**Mycobacterium leprae** DNA Associated with Type 1 Reactions in Single Lesion Paucibacillary Leprosy Treated with Single Dose Rifampin, Ofloxacin, and Minocycline

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**Abstract.** Leprosy affects skin and peripheral nerves, and acute inflammatory type 1 reactions (reversal reaction) can cause neurologic impairment and disabilities. Single skin lesion paucibacillary leprosy volunteers (N = 135) recruited in three Brazilian endemic regions, treated with single-dose rifampin, ofloxacin, and minocycline (ROM), were monitored for 3 years. Poor outcome was defined as type 1 reactions with or without neuritis. IgM anti-phenolic glycolipid I, histopathology, Mitsuda test, and *Mycobacterium leprae* DNA polymerase chain reaction (ML-PCR) were performed at baseline. χ² test, Kaplan-Meir curves, and Cox proportional hazards were applied. The majority of volunteers were adults with a mean age of 30.5 ± 15.4 years; 44.4% were ML-PCR positive. During follow-up, 14.8% of the patients had a poor clinical outcome, classified as a type 1 reaction. Older age (≥ 40 years), ML-PCR positivity, and lesion size > 5cm were associated with increased risk. In multivariate analysis, age (≥ 40 years) and ML-PCR positivity remained baseline predictors of type 1 reaction among monolesion leprosy patients.

**INTRODUCTION**

As reported by the World Health Organization (WHO) at the beginning of 2006, the registered global prevalence of leprosy was 219,826, with Brazil reporting the second largest number of both registered cases and newly detected cases during 2005. Newly detected cases in Brazil have shown only a modest decline over the last 5 years, indicating that active transmission of *Mycobacterium leprae* is still occurring despite more than two decades of effective multidrug therapy (MDT).¹

Acute inflammatory episodes before, during, or after MDT, known as type 1 (reversal reaction) and type 2 (erythema nodosum leprosum [ENL]) leprosy reactions, can lead to permanent disabilities and handicaps. The clinicopathologic characteristics of tuberculoid and lepromatous forms of leprosy and their associated reactions depend on TH₁ and TH₂-type immunity in skin lesions, respectively.²–⁴ To determine MDT regimens for treating patients, leprosy can be classified as paucibacillary (PB), defined by few localized skin and neurologic lesions with low bacterial load, and multibacillary (MB), characterized by high bacterial load and systemic clinical features. In 1997, the WHO proposed a single dose ROM regimen (rifampicin, ofloxacin, minocycline) to treat single skin lesion leprosy based on a clinical trial performed in India.⁵

Leprosy diagnosis is largely clinically based and would benefit from reliable, specific, and sensitive laboratory tests capable of diagnosing early paucibacillary forms of the disease.⁶ Anti-phenolic glycolipid I (PGLI) serology and acid-fast bacilli (AFB) staining techniques have low sensitivity for early PB leprosy diagnosis and, therefore, have been restricted to research applications.⁷–¹⁰ Detection of *M. leprae* DNA by polymerase chain reaction (PCR) in tissue samples has been shown to increase the sensitivity and specificity of early PB leprosy diagnosis¹¹; however, its usefulness for prognosis remains to be shown.

Single skin lesion, paucibacillary (SSL-PB) disease is considered one of the earliest clinical forms of leprosy. A multicentric prospective cohort of SSL-PB patients recruited in three Brazilian endemic settings and treated with one-dose ROM therapy was conducted, and the clinico-epidemiologic, histopathology, and cytokine features have been described previously.⁷,⁸,¹² This study describes the main clinico-epidemiologic and serologic features and the presence of *M. leprae* DNA detected by PCR associated with poor outcome, predominantly type 1 reactions, for a subgroup of this SSL-PB cohort during a 3-year clinical follow-up after ROM therapy.

**MATERIALS AND METHODS**

**Study population and setting.** Leprosy volunteers were recruited among outpatients attending health care units from three Brazilian endemic regions: north, Amazonas and Rondonia States; central-west, Goias State; southeast, Rio de Janeiro State. Written informed consent was obtained from all participants or legal guardians for volunteers < 18 years old. This study was approved by the National Ethical Review Board (CONEP-Brazilian Ministry of Health).

From November 1997 through December 1998, a total of 259 consecutive cases of single skin lesion leprosy patients (SSL-PB) were enrolled and treated with single-dose ROM therapy. Because of logistics, in two recruitment sites, the collection and storage of specimens for ML-DNA PCR started later. Therefore, for 135 volunteers, skin biopsies were available for ML-PCR, and for this reason, the results herein presented refer to a subgroup of patients.

All volunteers had a neuro-dermatological examination performed by dermatologists, with expertise in leprosy, to select eligible SSL-PB patients. Lesion size and the presence of a bacillus Clamette-Guérin (BCG) scar were reported for all patients at baseline as previously reported.⁷,⁸

**Inclusion and exclusion criteria.** Inclusion criteria were newly detected untreated single skin lesion leprosy patients without nerve involvement and classified as paucibacillary ac-
cording to bacterial index (BI) and histopathologic readings. Exclusion criteria were volunteers < 7 years of age, pregnant or breastfeeding women, and patients seropositive for HIV by history or medical record.

Clinical outcomes. The main outcome was type 1 reaction with or without neuritis that required steroid intervention. A type 1 reaction was diagnosed when a patient had erythema and edema of the skin lesion, which may be accompanied by neuritis and edema of the hands, feet, and face. The skin signs are obligatory; the nerve and general signs optional. For the purpose of analysis, a type 1 reaction was considered a poor clinical outcome. The secondary outcome was the shift from PB to MB classification as the only event (without neuritis or a type 1 reaction). The change from PB to MB disease classification was observed in two patients.

Follow-up. By December 2001, a 3-year clinical prospective follow-up was accomplished for the SSL-PB leprosy cohort treated with single-dose ROM. During the initial 6 months, monolesion leprosy patients were clinically assessed monthly, and thereafter, every 6 months until completion of follow-up. The follow-up was performed by the same team of dermatologists who decided the needs of intervention related to reactions and neuritis treatment, re-treatment by 6 months of the PB MDT regimen, or 12 months of the MB regimens. These clinical events required clinical intervention with either steroid or conventional MDT treatment. Time to event occurrence was measured from the date of ROM intake until the main outcome (type 1 reaction with or without neuritis) for the censored patients. Throughout the study period, the clinical follow-up in each site was performed by one dermatologist who was blinded to the histopathologic classification, the anti-PGLI1 serology, and *M. leprae* DNA PCR results. Standardized forms were used to collect data that were entered into our central computer database in Federal University of Goiás, Brazil, to ensure accuracy.

Histopathology. As part of this study, skin biopsies were collected at the rim area of the lesion and bisected, and one half was snap-frozen and stored at -80°C to perform *M. leprae*-DNA PCR. Histopathology was performed in the other half of the biopsy on paraffin sections (5 μm) stained with hematoxylin and eosin (H&E) and Fite-Faraco for AFB. Histopathologic readings were classified according to Ridley-Jopling criteria and classified as indeterminate (I), tuberculoid (TT), or borderline tuberculoid (BT). Approximately 50% of the skin biopsies (*N* = 66) were from north region patients, 42 samples were from the central-west, and 27 samples were from southeast region patients. Histopathology is not part of the routine leprosy control program.

*Mycobacterium leprae*-DNA PCR in skin lesion biopsies. Bisected skin biopsies were snap-frozen on collection and stored at -80°C, and genomic DNA was extracted using phenol/chloroform/isoamyl alcohol. *M. leprae* DNA was amplified in undiluted and in 1:5 diluted samples using primers for a 360-bp *M. leprae* DNA fragment of the 18-kd gene. Amplified products were detected by slot blot hybridization using a digoxigenin-labeled 212-bp DNA probe. Positive and negative control samples were included in each run. Duplicate samples were compared with positive and negative controls, and positivity was scored by visual observation of the 360-bp band on agarose gels and slot blot autoradiographs by two independent observers. Specimens were processed blinded to patient’s characteristics at the Tropical Pathology and Public Health Institute, Federal University of Goiás, Brazil.

Serology. Blood samples for anti-PGLI serology were collected at baseline before ROM therapy. ELISA tests for IgM anti-PGLI antibodies were performed as previously described. Briefly, PGLI synthetic disaccharide covalently coupled to bovine serum albumin (a gift from M. J. Colston, London, UK) was used, and the reaction was developed with O-phenylenediamine dye reagent (0.4 mg/mL; Sigma). The calculated cutoff for positivity using sera-endemic individuals was optical density (OD) > 0.2.

Mitsuda test. The Mitsuda preparation (40,000,000 *M. lepraecells/mL; Centro de Produção e Pesquisa de Imunobiológicos, Instituto de Saúde do Paraná, Brazil) was injected intradermally (0.1 mL), and the reaction at 3 weeks was measured. A positive result was considered for skin indurations ≥ 5 mm in diameter as previously described.

Statistical analysis. Initial analysis was performed with a χ² test for categorical variables and with a two-tail p value. The disease progression endpoint was defined as a poor clinical outcome during follow-up. Distributions to time to outcome were estimated by Kaplan-Meier methods. We applied Cox proportional hazard modeling to calculate the association between baseline predictors and outcome. This multivariate modeling was performed to control for potential confoundings, including variables associated with both exposure and outcome. Hazard ratios (HRs) were interpreted as relative risks. A two-tailed α level of 0.05 was used. Statistical analysis was performed with SPSS for Windows 11.0 (SPSS, Chicago, IL).

RESULTS

The main baseline characteristics of the 135 monolesion leprosy patients included in this study were as follows: mean age, 30.5 ± 15.4 years; 55.6% women; and 65.9% single skin leprosy lesions were ≤ 5 cm. Almost two thirds of the SSL-PB patients had a positive Mitsuda test (≥ 5 mm cut-off), and 12.6% had positive IgM anti-PGLI serology (> 0.2 OD). SSL-PB patients were evenly distributed among histopathologic groups: 32.6% were I, 32.6% were TT, and 34.8% were BT. Characteristics of the participants herein analyzed were similar to those of the entire cohort considering age and sex distribution and histologic results at baseline. The subgroup of SSL-PB patients tested for PCR also presented similar percentages of a type 1 reaction during 3-year follow-up compared with the entire cohort (14.8% vs. 19.4%; *P* = 0.5; data not shown).

Among 135 monolesion leprosy cases, *M. leprae* DNA was detected in skin biopsies by PCR in 44.4% (60/135; 95% CI, 35.9–53.2). In univariate analysis, patients positive for *M. leprae*-DNA PCR at baseline were older (≥ 40 years) and had larger lesion size (≥ 5 cm) than those testing negative by PCR. Data on sex, histopathology, anti-PGLI serology, Mitsuda test, and presence of BCG scar had similar proportions between patients positive and negative for *M. leprae*-DNA PCR.
During clinical monitoring, a total of 20 (14.8%) of the 135 patients presented with a type 1 reaction, 5 of them with neuritis. Two BT patients shifted from PB to MB leprosy. Single-lesion patients that tested positive by \textit{M. leprae}-DNA PCR at baseline presented consistently higher probability to progress to a poor clinical outcome than those testing negative (Figure 1B). The Kaplan-Meier curves were similar during the first 12 months, and thereafter, the difference between the two groups increased over time. The log-rank test results (log-rank, 5.4; \( P = 0.01 \)) indicated a statistically significant difference for poor outcome between the \textit{M. leprae}-DNA PCR-positive and -negative groups (Figure 1B). Event incidence increased strikingly after the second year of follow-up. The median time to the occurrence of poor clinical event was

![Kaplan-Meier curves](image.png)

**Figure 1.** Kaplan-Meier curves for poor outcome among single skin lesion paucibacillary leprosy patients stratified by (A) age < 40 and \( \geq 40 \) years; (B) \textit{M. leprae} PCR-positive and -negative groups; and (C) lesion size < 5 and \( \geq 5 \) cm.
In fact, in The shift from PB to MB forms was ex-
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< 0.01; Figure 1C). Mitsuda
PCR positivity at baseline were potential risk fac-
DNA at baseline had an increased HR
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patients, which has been reported to range from 34% to
with the sensitivity of
and type 1 reactions.
between PCR positivity in skin lesions of SSL-PB patients
leprosy diagnosis was a strong independent risk factor for
poor outcome compared with
patients 7–39 years of age (Table 1). Patients with skin lesions
for M. leprae DNA at baseline had an increased HR
poor outcome compared with M. leprae-DNA PCR-
negative patients (HR = 2.5; 95% CI, 1.2–6.8), and patients
with lesions ≥ 5 cm had increased risk of poor outcome. Table 1 shows that older age group and M. leprae-DNA PCR posi-
tivity remained independent predictors of event occurrence
after adjustment for potential confounders in the multivariate
model.

DISCUSSION

Our study showed that older patients (≥ 40 years), and M. leprae-DNA PCR positivity in patients were the most important
risk factors for poor clinical outcome among monolesion
PB leprosy patients from Brazil treated with single-dose
ROM therapy. We defined poor outcome as a type 1 reaction
with or without neuritis. A recent study conducted among
Vietnamese leprosy patients indicated that age > 15 years at
leprosy diagnosis was a strong independent risk factor for
type 1 reactions and sequelae.15 A positive BI and borderline
tuberculoid leprosy were also identified as risk factors for
a type 1 reaction in the Vietnamese cohort. The first of these
factors, positive BI, supports our finding of an association
between PCR positivity in skin lesions of SSL-PB patients
and type 1 reactions.

Among our cohort, M. leprae could be confirmed by PCR
in almost one half of the patients. This finding is compatible
with the sensitivity of M. leprae-DNA PCR among PB leprosy
patients, which has been reported to range from 34% to
74%.16 During clinical monitoring, ~14.8% of the 135 patients
presented with a type 1 reaction. M. leprae PCR positivity
in skin biopsies collected at baseline was associated with a
higher frequency of type 1 reactions and represented the only
laboratory test associated with poor clinical outcome during
the 3-year follow-up period. One fourth of M. leprae PCR-
positive patients developed a type 1 reaction compared with
9.3% among PCR-negative subjects (P = 0.03).

IgM anti-PGLI serology, known to reflect the bacillary
load, has lower sensitivity for PB disease.9,14 The monolesion
leprosy patients recruited in our study were PB, defined by
negative bacilloscopy, and thus, low levels of anti-PGLI an-
tibodies were expected in this cohort.10 Anti-PGLI seroposi-
tivity has been reported as a risk factor for a type 1 reaction17;
however, no association between anti-PGLI levels and type 1
reactions was seen among our cohort of single lesion leprosy
patients.

Type 1 reactions, considered the leading cause of neuro-
logic impairment in patients with leprosy, represented the
main poor clinical outcome in the SSL-PB patients. Leprosy
reactions are acute immune-inflammatory episodes that may
develop during the chronic course of leprosy, particularly
among the immunologically unstable BT forms.17–21 In our
study, BT leprosy was not statistically associated with a type
1 reaction compared with I or TT groups. Among the cohort
of SSL-PB leprosy cases, the in situ cytokine profile defined
by real time RT-PCR indicated effective TH1-type cell-
mediated immunity, which is compatible with both healing
and type 1 reactions observed during monitoring.12 In fact,
in this cohort, complete disappearance of the lesion was ob-
served in more than one half of the censored patients. This
result is statistically similar to the cure rate reported by the
WHO-sponsored clinical trial to evaluate one-dose ROM
therapy.5

It is worth mentioning that during the 3-year clinical follow-
up after ROM therapy, no severe reactive episode was ob-
served and that conventional steroid therapy was sufficient to
prevent nerve damage. The incidence of poor outcomes seen
in our study was similar to that observed among a PB cohort
from Ethiopia.22 The shift from PB to MB forms was ex-
tremely rare among monolesion cases and likely caused by
the strict eligibility criteria we adopted to increase specificity
of diagnosis before one-dose ROM therapy.

In summary, our findings indicate that age (≥ 40 years) and
M. leprae PCR positivity at baseline were potential risk fac-
tors for type 1 reactions among single lesion leprosy. Accord-
ingly, the value of M. leprae-DNA PCR positivity as a marker
for type 1 reactions deserves further evaluation in larger stud-
ies to address its potential as a marker of clinical prognosis.

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