548[1]
one scar	63.1	19.1	0.92	[0.67; 1.24]	
two scars	35.7	20.4	1.00	—	
Type of health service					
basic health service	44.2	25.3	1.00	—	**<0.001[1]**
regional referral service	33.2	17.9	0.64	[0.45; 0.9]	
state referral service	22.6	11.4	0.38	[0.24; 0.60]	
Age					
average	10.4	11.5*	1.07[3]	[1.03; 1.11][3]	**<0.001[2]**
standard deviation	4.6	4.4			
ML Flow					
negative	80.3				
positive	19.7				

[1]pearson Chi square test, [2]Student t test for two independent samples, [3]odds ratio and 95% CI calculated based on the logistic regression model for univariate binary observations.

*the average age of contacts with a negative ML Flow was 10.1 years-old.

CI: confidence intervals.

TABLE 3

Fitted logistic regression models for under-18 leprosy patients and household contacts, using the ML Flow result as dependent variable, Minas Gerais, Brazil, October 2002 to March 2004.

Patient sample (nº =108)*			Contact sample (nº =1,009)**		
Characteristics	Odds ratio	95% CI for odds ratio	Characteristics	Odds ratio	95% CI for odds ratio
Slit Skin smear			Index case Classification		
negative (baseline)	1.00	—	paucibacillary (baseline)	1.00	—
positive	18.09	[1.87; 175.23]	multibacillary	2.31	[1.53; 3.48]
Skin lesions (nº)			Type of health service		
≤ 5 lesions (baseline)	1.00	—	basic health service (baseline)	1.00	—
> 5 lesions	5.86	[1.85; 18.55]	regional referral service	0.70	[0.49; 0.99]
—			state referral service	0.35	[0.22; 0.56]
—			Age (years)	1.06	[1.03; 1.10]
P-value Hosmer − Lemeshow test	0.892		P-value Hosmer − Lemeshow test		0.828

*seven (6.1%) patients without skin smear results, **two (0.2%) contacts without information on the classification of index case, CI: confidence intervals.

a negative smear. Patients with more than five skin lesions had a 5.86-fold greater chance of being seropositive than those with less than five lesions.

For contacts, the variables associated with a positive ML Flow result were: index case classification, type of health service and age. Contacts of a MB index case were 2.3 times more likely to be seropositive than contacts of a PB index case. Contacts seen at regional referral service were 0.7 times less likely to test seropositive than those examined at basic health services, while those seen at State referral services were 0.35 times less likely to test seropositive. In relation to age, analyzed in years, the OR was 1.06; i.e., for every additional year, the chance of the contact presenting a positive ML Flow result was 1.06-fold greater.

The patient and contact models showed good fits using the Hosmer-Lemeshow test, with p-values of 0.892 and 0.828, respectively.

The patients classification tree revealed the following factors were associated with seropositivity: Madrid classification type, number of affected nerves, skin smear and age (**Figure 1**). The best splitting variable for the group of 115 patients was the Madrid classification. A 22% improvement in the homogeneity of the model was observed when this variable was introduced. No patient classified with indeterminate or tuberculoid leprosy presented a positive ML Flow test result. In patients with the borderline and lepromatous forms with up to three affected nerves and six-and-a-half years-old or younger, 33.3% tested positive in the ML Flow test. Among patients with three or fewer affected nerves and aged over 6.5 years-old, 84% presented a positive ML Flow. In patients with borderline or lepromatous forms, the group with

over three affected nerves and a negative skin smear, no patient presented a positive ML Flow while all patients with a positive smear were ML Flow-positive.

The contacts classification tree revealed that age, index case classification and the type of health service were all associated with seropositivity (**Figure 2**). The variable that best split the group of 1,011 contacts was age. Those younger than 8 years-old who were tested at basic health services showed a prevalence of ML Flow positivity of 19.6%. If the contacts were younger than 8 years-old and were attended at regional or State referral services, the prevalence of ML Flow positivity dropped to 6.8%. In contacts eight years-old or over, those with a PB index case showed 13.6% seropositivity in comparison with 26.7% in those with a MB index case.
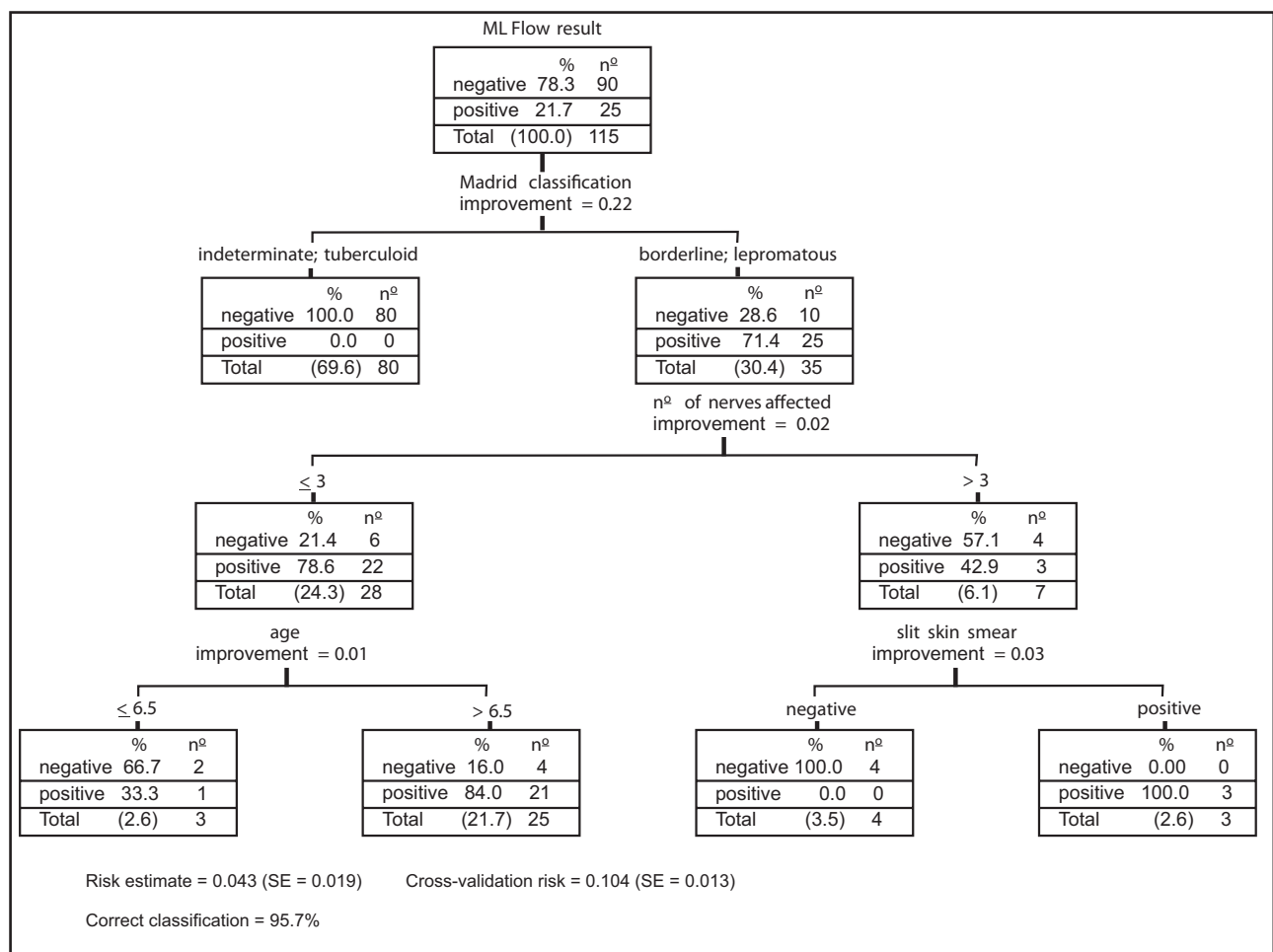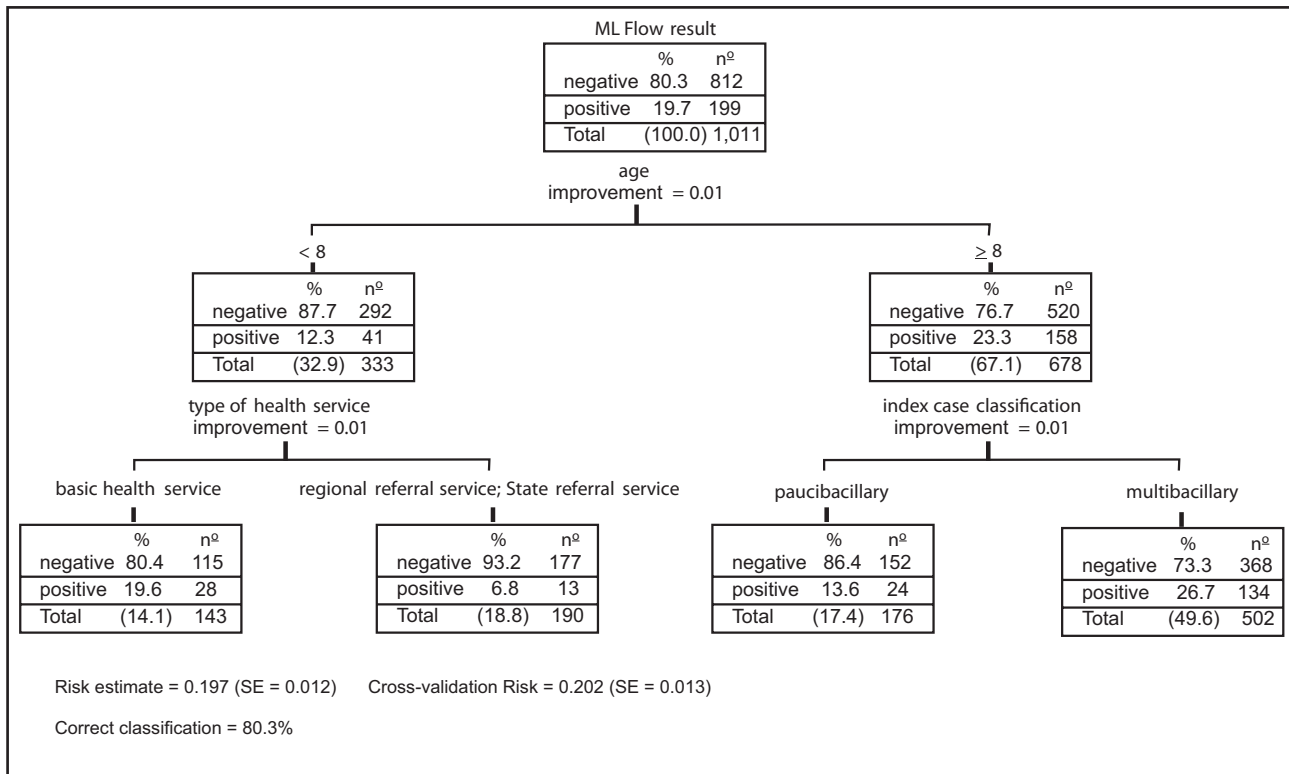

FIGURE 1

Classification tree (CART) of the 115 leprosy patients under the age of 18 years-old, Minas Gerais, Brazil, October 2002 to March 2004.

FIGURE 2

Classification tree (CART) of the 1,011 under-18 household contacts of leprosy patients, Minas Gerais, Brazil, October 2002 to March 2004.

DISCUSSION

The seropositivity observed among leprosy patients (21.7%) was lower than that found in the studies conducted by Bührer-Sékula *et al*[3], Grossi[8] and Lyon[14], which reported 72.9%, 50.7% and 57% respectively. In these investigations, the majority of patients were MB and were 15 years-old or over or age was not mentioned in the published articles. This study included only individuals under 18 years-old. The majority of cases were PB, which is typical for this age group[5 7 12 18].

The percentage of seropositivity (19.7%) observed among the contacts of this study falls between those obtained in Bührer-Sékula *et al*[3] and Calado *et al*[4], which were 28.6% and 15.6%, respectively. The Bührer-Sékula *et al*[3] study involved 42 household contacts and made no reference to the age of the individuals. Calado *et al*[4] studied household and peridomicile contacts, the majority of whom were older than 15 years-old.

The logistic regression indicated an association between ML Flow seropositivity in patients with a positive skin smear and more than five skin lesions, making skin smear the variable of greatest relevance. These findings are consistent with those reported in the literature, although the majority of patients in other studies were over 15 years-old[8 14].

The average age of seropositive patients was greater than those who were seronegative and the classification tree indicated an association between seropositivity and aged over 6.5 years-old. The increase in seropositivity with age demonstrates that bacterial load increases over time.

As for contacts, univariate analysis, multiple logistic regression and the classification tree all showed the same associations with ML Flow seropositivity, which were age, index case classification and type of health service used.

The likelihood of seropositivity in contacts increased with age, similar to that observed in the patient sample. Age was the variable of greatest relevance in the classification tree, showing higher positivity in those eight years-old or older. The age cutoff point in contacts, according to the classification tree, was higher (8 years-old) than that of the patients (6 1/2 years-old), which could be explained by sample size and differences in characteristics between the two groups.

The MB index case was another factor of association with ML Flow seropositivity in contacts. The proportion of seropositivity among contacts with a MB index case (22.6%) in this study was higher than that (18.4%) shown by Calado *et al*[4]. A study of the ML Flow test that included contacts conducted by Bührer-Sékula *et al*[3] made no mention of index cases.

Seropositivity was higher among contacts examined at basic health services. Regional and State referral services showed a lower likelihood of ML Flow seropositivity. The same effect was witnessed by Grossi[8]. This study, like that of Grossi[8], proved to be inconclusive regarding the association of ML Flow test seropositivity with the type of health service used, suggesting the need for further research on the subject.

Analyses showed that the factors that best explain ML Flow seropositivity in individuals younger than 18 years-old are those

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associated with a higher bacterial load, corroborating previous studies. In addition, observations in both the patient and contact groups showed that seropositivity increased with age, indicating the expansion of bacterial load over time.

In summary, the results showed similarities with other studies in the literature involving age groups older than the focus of this study. Therefore, this research supports the use of the ML Flow test in children as an additional tool for the correct classification of patients for MDT treatment purposes and to identify those contacts at greater risk of developing leprosy in the future.

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