

Serodiagnosis of leprosy and follow-up of household contacts using a commercial rapid test containing ND-O/LID-1 antigens

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Summary

Introduction: Early diagnosis of leprosy, including the diagnosis of sub-clinical disease in contacts of known cases, would be a major advance. The signs of early leprosy are often difficult to assess and a reliable diagnostic test could play an important role in identifying cases and thus reducing transmission.

Methods: Subjects were recruited at Centro de Saúde Jardim Guanabara, Rondonópolis, Mato Grosso State, Brazil. Sera were obtained from 174 recently diagnosed leprosy patients, 409 household contacts, 53 endemic controls and 12 patients with active TB. 186 of the household contacts were re-tested on subsequent visits. Both the NDO-LID® rapid test (Orange Life®, Rio de Janeiro, Brazil) and an anti-PGL-1 ELISA test were used on all samples for comparison.

Results: LID-1 was positive in 25 of 125 PB cases (20%), but in 41 of 49 MB cases (83.7%), while the anti-PGL-1 ELISA was positive in 8 (6.4%) and 35 (71.4%) cases, respectively. Specificity for LID-1 was 85% and for the anti-PGL-1 ELISA, 97%. Amongst household contacts, 9 of 409 (2.2%) were positive on the LID-1 test, and a further 51 (12.5%) were weakly positive; although 9 cases of leprosy were subsequently identified amongst these contacts, 6 had tested negative, 2 were weakly positive and only 1 had been positive.

Conclusion: The ND-O-LID-1 assay can be easily performed by the addition of serum to the assay device, and thus can be applied in resource-poor settings. The test was found to be useful for the detection of multibacillary cases, which in the long

term will help to reduce transmission of the disease. This is a specific test, but it is not very sensitive for early detection of leprosy in household contacts and paucibacillary forms.

Introduction

Leprosy is a chronic infectious disease with a wide spectrum of clinical forms. Until now there has not been a gold standard diagnostic test for leprosy, and its detection is mostly based on clinical signs and symptoms. According to the clinical spectrum, leprosy patients are classified as: tuberculoid (TT), borderline-tuberculoid (BT), mid-borderline (BB), borderline-lepromatous (BL), and lepromatous (LL).¹ The World Health Organization suggested a clinical classification to select the treatment regimen based on the number of skin lesions, bacterial positivity by slit skin smear and number of involved nerves.² This system classifies leprosy in two simplified categories: multibacillary (MB) leprosy (typically smear positive, or having more than one nerve involved or more than five skin lesions) and paucibacillary (PB) leprosy (smear negative, none or one nerve involved, maximum of five skin lesions).

Hyper-endemic areas and the high incidence of leprosy³ remain a cause for concern to the Leprosy Control Programme in Brazil. In addition, it has been shown that the earlier a leprosy patient is diagnosed and treated, better the outcome of the disease.⁴ In these endemic areas, sensitive and specific diagnostic laboratory tests would be of great use in the detection of leprosy at an early stage of the disease, or detection of disease during the evaluation of contacts.⁵ Serum antibodies levels against phenolic glycolipid-1 (PGL-1), a specific *M. leprae* cell wall antigen^{6,7} has been used in both enzyme-linked immunosorbent assay (ELISA) and rapid lateral-flow (LF) test formats, however, both showed limited sensitivity in the detection of PB leprosy.^{6,8,9} Nonetheless, anti-PGL-I response is strong among MB patients and reflects the bacillary load in leprosy patients.¹⁰

In 2006, Reece *et al.*,¹¹ described two novel antigenic proteins (ML0405 and ML2331) that clearly differentiated MB patients from healthy and *M. tuberculosis*-infected control individuals in endemic areas. Therefore, these antigens appear suitable for the development of serologic tests for leprosy. LID-1, a fusion of the ML0405 and ML2331 proteins, has proven to be strongly reactive with MB patients sera in many geographic locations.^{12–14} Also, a previous study suggested that the addition of LID-1 protein to PGL-I could improve sensitivity in a diagnostic test, since some patients lacking anti-PGL-I antibodies have antibodies that recognise LID-1, and vice versa.¹³ Thus, given the challenges of developing a diagnostic assay, a simple and rapid test is currently produced and commercialised in Brazil. It incorporates both the LID-1 and PGL-I antigens, immobilised on a nitrocellulose membrane for antibody detection in sera, requiring minimal personnel training for leprosy diagnosis.¹⁵

Leprosy is an insidious disease and it is very difficult to rely on clinical examination for early detection of the disease. Therefore, the validation of this recently developed commercial test would be useful for early leprosy diagnosis. In this work, carried out in a highly endemic area in Midwestern Brazil, we proposed to apply this rapid test in leprosy patients and their household contacts during 5 years of follow-up.

Material and Methods

SUBJECTS AND SAMPLES

This study was carried out in two phases: at diagnosis of leprosy patients (2009–2010) and follow-up of household contacts (2009–2014). Thus, between 2009 and 2010, leprosy patients (MB and PB), household contacts, endemic controls and pulmonary tuberculosis individuals were recruited at Centro de Saúde Jardim Guanabara, Rondonópolis, Mato Grosso State (Midwestern Brazil). All patients signed the informed consent forms, approved by the Ethics Committee of the Federal University of São Paulo (Protocol number 26803). Sera were obtained from 174 (78 female/96 male) recently diagnosed leprosy patients (LP), 409 (228 female/181 male) household contacts (HHC) and 53 (36 female/17 male) endemic controls (EC). We excluded patients with leprosy reactions. Patients were classified by bacterial, histological, and clinical observations carried out by qualified personnel, with the bacillary index (BI) of the skin biopsies recorded at the time of diagnosis (07 LL, 12 BL, 30 BB, 63 BT, 43 TT, 19 I). We also included 12 (07 female/05 male) tuberculosis (TB) patients to evaluate potential antigen cross-reactivity. Sera from TB patients were obtained after drawing blood from *Mycobacterium tuberculosis* sputum-positive, human immunodeficiency virus-negative individuals with clinically confirmed pulmonary TB who were undergoing treatment. All serum specimens were aliquoted and stored at -20°C or -80°C . From 2009 to 2014 the household contacts were contacted by telephone or via the Health Unit and invited to be re-evaluated. From the total, 186 individuals were examined two or three times and blood was collected for serology.

SEROLOGICAL ASSAYS

NDO-LID® rapid test

The NDO-LID® rapid test (Orange Life®, Rio de Janeiro, Brazil) was developed by impregnating nitrocellulose membranes with ND-O-LID-1, a conjugation of the ND-O (a synthetic mimetic of PGL-I disaccharide) and the LID-1 protein.^{12,15} The NDO-LID® test is provided as a ready-to-use kit to be performed by adding undiluted serum to be tested (10 μl) and two drops of running buffer into the sample well, causing the migration of the sample and colloidal gold beads loaded with anti-IgG and anti-IgM antibodies through the membrane and across a detection window. Interactions with the test and/or control lines are revealed as a red colour. Readings were performed 20 minutes after buffer addition.

Visual readings were performed by three independent readers. The results required the visualization of a clear control line in the detection window. A positive result was defined by the staining of both the control line and the test line (visual reading scores: 1+/2+/3+). Faint test line staining was considered as a weakly positive (score 0.5+) result, and it was considered a positive test response throughout the entire analysis. No test line staining was considered as a negative result.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Briefly, the wells of ELISA plates were coated with either the semi-synthetic antigen PGL-I-ND-O-BSA (natural disaccharide coupled to bovine serum albumin through an octyl group provided by John Spencer, Colorado State University, USA) (50 μl per well of 100 ng/ml

carbonate buffer, overnight at 4°C) or with coating buffer (control). After blocking with 1% milk-PBST (phosphate buffered saline tween 20), a dilution of each sample equivalent to 1:500 serum in PBST containing 10% normal goat serum (NGS) was included (50 ul per well, 37°C, 60 min) in four wells (duplicates of antigen coated and buffer coated). The plates were washed with PBST and incubated with 1:10,000 dilution of peroxidase-conjugated rabbit anti-human IgM/HRP (DAKO®, Denmark) in PBST-10% NGS (50 ul per well, 37°C, 60 min). After another wash, the colour reaction was developed with 50 ul of substrate solution containing O-phenylenediamine added to the wells (at room temperature for about 20 min). The reaction was stopped with 50 ul of 2.5 N H₂SO₄ and the absorbance was read at 490 nm. The mean absorbance of the BSA control wells was subtracted from that of the wells with ND-O-BSA, and the result was regarded positive if the optical density (OD) exceeded 0.150 (the mean absorbance plus three standard deviations of 100 healthy Brazilian control subjects).

STATISTICAL ANALYSIS

Differences in antibodies anti-ND-O-BSA and anti-ND-O-LID-1 levels between test groups were analyzed with the two-tailed Mann-Whitney and Kruskal Wallis tests for non-parametric distribution using GraphPad Prism version 5.01 for Windows (GraphPad Software®, version 5, USA). Pearson's correlation was used to calculate r^2 for ND-O-BSA versus ND-O-LID-1, and Spearman correlation was used to calculate r^2 for BI versus ND-O-LID-1, using GraphPad Prism version 5.01. Moreover, the sensitivity, specificity, positive and negative predictive values (PPV and NPV) were determined with 95% confidence interval (CI). Results were considered statistically significant for P values < 0.05.

Results

We analysed sera using both the ELISA leprosy test (based on the detection of IgM antibodies against the PGL-I mimetic ND-O-BSA antigen) and the ND-O-LID-1 rapid diagnostic test (based on the detection of IgM antibodies against ND-O and IgG antibodies against the LID-1

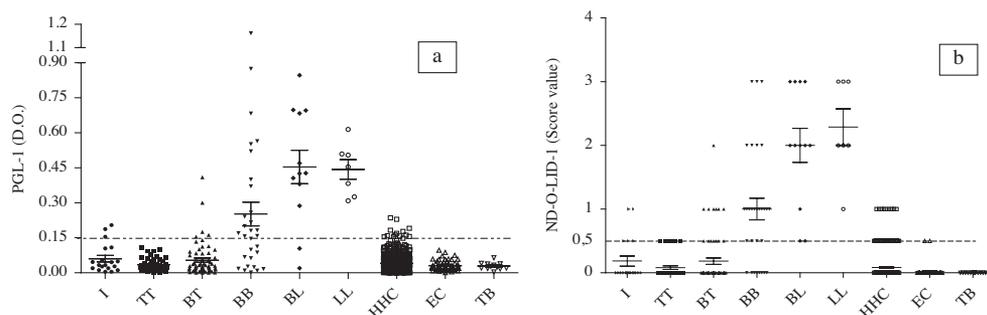


Figure 1. Distribution of antibody responses among participants. (a) IgM antibodies to phenolic glycolipid-1 (PGL-1) by ELISA, presented in optical density (OD). The traced horizontal line is the cut-off (OD \geq 0.150). (b) IgM/IgG antibodies to synthetic disaccharide glycolipid ND-O and to *Mycobacterium leprae* protein leprosy IDRI diagnostic-1 (LID-1) by immunodot tests, presented by visual reading of NDO-LID® test scored (0 = absent test line, positive result = 0,5,1,2,3+). Data grouped by clinical forms of leprosy patients groups (I, TT, BT, BB, BL, LL), endemic control (EC), household contact (HHC) and tuberculosis patients (TB).

Table 1. Serum detection of anti-ND-O-LID-1 IgG/IgM (ML0405 and ML2331 associated to ND-O) antibodies by *immunodot* and e anti-PGL-I (ND-O) IgM using ELISA, in 174 leprosy patients, classified according to Ridley and Jopling or Madrid's, with inclusion of the indeterminate group

Clinical Form (Sample n.)	NDO-LID-1 results					PGL-I results	
	<i>Negative</i>	<i>0.5+</i>	<i>I+</i>	<i>2+</i>	<i>3+</i>	<i>Pos</i>	<i>Neg</i>
I (19)	14	03	02	0	0	3	16
TT (43)	37	06	0	0	0	0	43
BT (63)	49	07	06	01	0	5	58
BB (30)	08	04	11	04	03	18	12
BL (12)	0	02	01	06	03	10	02
LL (07)	0	0	01	03	03	7	0
Total (174)	108	22	21	14	09	43	131

protein) to permit comparisons between these tests. The distribution of anti-PGL-I and anti-ND-O-LID-1 antibodies levels in sera of individuals from the different groups is demonstrated in Figure 1. In this study, among the MB leprosy (BB, BL and LL), the visual reading results indicated that ND-O-LID-1 tests produced a clear strong band.

Comparing the two serological test, the Pearson's correlation for MB was $r^2 = 0.7934$. Correlation of $r^2 = 0.5178$ was determined among PB patients (I, TT and BT). Comparing these assays for all leprosy patients a correlation of $r^2 = 0.8444$ was found, indicating good agreement in respect to serological status of the samples. The Spearman's correlation between bacterial index (BI) and antibodies against the ND-O-LID-1 resulted in $r^2 = 0.7382$ for MB leprosy patients and $r^2 = 0.1654$ for PB leprosy (data not shown).

Among 174 leprosy patients with diagnosis confirmed by clinical and Ridley and Jopling's histopathology classification, 22 (12.6%) weak positive results were found using the rapid test (Table 1).

The results of anti-PGL-I ELISA and ND-O-LID® were not always concordant (either positive, negative or weak positive) and three patients with Indeterminate leprosy were identified and presented with positive PGL-I: two ND-O-LID-1 positive and one ND-O-LID-1 weak positive. Among the five BT PGL-I positives, four were ND-O-LID-1 positive and one weak positive. The majority of the sera among 18 BB PGL-I positive patients were ND-O-LID-1 positive ($n = 16$) and two ND-O-LID-1 weak positive. However, in the BB group three PGL-I negative with two ND-O-LID-1 positive and one ND-O-LID-1 weak positive

Table 2. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the NDO-LID-1 and PGL-1 (serological evaluation)

	NDO-LID1	(%)		PGL-1	(%)	
	leprosy	MB leprosy	PB leprosy	leprosy	MB leprosy	PB leprosy
Sensitivity	37.93	83.67	20	24.71	71.43	6.4
Specificity	85.33	85.33	85.33	96.82	96.82	96.82
PPV	52.38	40.59	29.41	76.79	72.92	38.1
NPV	76.37	97.76	77.73	75.14	96.59	77.19

Table 3. Serum positivity of anti-ND-O-LID-1 rapid test and anti-PGL-I ELISA in 409 household contacts of leprosy patients, including the household contacts that were diagnosed with the disease

HHC of leprosy patients	N° sample	ND-O-LID-1 Test results			PGL-I ELISA Test results	
		Neg	Pos	Weak pos	Neg	Pos
LL HHC	(12)	12	–	–	12	–
BL HHC	(50)	46	1	3	49	1
BB HHC	(64)	56	1	7	63	1
BT HHC	(144)	119	3	22	138	6
TT HHC	(92)	79	3	10	88	4
I HHC	(47)	37	1	9	46	1
Total/group	409	349	9	51	396	13

HHC: Household contact; LL: lepromatous; BL: borderline-lepromatous; BB: borderline-borderline; BT: borderline-tuberculoid; TT: tuberculoid; I: indeterminate; Pos: positive; Neg: negative.

were found. Anti-PGL-I and anti-ND-O-LID-1 antibody levels varied significantly between patients and EC, HHC and TB ($P < 0.05$). No significant difference was found for EC, HHC and TB for both serological tests.

The sensitivity of ND-O-LID-1 test for leprosy patients was higher (37.93%) than the PGL-I ELISA test (24.71%). The sensitivity of the anti-ND-O-LID-1 was higher for MB and PB patients, compared to anti-PGL-I test. Specificity was high for both tests. On the other

Table 4. Serological evaluation of the semi-quantitative ND-O-LID-1 rapid test and ELISA PGL-I test, in household contacts of leprosy patients during 5 years of follow-up, Rondonópolis-MT, Brazil. Household contacts were categorized by social distance from the index patient

HOUSEHOLD CONTACTS BECAME PATIENTS 2009–2011					
N°	HHC Clinic	Kith and Kin	ND-O-LID-1 Test	PGL-I ELISA	Index Case Clinic
1	BT	son	0.5+	0.004	TT
2	TT	wife	neg	0.012	TT
3	I	daughter	neg	0.030	BT
4	TT	daughter	neg	0.005	LL
5	BT	son	neg	0.053	LL
6	TT	son	neg	0.074	TT
7	I	husband	neg	0.030	BB
8	BT	mother	0.5+	0.028	BT
9	BT	brother	1+	0.236	BT

HOUSEHOLD CONTACTS BECAME PATIENT 2012–2014					
N°	HHC Clinic	Kith and Kin	ND-O-LID-1 Test	PGL-I ELISA	Index Case Clinic
1	BB	son	0.5+	0.011	BT
2	BT	husband	0.5+	0.050	BT
3	TT	grandson	neg	0.038	BL
4	BB	brother	neg	0.068	BL
5	BT	grandmother	1+	0.075	BT
6	BT	mother	Neg	0.012	TT

hand, PPV was higher for the PGL-I ELISA than ND-O-LID-1 in leprosy patients. Both tests showed high NPV for leprosy and also for MB and PB patients (Table 2).

During initial evaluation of household contacts nine out of 409 contacts (2.2%) were diagnosed with leprosy, while 51 (12.5%) had weak positive results in the rapid test (Table 3).

The serological positivity responses among HHC was low to anti-PGL-I ELISA and anti-ND-O-LID-1 with result 1+, (3.2% and 2.2% respectively), in the first evaluation. Among these contacts, 349 individuals were ND-O-LID-1 negative and six of them became patients (2I, 3TT, 1BT); from the nine individuals ND-O-LID-1 positive, one became a patient (BT 1+) and among 51 individuals with weak positive ND-O-LID-1 result, two became patients (BT). One contact who developed BT leprosy presented positive ND-O-LID-1 and PGL-I ELISA test (OD > 0.150). Good correlation value was found for ND-O-LID1 and PGL-1 ELISA ($r^2 = 0.7717$) in HHC, EC and TB samples.

During the follow-up 25 household contacts were re-evaluated twice and 161, once. Among the 186 household contacts, 29 (17.16%) showed at least one initial positive serological exam. Positivity was repeated in 12 individuals at the second or third evaluations; however, they did not show any signs of leprosy during the remainder of the study. Six household contacts who attended on their own initiative at the Health Centre were diagnosed with leprosy, and only three of them presented positive serology at diagnosis (Table 4).

HHC also responded to the *M. leprae* anti-PGL-I ELISA (3.1% positivity) and rapid test ND-O-LID-1 (14.6% positive, including weak reaction). Amongst HHC, nine were diagnosed with leprosy almost at the same time as the index case. And among them, six were ND-O-LID-1 negative (2I, 3TT, 1BT), two weak positive (BT) and one positive (BT 1+).

From the EC, 51 individuals presented ND-O-LID-1 negative results and two of them showed weak positive test to ND-O-LID-1 resulting in specificity of 96.2%. The anti-PGL-I ELISA results were negative for all EC individuals. All the TB patients also presented negative results to both ND-O-LID-1 and PGL-I ELISA test.

Discussion

The World Health Organization set a global target reduction of Grade-2 disability in new cases at $\geq 35\%$ by the end of 2015 compared to baseline levels recorded at the end of year 2010.¹⁶ To achieve this goal, the leprosy control programmes would need to reduce the delay in disease diagnosis, administer MDT promptly, ensure that patients fully completed their treatment and confirm, during follow-up, that patients were cured. Together, these actions should impact in the transmission of *M. leprae* in the community and decrease the cases with disability.

Besides that, a tool for identifying leprosy cases (asymptomatic and any symptomatic form of leprosy) was identified as a prerequisite to elimination, thereby addressing the goals of the 2020 targets of the London Declaration on Neglected Tropical Diseases.^{17,18} Thus, in order to achieve this goal, studies are ongoing to develop a serological test that could be easily used in the field to identify those individuals at highest risk of acquiring leprosy. Such a test would improve early diagnosis and potentially break the chain of transmission to help Grade-2 disability rate reduction, besides facilitating the health workers during their evaluation of suspected cases.

In this context, recently, a lateral flow test based on IgM and IgG antibodies detection against the ND-O-LID-1 conjugate was developed. It provides results within 20 minutes of

sample addition, and has shown strong antibody responses in BB/BL/LL cases (87%),^{15,19} similar to the present study showing 83.6% of positivity for MB cases. According to Cardoso *et al.* (2013)¹⁵ the seropositivity of the ND-O-LID-1 test varied across the spectrum of leprosy: 97.4% for LL, 83.7% BL and 76.9% for BB patients. A similar distribution of positivity across the MB leprosy spectrum enrolled in the present study, varying from 100% for LL and 100% BL, to 73.3% for BB; also, when patients were compared with non-diseased individuals, the BB, BL and LL patients had significantly higher antibody titers against both anti-PGL-I ELISA and ND-O-LID-1. In the present study, the responses to ND-O-LID-1 and anti-PGL-I ELISA in PB individuals were weak, 20% and 6.4%, respectively. These results confirm other work in which leprosy IDRI diagnostic-1 (LID-1) and ND-O-BSA antigens used in combination appear to have utility as a biomarker for LL/BL leprosy forms (NDO-LID).²⁰

It is worth commenting that 12.2% of the samples from MB cases and 12.8% from PB patients resulted in ND-O-LID-1 weak positive. The manufacturer of the ND-O-LID-1 test suggests that tests with weak positive results should be considered negative, nevertheless, the weak positive result indicates that the patients may have antibody levels close to the threshold level of a positive result. This may be true for PB patients that have low bacillary loads, or even in early infection, in which the stimuli to IgG production against ND-O-LID-1 or IgM against PGL-I were not high enough to confirm diagnosis of leprosy. The antibody response of a weak positive result to ND-O-LID-1 was considered positive in order to achieve the goal of reducing the delay in disease diagnosis. In addition, the weak positive ND-O-LID-1 could help to improve the accuracy of the clinical assessment, since in the present study 22 patients showing weak positive test results (that otherwise would be considered negative by the manufacturer's instructions) indeed had a leprosy diagnosis confirmed.

Within the EC studied, specificity of 96.2% was calculated and corroborated the 97.4% specificity demonstrated by Duthie *et al.* (2014).¹⁹ The present study showed that among 53 EC, 51 presented negative ND-O-LID-1 results and two weak positive results, while all EC were negative for anti-PGL-I ELISA. The weak positive results suggest that individuals are infected with *M. leprae*, however, depending on their immune status, they will either evolve to disease, or else be able to control the infection before appearance of signs and symptoms.

When household contacts that became sick were distributed by inter family relationship, most of them were children (40.0%) or partners (20.0%) of the index case. These results corroborate the results of Dessunti *et al.*²¹ which showed 40.6% of children and 27.8% partners with disease among 1055 leprosy cases and 3394 household contacts in Londrina-PR, Brazil. In the present study children prevailed over partners and siblings as the most exposed relatives. The most frequent sources of infection were mothers and grandparents who constituted the relationship in 20% of the samples evaluated.

Matos²² notes that 11.19% of contacts in his study were already sick at the time of clinical evaluation and 14.29% were diagnosed during the follow-up. Douglas *et al.*,²³ however, reported that 33 new cases emerged among 559 contacts (27 cases during 5 years active surveillance and six cases during 10 years passive surveillance) in Cebu. In the present study 2.2% of the contacts had leprosy in the initial evaluation, while six among 186 HHC (3.2%) became sick during five years of follow-up. Similar results were obtained by Goulart *et al.*,²⁴ in Uberlândia-MG, Brazil, showing 2.0% of contacts with disease, 75% in the first year of surveillance. Also, the work of Amorin *et al.*,²⁵ showed that 332 HHC were followed and among those 12 (3.6%) were diagnosed with leprosy in a median time of 31 (3–79) months after recruitment, six of them presented with MB leprosy. In the present study, as a final result

15 (3.67%) HHC became sick, being 85% with PB leprosy. Interestingly, most of them were contacts of PB patients which suggests there is another source of infection in the surrounding family. As a control strategy, it is import to widen the concept of HHC to social or neighbourhood contacts, as they may be active players in the transmission chain.

Duthie *et al.* showed that IgG antibody response to ND-O-LID-1 was detected one year earlier than appearance of leprosy symptoms in seven out of 11 HHCs of MB patients (64%). The IgG response was strikingly more elevated and occurred much earlier than the increases in the anti-PGL-I IgM responses in the same individuals.¹² In the present study, this was not observed because most of the HHC that became sick were PB. This favours the idea that there may be a source of infection (undiagnosed MB) around the individual with positive serological exam, and supports the importance of evaluating contacts as a control measure for the disease.

Potential cross-reactivity is an important issue to be considered in the development of a new serologic test for leprosy, particularly for countries with a high incidence of TB, high BCG vaccination coverage and high levels of exposure to environmental mycobacteria. Thus, the ND-O-LID-1 negativity among TB and EC indicates that even with the previous BCG vaccination, there is no cross reactivity with the new rapid test. This indicates the high specificity of the test, so cross-reaction between *M. tuberculosis* and other mycobacterial antigens is not a significant concern.

Thus, the present data demonstrate not only the utility of the rapid test ND-O-LID-1 in detecting MB leprosy patients compared to control subjects, but also suggests exposure to or potential asymptomatic infection with *M. leprae*, reinforcing the findings of Cardoso *et al.*,¹⁵ Bobosha *et al.*,²⁶ and Wen *et al.*²⁷

The results of the present study showed good correlation between the two serological tests for confirmed leprosy cases, however, there were more ND-O-LID-1 positive tests than PGL-I positive, demonstrating higher sensitivity of the rapid test, that may be due to the presence of one glycolipid and two protein antigens in the ND-O-LID-1 that would be capable of detecting both, IgM and IgG antibodies. In spite of that, these results do not corroborate the statement that individuals with positive serology show eight times higher risk of developing leprosy,²⁸ since only a few positive individual became sick during 5 years of follow-up.

The ND-O-LID-1 assay can be easily performed by the addition of serum to the assay device, therefore, it can be applied in resource-poor settings, where specialised personnel and logistics are not available. Besides, this test was found to be useful for the detection of multibacillary cases, which in the long term will help to reduce transmission of the disease. This is a highly specific test, but it is not highly sensitive for early detection of leprosy in household contacts and paucibacillary forms. In spite of that, the findings support the need of stakeholders to continue promoting the concept of attaining zero transmission, measurable by *M. leprae* infection. Therefore, the investment in longitudinal research to identify biomarkers for the diagnosis of asymptomatic infected individuals, as well as for those at high risk of developing leprosy, such as contacts, should be continued.

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