



NDO-BSA, LID-1, and NDO-LID Antibody Responses for Infection and RLEP by Quantitative PCR as a Confirmatory Test for Early Leprosy Diagnosis

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Diagnostic tests for leprosy are limited, especially to identify early leprosy cases. We performed active case findings of leprosy to validate three potential antigen candidates and one molecular target. Cases were diagnosed by characteristic skin lesions, nerve enlargement, or skin sensation loss. Serum samples obtained from all subjects were tested by ELISA to assess antibody titers to three *Mycobacterium leprae* specific antigens: NDO-BSA, LID-1, and NDO-LID. Most of the field cases on Mosqueiro Island, northern Brazil, also collected slit skin smear for qPCR. Active case finding diagnosed 105 new cases of leprosy out of 894 subjects (11.7%), revealing a high prevalence of *M. leprae* in the region. With the use of amplification of the *M. leprae*-specific RLEP by qPCR, 68/79 (86.07%) of these cases were positive, confirming leprosy in subjects diagnosed in the field. Patients diagnosed at the leprosy reference center showed much higher antibody titers to all three antigens, while titers of patients from the field were significantly lower. Our results support previous findings that active surveillance by experienced leprologists can diagnose additional cases based on clinical findings, that many would not be identified using ELISA assay with the available biomarkers, and that RLEP qPCR may be used to confirm the majority of the field cases.

Keywords: leprosy, active case finding, schoolchildren, serology, RLEP, qPCR

INTRODUCTION

The lack of diagnostic tools to detect early oligosymptomatic or even late well-defined cases contributes to the hidden endemic of leprosy, maintaining active foci of the disease in mostly tropical and developing countries (1). Pará state, located in the Brazilian Amazon region, is classified as hyperendemic for leprosy (29.82 new cases per 100,000 population in 2019). Considering the rate among children less than 15 years old, Pará is also classified as hyperendemic (9.60 new cases per 100,000 population), which is 3.1 times higher than Brazil's national rate according to the National Notifiable Diseases Information System (SINAN) in 2019. Children diagnosed with leprosy are robust epidemiological indicators since the disease in people under 15 years old indicates recent disease spread and the existence of multiple active foci within communities (2). Untreated multibacillary (MB) cases are thought to be the main source of *Mycobacterium leprae* transmission in households (3); therefore, treatment of all diagnosed index cases and surveillance and follow-up of household contacts (HCs) are essential strategies for the early detection of leprosy cases to block transmission and to prevent irreversible nerve damage, disabilities, and deformities (4, 5).

Leprosy diagnosis is based mainly on well-accepted clinical signs/symptoms of loss of sensation on hypochromic or infiltrated skin lesions that can be accompanied by muscle weakness, nerve damage, pain, or loss of function. Since there is no laboratory diagnostic test, and detection of acid-fast bacilli is not very sensitive, molecular methods to detect *M. leprae* DNA in an earlobe slit skin smear (SSS) or a biopsy (6) using the repetitive element RLEP by PCR have shown promise as an early diagnostic tool (7).

The use of a simple ELISA to detect antibodies to the *M. leprae*-specific phenolic glycolipid I (PGL-I/NDO-BSA) antigen (8) has been in use for over 30 years. Antibody titers correlate well with the bacillary load and are almost universally positive in patients at the lepromatous end of the spectrum (borderline-lepromatous [BL] and lepromatous-lepromatous [LL]), although positive responses in paucibacillary (PB) patients are not frequent (9). Additional antigens used in serological tests include the fusion protein LID-1 (leprosy immune diagnostic 1, a combination of ML0405 and ML2331) (10), which, similar to PGL-I, detects positive responses in virtually all MB patients, and the glycoprotein NDO-LID, a combination of LID-1 and a disaccharide mimetic of PGL-I, which aim to be more sensitive than NDO-BSA (11).

Research with point-of-care (POC) tests have an increasing trend since it is an easy-to-perform method, which may allow diagnoses in the field without expert technical skills and support (12). PGL-I/NDO-BSA (12–14), LID-1 (15, 16), and NDO-LID (17–19) lateral flow assays showed feasibility to detect classic MB cases of leprosy, similar as ELISA method; however, its use for PB and, consequently, also for early leprosy cases is limited, impacting on its applicability.

Based on these findings, we decided to evaluate the antibody titers to these three antigens (NDO-BSA, LID-1, and NDO-LID)

along with the molecular detection of RLEP by qPCR in leprosy patients, schoolchildren (SC), and HCs from the hyperendemic Mosqueiro Island in Pará state, Brazilian Amazon.

METHODS

Study Design and Population

The present cross-sectional study was conducted on Mosqueiro Island, a district belonging to Belém, the capital of Pará state, Brazilian Amazon, which has a total population of approximately 30,000 inhabitants. The field studies were performed during a 1-week period from May 12 to 16, 2014. SC was randomly selected from four public schools (three elementary and one middle school). Newly diagnosed cases in children were followed up by visits to the households to examine HC. Blood was obtained from all SC and HC to assess antibody titers to the three different antigens. Earlobe SSSs were obtained from 79/105 new cases from the field to perform RLEP qPCR. As a control group, leprosy patients newly diagnosed at Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará (URE) located in Marituba, Pará, and their corresponding HC were examined, and blood samples were obtained.

This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethics Committee from Pará Federal University (CAAE 26765414.0.0000.0018 CEP-ICS/UFPA). All individuals involved agreed voluntarily to participate and signed an informed consent form after receiving information about the study. Parents of minors or responsible adults signed their consent, allowing them to participate. All data analyzed were anonymized to protect the privacy of participants.

Clinical Diagnosis

All subjects were examined and evaluated by experienced leprologists. The diagnosis of leprosy was based solely on well-accepted clinical signs and symptoms, including the detection of hypopigmented or infiltrated skin lesions with loss of sensation assessed with standard graded Semmes–Weinstein monofilaments (20) and the palpation of peripheral nerves to identify characteristic pain associated with inflammation or swelling. Each newly diagnosed patient was classified into one of the five clinical forms defined by Ridley–Jopling, except in cases where there was only a single hypopigmented macule without nerve involvement, indicating an early indeterminate form of leprosy. Indeterminate (I) and tuberculoid-tuberculoid (TT) forms were operationally categorized as PB, while all other forms (borderline-tuberculoid [BT], borderline-borderline [BB], BL, and LL) were grouped together as MB. Primary neural leprosy (PNL) was diagnosed if there was nerve enlargement associated with functional or sensory loss without any skin lesions. If only one nerve was affected, the case was classified as PB, whereas two or more enlarged nerves indicated MB. The DG was ranked from 0 to 2 (DG0, no disability; DG1, loss of sensation in the hand or foot; DG2, visible damage, deformity or disability) as determined during a clinical examination of

sensory-motor functions based on a WHO standardized neurological evaluation (21).

ELISA for NDO-BSA, LID-1, and NDO-LID

All participants had blood samples collected in EDTA tubes after clinical evaluation. For antibodies titration, high-affinity polystyrene Immulon IV 96-well ELISA plates (Dynex Technologies, Chantilly, VA, USA) were coated overnight with 50 ng/50 μ l of carbonate/bicarbonate sodium buffer with NDO-BSA, LID-1, or NDO-LID. The plates were blocked with blocking solution (phosphate-buffered saline [PBS], pH 7.2 with 1% bovine serum albumin [BSA], and 0.05% Tween-20 [T]) for 1 h. Serum samples were diluted 1:300 with a blocking solution and tested in duplicate for 2 h of incubation. The secondary anti-human IgM (Sigma-Aldrich, St. Louis, MO, USA, A0420) antibodies were used for NDO-BSA (dilution 1:10,000), while secondary anti-human IgG (Rockland, Gilbertsville, PA, USA, #609-1316 2 mg) were used for LID-1 (dilution 1:20,000), and both antibodies were used for NDO-LID. The reaction was developed by the insertion of an antibody conjugated with peroxidase in PBS/T for 1.5 h, and then the peroxidase color substrate *o*-phenylenediamine dihydrochloride (SIGMAFAST™ OPD, Sigma-Aldrich P9187, USA) was added to each well for 15 min. To stop the reaction, 50 μ l/well of H₂SO₄ at 4 N was added and immediately read at 490 nm using an MRX Revelation 4.25 microplate reader (Dynex Technologies, Chantilly, VA, USA).

To control intra- and inter-test variations, positive (LL patient) and negative (non-endemic control [NEC]) controls were added to each plate. All samples were tested in duplicate plus a blank control well coated only with BSA.

Cutoff values were established using the mean of results plus 3 times SD of optical density (OD) of health endemic and non-endemic subjects. Results were considered from the mean of the duplicates less the blank value. *Cutoff* for anti-NDO-BSA and anti-LID-1 was 0.295 and for anti-NDO-LID was 0.475.

RLEP Detection by Quantitative PCR

A wide range of molecular targets were evaluated for the detection of *M. leprae* (7); one of them is an RLEP family, which, despite a variable structure and unknown function, has 29 repetitions scattered on the *M. leprae* chromosome; for this reason, the RLEP region is easier to amplify, expanding the possibility of detection even with small amounts of sample (22). SSS samples of both earlobes were collected, immediately fixed in 1 ml of 70% ethanol, and stored for up to 30 days at room temperature before extraction.

DNA extraction was performed following the manufacturer's recommendations (Qiagen DNeasy Blood and Tissue Kit, Germantown, MD, USA). RLEP detection was established with 10 ng of total DNA using 5'-GTGAGGGTAGTTGTT-3' (LP1) and 5'-GGTGCGAATAGTT-3' (LP2) primers (IDT, Integrated DNA Technologies, IA, USA) amplifying a 129-bp fragment detected by SYBR Green fluorescent DNA-binding dye using Applied Biosystems 7500 Real-Time PCR Systems. Each reaction was conducted in a total of 20 μ l (4 μ l of DNA template, 5 μ l of water, 10 μ l of SYBR, and 1 μ l of primers). The reaction occurred

with the following specifications: Uracil-DNA glycosylase (UDG) at 50°C for 2 min, prior 95°C for 2 min for initial denaturation followed by 45 cycles, each cycle consisting of denaturation at 95°C for 15 s, annealing at 58°C for 15 s and extension at 72°C for 1 min, and melting curve running in each experiment. To determine qPCR positivity, a standard curve was prepared from purified *M. leprae* DNA extracted from nude mouse footpads, and then five points of standard dilution (1:50, 1:100, 1:1,000, 1:2,000, and 1:5,000) of this DNA were included in each plate, plus three negative points as a template control (see **Supplementary Figure 1** and **Supplementary Table 1**).

A melt curve was used to analyze the specificity of the amplified products. Samples were considered positive when the fluorescent signal intersected the automatically calculated threshold line. Amplifications with a cycle threshold (Ct) \leq 45 were considered positive for RLEP. Pearson's coefficient (r^2) is calculated for each plate, and the plate results only were accepted if $r^2 \geq 0.95$. We perform qPCR experiments according to the MIQE checklist (see **Supplementary Table 2**).

Statistical Methods

A two-tailed Mann-Whitney U test was used for the comparison of antibody titers between two groups. All results were considered statistically significant when $p < 0.05$. To verify the antigen diagnostic performances, we calculated the area under the curve (AUC) by receiver operating characteristic (ROC) curve. The results above 0.7 were considered able to discriminate HC from patients.

Ethics Statement

This study was performed in accordance with the recommendations of the Brazilian National Ethics Committee (CONEP) guidelines approved by Pará Federal University Ethics Committee number CAAE 26765414.0.0000.0018 with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Pará Federal University Ethics Committee.

RESULTS

Active Case Finding of New Cases

A total of 894 individuals from Mosqueiro Island were surveyed, 706 SC were examined for clinical signs/symptoms of leprosy (**Figure 1**), and 65 (9.2%) were SC diagnosed as a new case of leprosy (SC_{NC}), presenting mainly hypochromic skin lesions with loss of sensation, with the number of lesions ranging from 0 to 9 (median 2). Subsequently, 188 HC of SC_{NC} were evaluated at their homes during follow-up visits (range 1–9 and median of 2 HC per household), and 40 (21.2%) HCs were diagnosed as a new case of leprosy (HC_{NC}). HC_{NC} showed a median of three (range 0–21) skin lesions, and nine (22.5%) presented with disability grade 1 (DG1) (**Table 1**). In summary, active case finding diagnosed leprosy in 105 subjects (105/894,

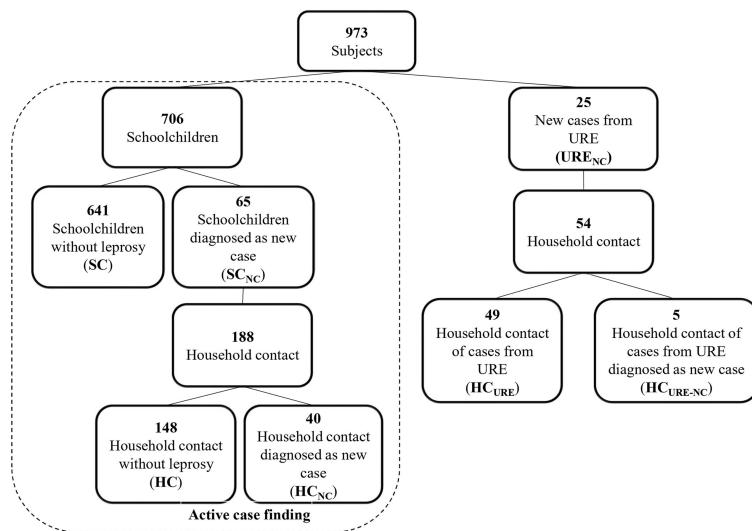


FIGURE 1 | Diagram of study design. A total of 973 subjects were examined, including 706 SC and 188 HC of SC_{NC}, resulting in 894 subjects. Furthermore, 25 URE_{NC} and 54 HC were also examined. URE, Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará. SC, schoolchildren; SC_{NC}, schoolchildren diagnosed as new case; HC, household contacts; HC_{NC}, household contacts diagnosed as new case; URE_{NC}, new cases from URE; HC_{URE}, household contact of cases from URE; HC_{URE-NC}, household contact of cases from URE diagnosed as new case.

11.7%), with 65 SC and 40 HC. Although 19 (18.1%) presented with DG1, there was no case with disability grade 2 (DG2).

Antibody Titers in Healthy Subjects and Leprosy Cases From Mosqueiro

NDO-BSA, LID-1, and NDO-LID were quantified by ELISA for each group (SC, SC_{NC}, HC, and HC_{NC}), and seropositivity was characterized based on the *cutoff* defined for each antigen. There was no significant difference between the SC median versus the SC_{NC} median for any of the antigens (*p*-value: 0.254, 0.955, and 0.282 for NDO-BSA, LID-1, and NDO-LID, respectively), with an equivalent OD range within each group. HC and HC_{NC} also did not show differences for the three antigens tested (*p*-value: 0.968, 0.480, and 0.262 for NDO-BSA, LID-1, and NDO-LID, respectively). The seropositive percentages were slightly higher for HC than SC for NDO-BSA (70.9% and 68.8%, respectively) and NDO-LID (29.5% and 20.3%, respectively). Similarly, the seropositivity profile of newly diagnosed cases was the highest for

NDO-BSA (73.3%), followed by NDO-LID (33.3%) and LID-1 (5.7%).

Use of Quantitative PCR in New Cases Diagnosed in the Field

To ratify the clinical diagnosis, amplification of the RLEP region using qPCR was performed on 79/105 new cases diagnosed in the field. Out of the 49 SC_{NC}, 43 (87.7%) were found to be positive, while of the 30 HC_{NC}, 25 (83.3%) were positive. In total, 68/79 (86.07%) new cases were found to be positive, with a Ct median of 41.1 cycles (Figure 2A).

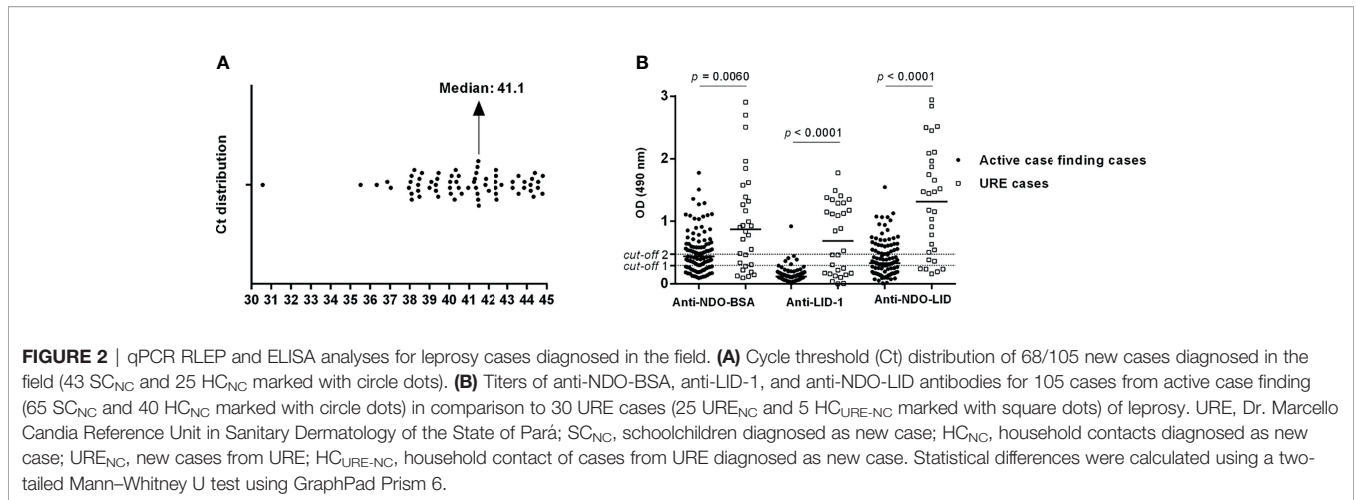
Reference Unit Cases and Their Household Contacts

In comparison to the field diagnosis from Mosqueiro, we randomly selected 25 patients (24 MB and 1 PB) who were diagnosed at Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará (URE). These patients, new

TABLE 1 | Epidemiological characteristic of cases.

New Cases	n	Sex M/F	Age median (range)	Ridley and Jopling Classification							Disability		
				PNL	I	TT	BT	BB	BL	LL	0	1	2
SC _{NC}	65	22/43	12 (6-18)	3 (4.6%)	11 (16.9%)	0 (0%)	39 (60%)	12 (18.4%)	0 (0%)	0 (0%)	55 (84.6%)	10 (15.4%)	0 (0%)
HC _{NC}	40	15/25	21 (4-58)	1 (2.5%)	3 (7.5%)	2 (5.0%)	21 (52.5%)	13 (32.5%)	0 (0%)	0 (0%)	31 (77.5%)	9 (22.5%)	0 (0%)
URE _{NC}	25	16/9	35 (8-84)	0 (0%)	0 (0%)	1 (4.0%)	14 (56.0%)	0 (0%)	5 (20.0%)	5 (20.0%)	15 (60.0%)	8 (32.0%)	2 (8.0%)
HC _{URE-NC}	5	3/2	39 (28-59)	1 (20.0%)	1 (20.0%)	0 (0%)	0 (0%)	0 (0%)	1 (20.0%)	2 (20.0%)	3 (60.0%)	1 (20.0%)	1 (20.0%)

SC_{NC}, schoolchildren diagnosed as new case; HC_{NC}, household contacts diagnosed as new case; URE, Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará; URE_{NC}, new cases from URE; HC_{URE-NC}, household contacts from URE diagnosed as new cases; PNL, primary neural leprosy; I, indeterminate; TT, tuberculoid-tuberculoid; BT, borderline-tuberculoid; BB, borderline/borderline; BL, borderline-lepromatous; LL, lepromatous-lepromatous.



cases from URE (URE_{NC}), come from the surrounding region, referred by other basic health units or physicians for confirmation of suspected leprosy. Often, these individuals exhibit more advanced disease due to delays in diagnosis or misdiagnosis (23), which can manifest itself with an increased bacillary index, additional nerve damage, and increased levels of disability. Along with these 25 patients from URE, 54 of their HCs (HC_{URE}) were also enrolled. Upon examination, five of these (9.2%) were found to have clinical symptoms of leprosy, and these HCs of cases from URE were diagnosed as a new case (HC_{URE-NC}).

Antibody Titer Discrepancy for Mosqueiro and Reference Unit Cases

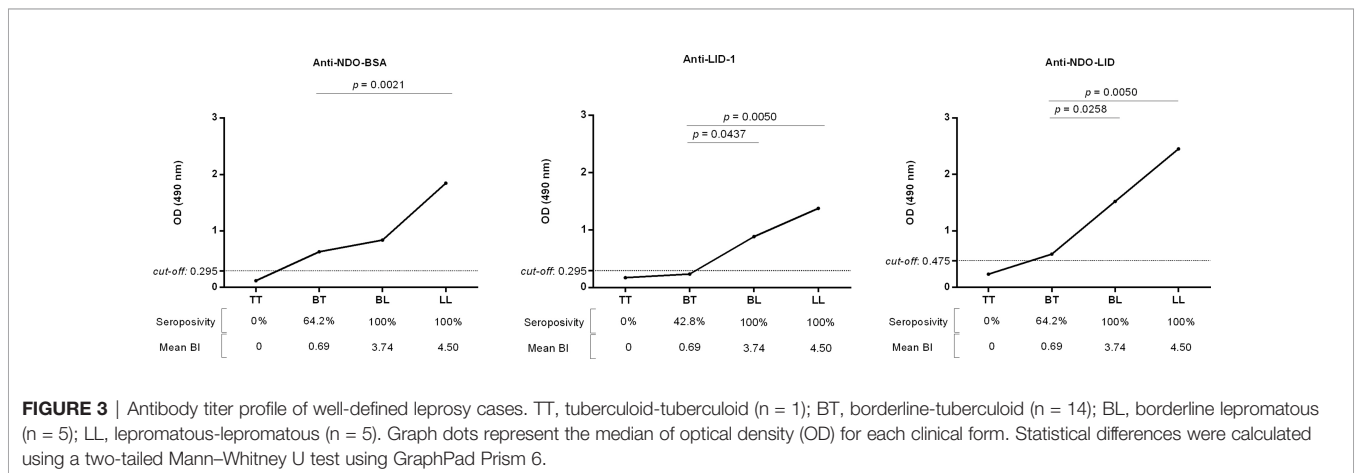
The antibody titers against the three antigens were determined for all PB (n = 17) and MB (n = 88) cases from Mosqueiro (SC_{NC} and HC_{NC}) versus PB (n = 1) and MB (n = 24) cases from URE. All cases from Mosqueiro demonstrated antibody titers significantly lower than those from URE cases, especially for anti-LID-1 and anti-NDO-LID antibodies (Figure 2B). When the titers were analyzed based on clinical form, there was a clear increase in the mean OD as subjects progressed from TT to LL,

with 100% of BL (n = 5) and LL (n = 5) patients being positive against all three antigens (Figure 3).

Comparing exclusively subjects without leprosy from Mosqueiro and URE (SC, HC, and HC_{URE}), we observed that only anti-NDO-BSA demonstrated different levels of antibody titers between groups, with a high median on HC from Mosqueiro, followed by SC and HC_{URE} from URE (Figure 4).

Diagnostic Validation of the NDO-BSA, LID-1, and NDO-LID Biomarkers

To evaluate the validity of NDO-BSA, LID-1, and NDO-LID as diagnostic tools, parameters of sensitivity, specificity, and accuracy were calculated for both classes of leprosy patients. No biomarker detected all cases of leprosy, independent of the disease progression at the time of diagnosis. The ability to identify leprosy cases through serology (sensitivity) was higher for NDO-BSA, followed by NDO-LID and LID-1. Anti-NDO-BSA antibodies presented the highest sensitivity either in the field or at URE; however, specificity and accuracy significantly decreased for early leprosy cases diagnosed in the field, limiting anti-NDO-BSA applicability as a diagnostic biomarker for cases in the initial



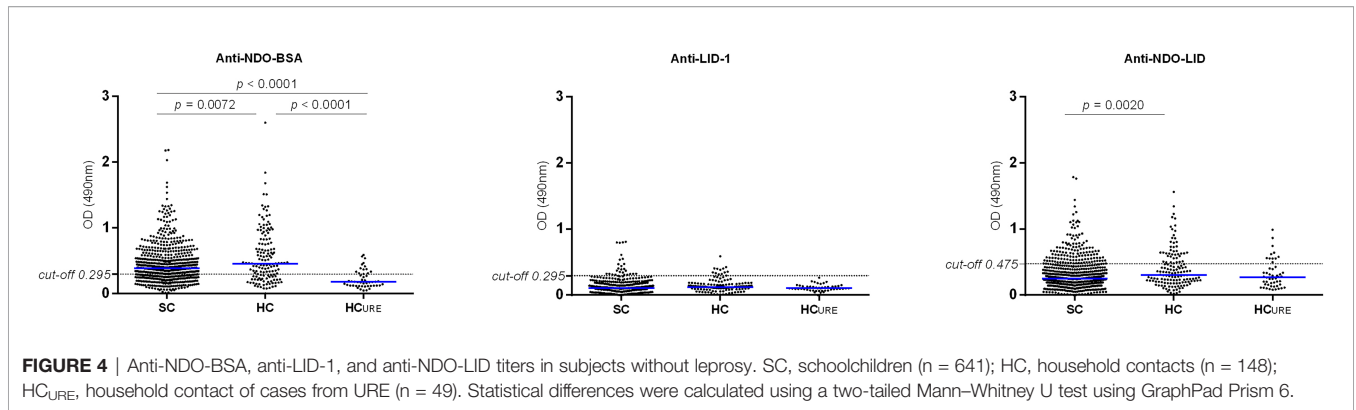


TABLE 2 | Parameters of diagnostic validation of biomarkers tested.

		NDO-BSA	LID-1	NDO-LID
Active case finding	Sensitivity	73%	6%	33%
	Specificity	31%	95%	78%
	Accuracy	36%	85%	73%
URE cases	Sensitivity	77%	63%	77%
	Specificity	71%	100%	78%
	Accuracy	73%	88%	77%

Sensitivity, specificity, and accuracy were calculated using 105 cases from active case finding (65 SC_{NC} and 40 HC_{NC}) compared with subjects without leprosy (641 SC and 148 HC) and 30 URE cases (25 URE_{NC} and 5 HC_{URE-NC}) compared with subjects without leprosy (49 HC_{URE}).

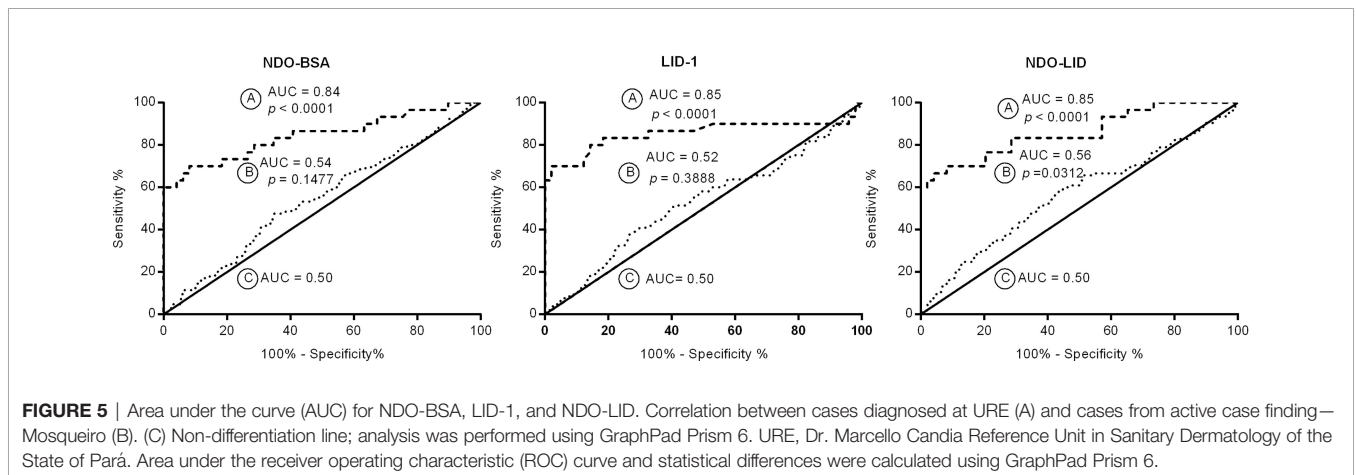
stages. Anti-NDO-LID displayed equivalence to anti-NDO-BSA in all percentages for well-defined patients, while for field subjects, the sensitivity was notably lower. On the other hand, anti-LID-1 was the most effective for differentiating subjects with or without leprosy (specificity), even for cases diagnosed with initial clinical manifestations, showing 100% specificity for URE cases and 95% specificity for Mosqueiro cases. Unfortunately, anti-LID-1 antibodies showed very limited sensitivity, particularly for early diagnosis (Table 2).

Analyzing the correlation of laboratory test parameters by ROC curve, we verified a large difference in ROC curve power comparing well-defined URE cases or oligosymptomatic field

cases. URE subjects presented a high AUC for all biomarkers, indicating the applicability of the use of these biomarkers for well-defined leprosy patients. In contrast, anti-NDO-BSA, anti-LID-1, or anti-NDO-LID antibodies did not discriminate serological cases and non-cases based on the AUC test (Figure 5).

DISCUSSION

Active case finding in schools is not a recent strategy (24); however, it became an important approach to find leprosy



cases after inclusion in the WHO global leprosy strategy 2016–2020 (25). In our work, 706 students from Mosqueiro Island were clinically examined, resulting in 65 (9.2%) new cases being diagnosed. This percentage is even superior to our previous studies (5, 23), corroborating the fact that leprosy in Pará has a high number of hidden cases. Mosqueiro is one of the 33 poor islands on the periphery of the capital, with less than 20% coverage by the family health strategy.

Among the examined HC at the island, 40/188 subjects (21.2%) were diagnosed with leprosy, while at URE, only 5/49 (9.2%) were diagnosed with leprosy, corroborating that the field approach is more effective in finding new cases (5, 23, 26). Furthermore, no DG2 cases were found during active case finding on 105 new cases, while for the 5 new cases diagnosed at the URE, 2 had disabilities, 1 DG1 and 1 DG2, indicating that spontaneous demand may diagnose people with higher disability rates, also confirming previous findings (26).

Studies demonstrating the applicability of NDO-BSA (8, 27), LID-1 (18, 28), and NDO-LID (18, 29) to identify and differentiate PB and MB leprosy cases diagnosed at health centers have been published. Although there are divergent concepts of subclinical infection in relation to serology for the determination of infection rate in a community (30), our data ratify the hypothesis that antibody quantification can be an indicator of endemicity (5, 23). High levels of anti-NDO-BSA were observed in HC from Mosqueiro, indicating an increased risk of developing leprosy. HCs positive to PGL-I/ND-O-BSA have 3- to 8.6-fold more chance to progress to disease in relation to seronegative subjects (30–32). HC_{URE} who presented initially high OD for anti-NDO-BSA were followed up and diagnosed (HC_{URE-NC}, n = 5).

Previous works showed that HC anti-LID-1 positivity resulted in diagnosis with leprosy after 6–15 months (28, 33) of follow-up, indicating a very high predictive value. However, although all BL/LL from URE were positive for anti-LID-1 antibodies, only 6/105 (5.71%) of cases from active case finding were positive, indicating that this biomarker was not positively correlated with oligosymptomatic leprosy cases diagnosed in the field. Moreover, 26/641 healthy SC (4.05%) were seropositive with no clinical signs of leprosy and need follow-up to identify possible development of leprosy in the future.

No serological biomarker presented enough sensitivity to identify all cases of leprosy, either well-defined or oligosymptomatic; although anti-NDO-BSA antibodies presented a sensitivity closer to 75%, they showed low specificity, especially in early cases. Diagnostic validation parameters display a large range of sensitivity (2% to 63.8%) and specificity (66% to 98%) when comparing healthy subjects and patients (30, 34). Despite the strong correlation of anti-NDO-BSA titers with MB patients at URE with well-defined late diagnosed cases, this effect is lost in the field, even with MB patients, probably because *M. leprae* is highly circulating in the community, resulting in infection, not disease. This hypothesis is supported by the increased probability of detecting new cases in people living in houses where at least one seropositive dweller

lives in comparison to people living in houses where nobody is positive for anti-PGL-I antibodies (35).

LID-1 and NDO-LID performance described in the literature generally refers to them as useful biomarkers for leprosy diagnosis. However, most of the data include well-defined cases, and relevant sensitivity, specificity, or AUC is observed mostly for classic MB patients (11, 18). Our findings for LID-1 presented 100% and 95% specificity for well-defined and oligosymptomatic cases. The main drawback for LID-1 is the poor sensitivity, with no more than 6% for oligosymptomatic early cases.

Another useful tool extensively applied for the detection of various bacterial diseases is qPCR, which was tested for diagnoses of patients or for monitoring HCs of leprosy cases (36). We observed 86.07% qPCR positivity in cases diagnosed in the field, similar to previous data using well-defined leprosy cases, corroborating the use of RLEP qPCR as a complementary tool to confirm the disease, even in early oligosymptomatic cases (37). These data are just confirming our previous work showing 83.9% positivity of RLEP PCR on new cases, in fact very near of the 86.07% that we had now, against 27.4% of contacts positive for RLEP PCR (38).

Taken together, our data indicate that 1) NDO-BSA, LID-1, and NDO-LID, for specificity or sensitivity reasons, are not reliable for use as POC diagnostic tools, especially for oligosymptomatic cases that are the main targets for leprosy control in the remaining endemic regions, and that 2) qPCR using RLEP is a reliable tool to use as a complementary laboratory exam for leprosy diagnosis, even in patients diagnosed early in the field.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institute of Health Sciences Research Ethics Committee from Pará Federal University (CAAE 26765414.0.0000.0018 CEP-ICS/UFPA). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

The study concept was designed by AG, JB, MS, and CS. Active case-finding strategy was designed and supervised by JB. Clinical examination of sensory-motor functions was performed by MF and CS. The database software with all clinical and epidemiological information was created and managed by GC. Serological experiments were performed and analyzed by AG, TM, PC, and JS. Antigens used in serological

experiments were generously provided by MD and JS. Molecular experiments were designed, managed, and performed by AR-d-S, RB, PP, and MS. The manuscript was primarily written by AG with substantial critical revision by JB, AR-d-S, MD, MS, JS, and CS. The final manuscript was read and approved by all co-authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ftd.2022.850886/full#supplementary-material>

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Conflict of Interest: Author MD is employed by HDT Biocorp. He has provided antigens to commercial companies for the manufacture of diagnostic tests.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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GLOSSARY

AUC	area under the curve
BB	borderline-borderline
BL	borderline-lepromatous
BT	borderline-tuberculoid
Ct	cycle threshold
DG	disability grade
HC	household contact
HC _{NC}	household contact diagnosed as a new case of leprosy
HC _{URE}	household contact of cases from URE
HC _{URE-NC}	household contact of cases from URE diagnosed as new case
I	indeterminate
LID-1	leprosy immune diagnostic 1
LL	lepromatous-lepromatous
MB	multibacillary
NDO-BSA	natural disaccharide O-linked bovine serum albumin
NDO-LID	natural disaccharide octyl—leprosy IDRI diagnostic-1
OD	optical density
PB	paucibacillary
PGL-I	phenolic glycolipid I
PNL	primary neural leprosy
qPCR	quantitative PCR
ROC	receiver operating characteristic
SC	schoolchildren
SCNC	schoolchildren diagnosed as new case of leprosy
SSS	slit skin smear
TT	tuberculoid-tuberculoid
URE	Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará
URE _{NC}	new cases from URE