

Bacterial load in the nose and its correlation to the immune response in leprosy patients

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Accepted for publication 27 February 2013

Summary

Introduction: Leprosy, whose etiologic agent is *M. leprae*, has its clinical manifestations correlated with distinct immunologic forms. The mechanism of infectivity and dissemination of the disease are not completely known, although the nasal mucosa is supposed to have an important role in pathogenesis.

Objective: To correlate the clinical and bacteriological parameters with that of nasal biopsy and immunological tests, such as lepromin and ML-Flow results, in untreated leprosy patients.

Material and Method: Two hundred and twenty-two patients were evaluated, clinically classified and subjected to skin smear, nasal biopsy, ML-Flow, and Mitsuda test.

Results: 68.9% of the cases were borderline cases. Nasal biopsy revealed 91.4% positivity in those who had specific antibodies against *M. leprae* on blood sample. Lepromatous leprosy cases were 100% positive on ML-flow test, had a large involvement in the nasal mucosa (91%), positive skin smears (100%) and negative Mitsuda test. Nasal bacillary index showed a good correlation with ML-Flow and had

similar results when compared to skin smear. The tests agreement was good, revealing that nasal biopsy can be reliable in the diagnosis of multibacillary clinical forms and in the evaluation of the immunological status of leprosy patients.

Conclusion: The presence of disseminated bacilli in the nasal mucosa was similar to skin involvement, when correlated with Mitsuda test and ML-Flow. As a result, the role of nasal bacillary index may play an important role in the clinical and immunologic characterization of leprosy patients.

Introduction

Leprosy is a public health problem in the world, causing a socioeconomic impact. Brazil, India, Nepal, Congo, and Tanzania are responsible for the main endemic areas in the world.¹⁻⁴

According to the official data from 118 countries and territories, the world prevalence for the year of 2008 was 212,802 cases, and the number of new cases is falling dramatically, about 20% in the last 4 years. Early treatment is an important element towards the elimination of the disease.^{1,4}

On the other hand, the infectivity and dissemination of leprosy are not completely elucidated. Previous researches have shown that the upper respiratory tract, mainly the nasal mucosa, has an important role in pathophysiology of leprosy, participating directly as a port of entry and exit. Further, nasal disease can precede skin lesions by months or years, depending on the cellular and the humoral immunological status of the hosts.⁵⁻⁸

So far, the tools available to evaluate the nasal involvement and the immunologic status of the patient are the nasal mucosa biopsy, the Mitsuda test, and the ML-flow test. The application of nasal biopsy for diagnosis is mainly used for scientific purposes.^{5,7} Mitsuda test consists of an intradermic inoculation of bacilli suspension that evaluates the cellular response of the host and the progression of disease.^{8,9} ML-Flow test evaluates humoral immunologic response, detecting IgM against PGL-1 (Phenolic glycolipid on cell wall of *Mycobacterium leprae*).^{8,10-12}

The diagnostic methods that can determine and quantify the presence of *M. leprae* in the nasal mucosa, in association with methods that determine the specific humoral response against the bacilli, ML-Flow, and the cellular immune response, Mitsuda test, may considerably enhance the accuracy in early diagnosis, treatment decision, and disability prevention.⁸

Therefore, we aimed to correlate the clinical and bacteriological parameters with that of nasal biopsy and immunology as lepromin and ML-Flow results of untreated leprosy patients.

PATIENTS AND METHODS

Two hundred twenty-two leprosy patients from the Leprosy Ambulatory, at the National Reference Center for Sanitary Dermatology, Federal University of Uberlandia, MG, Brazil, were enrolled in this study. This study had the approval of the Federal University of Uberlandia Ethics Committee, protocol number 099/2003 and all patients in this study agreed with and signed an informed consent form.

Clinical and histopathological classification was based on Ridley and Jopling (1966): indeterminate (I), tuberculoid (TT), borderline tuberculoid (BT), borderline-borderline

(BB), borderline lepromatous (BL), and lepromatous leprosy (LL). All patients were subjected to skin smear, nasal mucosa biopsy, Mitsuda and ML-Flow tests.^{13,14}

SKIN SMEAR

Skin smears for bacilloscopy were 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 oil immersion at $100\times$. The BI was calculated according to Ridley's logarithmic scale from 0 to 6+.^{6,14}

BIOPSY OF THE NASAL MUCOSA

We used a nasal speculum number 3 and headlight for the nasal mucosa biopsy. Before the procedure, a small pledget of cotton wool soaked in 0.5 mL of 2% tetracaine solution and 0.5 mL of 1:1.000 epinephrine was placed along the inferior nasal turbinate for 10 minutes; 0.5 mL of lidocaine 2% with 1:200.000 epinephrine was then injected at the head of the inferior turbinate or at the intranasal ulcer (only present in some LL patients). The biopsy was then performed with a small oval punch forceps, and a specimen of approximately $2.0 \times 1.5 \times 0.5$ mm of the nasal mucosa was extracted and fixed using 10% buffered formalin. The slides were stained using the Ziehl-Neelsen method and were examined in oil immersion at $100\times$.^{5,7} The BI was calculated according to Ridley's logarithmic scale from 0 to 6.

MITSDA TEST

The Mitsuda test was read by an experienced leprosy specialist 28 days after an intradermal injection of 0.1 ml lepromin suspension (6.0×10^7 bacilli/ml, heat killed, supplied by the Instituto Lauro de Souza Lima, Bauru, Sao Paulo, Brazil) in the upper one-third of the anterior aspect of the right forearm. Results were measured in millimeters for quantitative and qualitative analysis.¹⁵ Then, they were divided into two categorical classes according to WHO recommendations¹⁶: 'negative' for readings of ≤ 7 mm, which consisted of negative and weakly positive reactions, and 'positive' for readings of > 7 mm, which consisted of positive reactions, and strongly positive reactions or with the presence of pustular lesion and/or ulceration.^{8,9}

ML-FLOW

The reactions of the Lateral Flow test for *M. leprae* (ML-Flow) were performed with the ML-Flow kit that uses synthetic antigen DBSO. A lancet was used to collect five micro-liters of blood sample from the left fingertip. The ML-Flow test was recorded qualitatively (positive or negative) and semi-quantitatively (zero, 1+, 2+, 3+ or 4+).^{8,10-12}

STATISTICAL ANALYSIS

Quantitative data were analysed as mean and standard deviation and a nonparametric test compared independent groups. Pearson's linear correlation (r) compared positivity within the tests. The κ -test was performed to compare the tests agreement.

Results

The study included 222 untreated patients, 129 (58%) male and 93 (42%) female. The mean age of the patients was 46.21 ± 16.28 (age range, 9–87 years). They were clinically classified as 0.9% I, 8.1% TT, 38.3% BT, 17.1% BB, 13.5% BL, and 22.1% LL.

One hundred fifty-five (69.8%) of 222 cases were positive in the ML-Flow test, 67 patients (30.2%) were positive in the Mitsuda test, 101 (45.5%) were positive in the nasal biopsy, and 124 (55.8%) were positive in the skin smear. The only unexpected result was one TT patient (0.6%) who was ML-Flow test positive (Table 1).

One hundred and one (45.4%) patients were positive in nasal biopsy and ML-Flow. One patient was negative in the nasal biopsy and positive in the ML-Flow test. Also none of the patients who were positive on nasal biopsy had a negative result for ML-Flow. Thus, this resulted in an extremely high concordance between these tests (Kappa = 0.9909; $P < 0.0001$). Forty-seven (21.2%) patients were positive in both Mitsuda test and nasal biopsy. One hundred and seventeen (52.7%) patients were positive in nasal biopsy and negative in Mitsuda test. Twenty patients (9.0%) were positive in the Mitsuda test and negative in nasal biopsy. This resulted in a low concordance between these tests (Kappa = -0.0378; $P = 0.2031$).

Nasal BI and ML-Flow test (humoral immunologic response) were correlated and showed a direct association between them ($r = 0.8383$) (Graph 1).

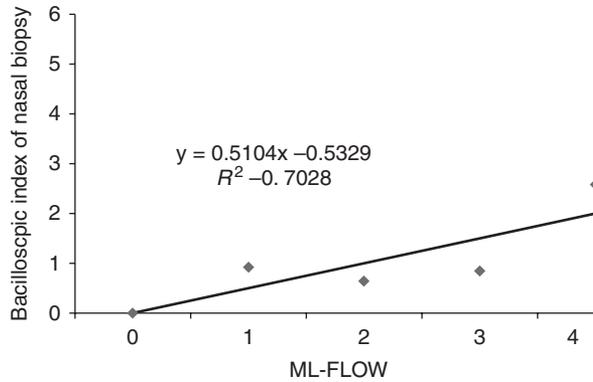
In addition, nasal BI was correlated to Mitsuda test (cellular immunologic response) demonstrating an inverse correlation ($r = 0.7123$) (Graph 2).

Discussion

M. leprae most likely enters the body via the nose and then spreads to skin and nerves via circulation.^{5,7} The immunological response of the host dictates the clinical phenotype that will develop. Experimentally, the polar forms of the disease behave according to an immunological paradigm. Tuberculoid disease is the result of high cell-mediated immunity with a largely Th1 type response. Lepromatous leprosy, however, is characterised by low cell-mediated immunity with a humoral Th2 response.^{6,8,17} The nasal biopsy can be

Table 1. Correlation of positive results between nasal biopsy, ML-Flow, Mitsuda test and skin smear according to clinical forms of leprosy

FC	Nasal Biopsy		ML-Flow		Mitsuda Test		Skin Smear	
	%	N	%	N	%	N	%	N
I	0	(0/2)	0	(0/2)	100	(2/2)	0	(0/2)
TT	0	(0/18)	5,5	(01/18)	100	(18/18)	0	(0/18)
BT	8,5	(07/85)	43.5	(37/85)	55,2	(47/85)	9.4	(8/85)
BB	56,7	(21/38)	100	(38/38)	0	(0/38)	97.8	(37/38)
BL	92,8	(26/30)	100	(30/30)	0	(0/30)	100	(30/30)
LL	91,4	(43/49)	100	(49/49)	0	(0/49)	100	(49/49)
Total	45,4	101/222	69,8	155/222	30,2	67/222	55,8	124/222

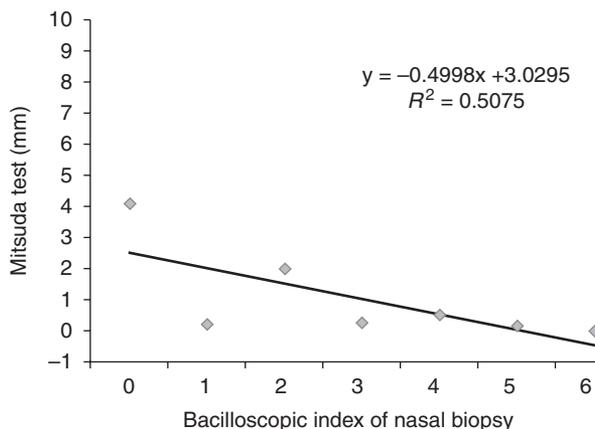


Graph 1. Concordance between bacillary index of nasal biopsy and ML-Flow results.

correlated with early diagnosis and immunological response, predicting clinical features and preventing disabilities and impairments.¹⁸

The clinical features of the disease are determined by the host response to *M. leprae*. The Mitsuda test is 100% positive in tuberculoid leprosy, demonstrating a high cell-mediated immune response with a largely Th1 response, determining a negative nasal biopsy, skin smear and ML-flow, consequently the disease has a low infectivity and higher predilection to skin and nerves. The lesions are frequently scaly, dry, hairless, and hypoesthetic. The anesthesia is due to destruction of dermal nerve fibres. This form carries a good prognosis and lesions will often self-heal. Damage to peripheral nerves is limited.^{5,8,18}

The lepromatous leprosy showed 100% positive on ML-flow test illustrating a high humoral Th2 response, resulting in a large involvement in the nasal mucosa (91%) and skin smear (100%) with a disseminated bacilli infiltration, higher infectivity, in contrary to negative Mitsuda test. Present many years before diagnosis, the early skin changes are widely and symmetrically distributed macules, and poorly defined with mild hypopigmentation and erythema. Infiltration of nasal structures may lead to sensation of nasal stuffiness, epistaxis, saddle deformity due to septal perforation and destruction of the anterior nasal spine.¹⁹⁻²²



Graph 2. Concordance between bacillary index of nasal biopsy and Mitsuda test results.

The borderline part of the spectrum is immunologically dynamic, and movement between the two polar forms occurs. These shifts in the immunological response underlie the reactions that are a feature of the borderline states. Therefore, the test results of BL form is similar to LL form and BT is similar to TT clinical type. All results are better comprehended when the linear correlation is performed between the nasal biopsy, the Mitsuda test, and ML-Flow. A direct correlation within the nasal biopsy and the ML-flow was found (Graphic 1) proving that the nasal disease indicates a high humoral response with a low cell-mediated response, more evident on lepromatous spectrum (BL and L). An inverse correlation between the nasal biopsy and the Mitsuda test was obtained (Graphic 2), thus confirming that low nasal infiltration is associated with larger Th1 type response and higher cell-mediated immunity, foreseeing the tuberculoid spectrum (BT and TT).^{5,7,8,15,16}

Leprosy eradication has not been achieved despite 20 years of MDT. The use of any classification system has its limitations, especially those that oversimplify a complex disease such as leprosy. The correlation between nasal biopsy, immunological status, and skin smear can improve the early diagnosis and treatment decision, minimising the impact of the disease on physical, psychological, and social well-being.

Conclusion

The present study showed that the presence of bacilli disseminated in the nasal mucosa occurs similar to its presence in the skin, as demonstrated by the extremely high concordance and the high correlation between ML-Flow test and nasal biopsy, and by the inverse correlation between Mitsuda test and nasal biopsy. Therefore, the nasal mucosa plays an equally important role as the skin smear and the skin biopsy in the clinical and immunological characterisation of leprosy patient and might aid to enhance early and correct diagnosis, and to prevent disabilities.

Acknowledgements

The authors are grateful to all the staff of the National Reference Center of Leprosy of the Federal University of Uberlândia for fundamental support, to Maria Esther Salles Nogueira of the Lauro de Souza Lima Institute, Bauru, SP, Brazil, for providing the Mitsuda antigen, and to the NLR (Netherlands Leprosy Relief) for providing the ML-Flow tests. This work was supported by grants from FAPEMIG, CNPq, CAPES, FINEP, and the Ministry of Health of the Brazilian Government.

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