Anti-PGL-I seroepidemiology in leprosy cases: household contacts and school children from a hyperendemic municipality of the Brazilian Amazon

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Summary

Objective: We investigated the prevalence of antibodies against PGL-I in people affected by leprosy (PAL) who were diagnosed and treated between 2004 and 2010, their household contacts (HC) and school children (SC) from a hyperendemic municipality in the Brazilian Amazon, and determined the prevalence of previously undiagnosed leprosy (PPUL) among both the HC and SC.

Design: We conducted a cross-sectional study involving 87 PAL, 302 HC and 188 SC. The subjects were clinically assessed, and their levels of anti-PGL-I antibodies were determined by ELISA. The subjects were also interviewed to determine their demographic and socio-economic characteristics.

Results: For PAL, a mean of 44 (SD = 21.8) months had passed since their initial diagnosis, and 34 (39%) of them remained seropositive. The level of anti-PGL-I antibodies was significantly higher in multibacillary (MB) than in paucibacillary (PB) cases ($P < 0.05$). Thirty-nine percent of HC were positive for anti-PGL-I, and we detected eight (2.6%) new cases among these individuals. One hundred and twenty-five SC (66.5%) were seropositive, and we detected nine (4.8%) new cases of leprosy (eight PB and one MB) in this group. When we visited the homes of SC affected by...
leprosy, 31 contacts were clinically examined, and three (10%) new cases were detected (one PB and two MB). The mean age of students with leprosy was 14.1 years (SD = 2.5; min = 10, max = 18).

Conclusion: The seroepidemiology of anti-PGL-I and the PPUL among both HC and SC suggests that there are many active foci of infection and that Mycobacterium leprae is circulating among this population.

Introduction

Leprosy, a chronic infectious disease caused by Mycobacterium leprae, is under control in developed countries; however, 228,474 new cases were detected worldwide in 2010, mostly in developing countries. The registered prevalence at the first quarter of 2011 was 192,246 cases. Brazil, with 34,894 new cases detected in 2010, has the highest prevalence rate of leprosy in the world (1.56/10,000 people), registered at the end of the first quarter of 2011, and has yet to reduce its rate below the threshold of 1/10,000 people – the level at which leprosy would be considered eliminated as a public health problem. The geographic distribution of leprosy is heterogeneous in Brazil. The more developed states in the South, such as Rio Grande do Sul, have already achieved the elimination target, whereas there are clusters of high endemicity in the North, Central-West and Northeast, suggesting a high concentration of leprosy cases in parts of Brazil. Most of the areas with clusters are in the Brazilian Amazon, which has been recognised as a highly endemic leprosy area.

Case detection among children under 15 years old is correlated with recent disease and active foci of transmission in the community, reflecting the efficiency of local control programmes. The state of Pará in the Amazon region is hyperendemic in this age group, with an annual case detection rate of 20.4/100,000 people in 2008, much higher than the Brazilian average of 5.8/100,000 people, indicating that M. leprae is circulating among this population of children.

Household contacts (HC) of untreated multibacillary (MB) leprosy patients are considered the main group at risk of contracting the disease. Early case detection and multidrug therapy (MDT) administered regularly and completely are the key components of a leprosy elimination programme. The diagnosis of leprosy is based mainly on clinical examination, and there is no laboratory test that detects all forms of leprosy. Since phenolic glycolipid-I (PGL-I), a species-specific antigen from the M. leprae cell wall, was isolated and characterised, various studies have noted the potential use of serology to detect antibodies against PGL-I to classify patients for treatment purposes, case monitoring, identifying the risk of relapse, and in the selection of HC at a higher risk of contracting the disease. A positive test for anti-PGL-I is associated with an 8.6-fold higher leprosy risk in HC and a 4.4-fold higher risk in non-contacts. Therefore, screening tests to determine the seroepidemiology in hyperendemic areas may be useful to identify subclinical infection among HC and children in the general population, although there are no conclusive data confirming this approach.

The objectives of this study were to: (1) detect the prevalence of IgM antibodies against PGL-I among people affected by leprosy (PAL), their HC and school children (SC) from a hyperendemic municipality in the Brazilian Amazon; (2) detect the prevalence of new leprosy cases among this population, and (3) identify clinical, demographic and socioeconomic characteristics that contribute to seropositivity and disease.
Methods

This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethics Committee from the Federal University of Pará (protocol number 197/07 CEP-ICS/UFPA).

Study Design, Setting and Population

We conducted a cross-sectional study in the municipality of Castanhal, state of Pará, Brazil, in June 2010. Castanhal is 68 km from Belém, the state capital, and its total population was 173,096 inhabitants in 2010. There were 82 public elementary and high schools with a total of 39,331 students in 2010. According to the municipal secretary of health, there were 633 newly detected leprosy cases from 2004 to February 2010. The annual case-detection rate among the general population was 48.2/100,000 inhabitants in 2009; among children under 15 years old, the case-detection rate was 10.3/100,000, classifying the municipality as hyperendemic according to the parameters designated by the Brazilian Ministry of Health.

Participants

Leprosy is a compulsory notifiable disease in Brazil; thus, all patients have their clinical data and addresses registered in the national notifiable diseases information system (SINAN). We identified patients’ addresses in cooperation with the municipal secretary of health. A random sample of 100 subjects from 11 urban neighbourhoods and one rural village, identified as leprosy cases from 2004 to February 2010, were electronically selected according to a sequence generated by BioEstat 5.0 software (Sociedade Civil Mamirauá, Amazonas, Brazil). These individuals were visited at their homes by a team of health care professionals with experience in treating leprosy patients, including a dermatologist, a nurse, a physical therapist, a lab technician and a local community health agent. The subjects and their HC were clinically assessed for signs and symptoms of leprosy, and a sample of peripheral blood from each person was collected to identify the prevalence of IgM antibodies against PGL-I. They were also interviewed about their demographic and socioeconomic characteristics using a standard questionnaire.

Eligibility criteria were as follows: (1) residing at the same address as when they were diagnosed, (2) being home at the time of health care team visit, and (3) providing written, informed consent to participate in the study. When a participant was younger than 18 years old, consent was obtained from parents or another responsible adult in the family. There were no restrictions on gender, skin colour, age group, or comorbidity. When at least one eligibility criterion was not met, another patient home in the same neighbourhood was selected from a randomly generated reserve list.

In addition to evaluating cases and contacts, we also randomly selected 200 students between six and 18 years old from four public schools (two elementary and two high schools) located in four different peripheral neighbourhoods. Teachers, selected students and their parents received information about general aspects of leprosy, followed by visits from the team of health care professionals, who clinically evaluated the students and collected blood samples from those who consented to participate in the study. When a new case was detected among the students, we went to the student’s home to evaluate household contacts.
The diagnosis of a new case among PAL, HC or SC was done by the finding of a skin lesion with loss of sensation. After that, the case was classified as indeterminate leprosy when there was only a hypopigmented macule, with no detection of nerve involvement (PB cases), or as one of the clinical forms defined by the Ridley-Jopling classification (TT as PB cases, while BT, BB, BL or LL were classified as MB cases).

LABORATORY PROCEDURES

Peripheral blood samples (4 ml) were collected aseptically in EDTA tubes by an experienced technician; plasma was separated by centrifugation (2000 rpm/5 min) and then stored at −80°C until use. For analysis, samples were defrosted and immediately tested at the Dermato-Immunology Laboratory in Marituba, Pará, Brazil. Seropositivity was determined by enzyme-linked immunosorbent assay (ELISA).

The following ELISA protocol was used: native PGL-I (batch 9/10/10 pool 16/20), generously provided by Dr. John Spencer of Colorado State University (USA), was coated onto 96-well, flat polystyrene microtitre plates (Kartell S.P.A., Novigilio, Milan, Italy) using 0.5 μg/well in 50 μl of ethanol, and plates were stored overnight in a fume hood at room temperature (RT) to evaporate the ethanol from the wells. The plates were blocked for one hour using 200 μl/well of PBS, pH 7.2, containing 3% bovine serum albumin (BSA, Sigma-Aldrich A7906, St. Louis, MO, USA) (blocking solution), then washed twice with the same solution. Two microlitres of each plasma sample was diluted 1:200 in 400 μl blocking solution, and 100 μl of this solution was added to the wells in duplicate and to one blank control well (not coated with PGL-I) for each subject and incubated for 2 hours at RT. After incubation with plasma, the wells were washed five times with 0.3% BSA-PBS (washing solution) using an automatic plate washing machine (Ultrawash plus, Dynex Technologies, Chantilly, VA, USA) and then one more time with blocking solution. Then, 100 μl/well of secondary anti-human IgM antibody (μ-chain specific) conjugated with peroxidase (Sigma-Aldrich A0420, St. Louis, MO, USA) at a 1:10 000 dilution in blocking solution was added and incubated for 2 hours at RT. After incubation with the conjugate, the wells were washed five times with washing solution, one time with blocking solution, and then two more times with PBS only. After washing, 100 μl of o-Phenylenediamine dihydrochloride substrate (SIGMAFAST™ OPD, Sigma-Aldrich P9187, St. Louis, MO, USA) was added. After 30 minutes, the reaction was stopped with 50 μl of sulphuric acid solution (4 N), and after 10 minutes, the absorbance was read at 490 nm using an MRX Revelation 4.25 microplate reader (Dynex Technologies, Chantilly, VA, USA). The cut-off for positive results was arbitrarily established at an optical density (OD) of 0.295, based on the average plus three times the standard deviation of the test results from 14 healthy subjects from the same hyperendemic area. The final OD value of each plasma sample was determined by subtracting the OD background of the blank control well from the mean of the duplicates from each respective subject. To control for each plate, a positive plasma sample from a high bacilloscopic index (BI) lepromatous leprosy patient and a negative sample from an US-born healthy person with no known exposure to leprosy who lives in Colorado (USA) were included in duplicate on each plate. An external validation was performed to assure the quality of the laboratory procedures and reagents used.
DATA ANALYSIS

The collected data were submitted to descriptive analysis and methods of statistical inference using BioEstat 5.0 software. Statistical significance was assessed using a significance level of 0.05 (two-tailed). Student’s t-test or Mann-Whitney U test was used to assess quantitative data from independent samples. A Chi-squared or Fisher’s exact test was used to compare proportions between different groups when appropriate.

Results

Of the 100 randomly selected PAL, 87 were visited at their homes by the team of health care professionals and were included in the present study. During these visits, we were also able to evaluate 302 HC. Thirteen patients were excluded because we were unable to find their residences; they had moved away, had died or did not consent to participate. Of the 200 students selected for the examination of SC, 188 were evaluated. Twelve students were excluded because their parents did not attend the meeting with the researchers to provide written consent for their children’s participation.

The mean age of PAL included in this study was 39.8 years old (SD = 17.6; min = 12, max = 91). At the time of clinical examination and blood collection, a mean of 44 (SD = 21.8; min = five max = 84) months has passed since the initial diagnosis of these individuals; 34 (39%) of them were positive for anti-PGL-I; 81 (93%) lived in an urban area; 32 (37%) were illiterate or functionally illiterate; 31 (36%) had completed elementary school and 24 (28%) had completed high school; 21 (24%) indicated that they had experienced starvation at least once, as defined by a full day without meals, because of the absence of resources to buy food [IBGE]; 65 (75%) had family incomes of up to twice the Brazilian minimum wage (approximately 695 US dollars), including 56 (64%) that received some kind of governmental financial assistance, such as family allowance and retirement benefits; and 35 (40%) lived in a house with more than two people per bedroom.

According to the degree of physical disability at the time of diagnosis, discharging by cure and the day of evaluation in this study, 41 people (47%) started and finished treatment with no disability, 26 (30%) were diagnosed with some degree of disability and improved during or after the MDT, and 13 (15%) patients experienced diminished functional capacity after discharge by cure. Table 1 shows the seroprevalence in PAL according to sex, age group, WHO classification, the number of BCG scars and the duration since diagnosis.

The levels of anti-PGL-I antibodies were significantly higher in MB than in PB cases (Figure 1), but there was no difference between those who became more physically impaired and those who remained well or experienced improvements in their levels of disability with MDT (P = 0.160; 95% CI = −0.63 to 0.10). The mean elapsed time since diagnosis among seronegative and seropositive PAL was 46.2 and 40.7 months, respectively, and there was no significant difference between these groups (p = 0.272; 95% CI = −15.44 to 4.42).

The mean age of HC was 28.8 years old (SD = 17.6; min = three, max = 81), and 126 (42%) were male. Table 2 presents the seroprevalence among contacts according to sex, age group, index case classification and the number of BCG scars.

Of the 302 HC evaluated, 118 (39%) were positive for anti-PGL-I; six (2%) were previously affected by leprosy and completed the MDT. Furthermore, we detected eight new
cases among these individuals, corresponding to 2.6% of all of the HC clinically examined (four PB and four MB).

Among SC, 125 (66.5%) tested positive for anti-PGL-I. We detected nine new cases in students (eight PB and one MB, 88.9% anti-PGL-I positive) and visited seven of them at their homes (two addresses were not found). During these visits, 31 HC of the newly diagnosed students were also clinically examined, and three (10%) additional new cases were detected.

Table 1. The seroprevalence in leprosy cases* according to sex, age group, WHO classification, the number of BCG scars and the elapsed time since diagnosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategory</th>
<th>Seropositive</th>
<th>Seronegative</th>
<th>OR (95% CI)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>14 36.8</td>
<td>24 63.2</td>
<td>0.84 (0.35–2.02)</td>
<td>0.706</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>20 40.8</td>
<td>29 59.2</td>
<td>1.18 (0.49–2.82)</td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td>&lt;15 years old</td>
<td>1 20</td>
<td>4 80</td>
<td>0.37 (0.04–3.47)</td>
<td>0.347</td>
</tr>
<tr>
<td></td>
<td>≥15 years old</td>
<td>33 40.2</td>
<td>49 59.8</td>
<td>2.69 (0.28–25.1)</td>
<td></td>
</tr>
<tr>
<td>Classification</td>
<td>Paucibacillary</td>
<td>8 30.8</td>
<td>18 69.2</td>
<td>0.59 (0.22–1.58)</td>
<td>0.299</td>
</tr>
<tr>
<td></td>
<td>Multibacillary</td>
<td>26 42.6</td>
<td>35 57.4</td>
<td>1.67 (0.63–4.43)</td>
<td></td>
</tr>
<tr>
<td>BCG scar1</td>
<td>None</td>
<td>16 42.1</td>
<td>22 57.9</td>
<td>1.22 (0.50–2.99)</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td>One</td>
<td>14 34.1</td>
<td>27 65.9</td>
<td>0.63 (0.25–1.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Two</td>
<td>2 100</td>
<td>——</td>
<td>——</td>
<td></td>
</tr>
<tr>
<td>Elapsed time since diagnosis</td>
<td>≤ 36 months</td>
<td>18 47.4</td>
<td>20 52.6</td>
<td>1.85 (0.77–4.44)</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>&gt; 36 months</td>
<td>16 32.7</td>
<td>33 67.3</td>
<td>0.54 (0.22–1.29)</td>
<td></td>
</tr>
</tbody>
</table>

* Cases detected from 2004 to February 2010, evaluated cross-sectionally in June 2010.
# Chi-squared test.
1 The odds ratio could not be calculated because of the null frequency of the matrix.

Figure 1. The levels of anti-PGL-I in people affected by leprosy according to WHO classification. Note: WHO = World Health Organisation. PB = Paucibacillary. MB = Multibacillary. OD = Optical Density. Statistical differences between groups were calculated using Student’s t-test (2-tailed), 95% CI = −0.67 to −0.03.
(one PB and two MB). Only 18 (58%) of the students’ HC were BCG vaccinated, and nine (29%) were positive for anti-PGL-I, including two of the three new cases. The mean age of students with leprosy was 14.1 years old (SD = 2.5; min = 10, max = 18), and none of them had disabilities at the time of diagnosis. Table 3 presents the demographic characteristics, BCG vaccination history, rate of new case detection, and seroprevalence in students per visited school.

There was no correlation between SC age and seroprevalence (Pearson’s correlation coefficient, $P = 0.142$; 95% CI = −0.04 to 0.25), but when we grouped HC and SC, there was a significant difference between the proportion of BCG-vaccinated individuals who were seropositive compared to unvaccinated subjects (Figure 2).

Table 3. The demographic characteristics, BCG vaccination, rate of new case detection, and seroprevalence of students per school

<table>
<thead>
<tr>
<th>School</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled students in 2010</td>
<td>660</td>
<td>504</td>
<td>755</td>
<td>1,477</td>
<td>3,396</td>
</tr>
<tr>
<td>Evaluated students</td>
<td>49</td>
<td>47</td>
<td>42</td>
<td>50</td>
<td>188</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>8.8 (1.3)</td>
<td>11.1 (1.8)</td>
<td>16.5 (1.9)</td>
<td>16 (1.5)</td>
<td>13 (3.6)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>20/29</td>
<td>29/18</td>
<td>17/25</td>
<td>9/41</td>
<td>75/113</td>
</tr>
<tr>
<td>BCG vaccination (%)</td>
<td>45 (92)</td>
<td>46 (98)</td>
<td>41 (98)</td>
<td>47 (94)</td>
<td>179 (95)</td>
</tr>
<tr>
<td>New cases detected (%)</td>
<td>0 (0.0)</td>
<td>3 (6.4)</td>
<td>3 (7.1)</td>
<td>3 (6.0)</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td>Seroprevalence (%)</td>
<td>31 (63.3)</td>
<td>27 (57.4)</td>
<td>31 (73.8)</td>
<td>36 (72)</td>
<td>125 (66.5)</td>
</tr>
<tr>
<td>OD of seropositives, median (IQR)</td>
<td>0.453 (0.284)</td>
<td>0.446 (0.335)</td>
<td>0.593 (0.211)</td>
<td>0.581 (0.385)</td>
<td>0.543 (0.287)</td>
</tr>
<tr>
<td>OD of seronegatives, median (IQR)</td>
<td>0.178 (0.035)</td>
<td>0.208 (0.078)</td>
<td>0.185 (0.107)</td>
<td>0.230 (0.095)</td>
<td>0.191 (0.083)</td>
</tr>
</tbody>
</table>

Note: IQR = interquartile range. M/F = male/female. BCG = Bacillus Calmette-Guérin. OD = optical density read at 490 nm. There were no statistically significant differences in the proportions of BCG vaccination, new case detections or seroprevalence among the different schools (Chi-squared test, $p > 0.05$).
Discussion

The former team leader of the WHO’s Global Leprosy Programme, Vijaykumar Pannikar, has indicated that Brazil’s control efforts focus almost entirely on urban areas, to the detriment of poor northeastern and Amazon regions, where leprosy is particularly acute.\textsuperscript{10} Our study demonstrates that \textit{M. leprae} infection is widely spread in this sample of the Amazon population, especially among school children. Official data indicate that the State of Pará has the highest known number of new cases detected among children less than 15 years old in Brazil (8,938 cases, from 1994 to 2007).\textsuperscript{11} Furthermore, there is evidence that in highly endemic areas, the prevalence of previously undiagnosed leprosy in the general population is six times higher than the registered prevalence.\textsuperscript{12}

According to the Brazilian Leprosy Control Programme (PNCH), all HC of newly detected cases should be examined, but the average proportion of examinations, which has been considered precarious since 2002, was only 41% in Pará.\textsuperscript{3} Case detection is based mainly on spontaneous demand at health facilities. When we visited the houses of PAL and evaluated their HC, we detected a number of new cases that would correspond to a prevalence

\begin{table}
\centering
\caption{The optical density (OD) values in leprosy cases*, household contacts and school children}
\begin{tabular}{lllll}
\hline
Category & Median OD (IQR) & Subcategory\textsuperscript{a} & Median OD (IQR) & \textit{p}-value\textsuperscript{b} \\
\hline
People affected by leprosy\textsuperscript{*} & 0.224 (0.324) & Seropositive & 0.543 (0.866) & <0.0001 \\
 & & Seronegative & 0.152 (0.116) & \\
Household contact & 0.216 (0.290) & Seropositive & 0.467 (0.302) & <0.0001 \\
 & & Seronegative & 0.128 (0.124) & \\
School children & 0.377 (0.365) & Seropositive & 0.543 (0.287) & <0.0001 \\
 & & Seronegative & 0.191 (0.083) & \\
\hline
\end{tabular}
\textsuperscript{a} Cases detected from 2004 to February 2010, evaluated cross-sectionally in June 2010.
\textsuperscript{b} The cut-off for positivity = 0.295 OD.
\textsuperscript{c} Mann-Whitney \textit{U} test (two-tailed).
Note: OD = optical density read at 490 nm. IQR = interquartile range.
\end{table}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{seroprevalence.png}
\caption{The seroprevalence of anti-PGL-I according to BCG vaccination status. Note: BCG = Bacillus Calmette-Guérin. This sample represents all household contacts and school children evaluated. Statistical differences between groups were calculated using Fisher’s exact test (2-tailed); 95% CI = 1.06 to 3.39.}
\end{figure}
rate of 265/10,000 people in this specific group. These clinical findings, along with the high proportion of seropositivity, confirm that the HC of newly detected cases must be examined and also indicate that HC should be examined not only at the time of index case detection but also periodically, perhaps annually, during the average period of disease incubation.

In June 2010, we examined 13.7% of all PAL diagnosed from 2004 to February 2010 in Castanhal in a cross-sectional study. It is expected a reduction in the serum levels of antibodies against PGL-I after the start of MDT. This decline is typically 25 to 50% per year, but in some patients, the decline is slower, and seropositivity can remain for years after an official cure.\textsuperscript{13–15} The high rate of seroprevalence observed in this sample of former patients could be the result of: (1) dead or inactive bacteria that are still present in PAL; (2) constant contact with an untreated source of infection; or (3) a first indicator of a relapse, given that seropositivity is a risk factor in the future development of relapses, especially in those groups of patients who have received a shorter than usual course of treatment.\textsuperscript{16,17} However, we did not evaluate the antibody levels at the beginning of treatment, thus it is possible that those values were higher than that at the moment of examination, meaning that the anti-PGL-I titers is simply gradually declining after MDT.

Despite little change in incidence rates of leprosy, one study shows that the disease is more common in people with insufficient education in lower social classes than in other population groups, and that this reality is more stark and systematic in the Amazon region of Brazil than in other areas of this country.\textsuperscript{18} This characteristic was observed in our study. Notably, Pará has one of the highest population densities per domicile in Brazil (4.1 people per residence),\textsuperscript{19} and we found almost half of PAL living in a home with more than two people per bedroom, with up to 15 people living in a single impoverished house. This fact probably contributes to the dissemination of communicable diseases like leprosy; it has been shown that people living in households with more than seven members have a 3.1-fold higher risk of contracting disease than people living in households of 1–4 members.\textsuperscript{20}

In addition, Feenstra and colleagues\textsuperscript{21} stated that a recent period of food shortage was significantly associated with clinical manifestation of leprosy. We detected that the proportion of PAL who experienced starvation (24%) was much higher than that of the general population in the North of Brazil (9%), and almost five fold higher than the national average (5%).\textsuperscript{22} Subnutrition could impair the effectiveness of immunological system,\textsuperscript{23} and should be target as part of the leprosy control strategy.

Some PAL experienced a worsening in their levels of disability after finishing MDT. It has been observed in other studies that approximately 5% of PB and 20% of MB patients develop sensitivity losses after their official cure.\textsuperscript{24} We failed to find any correlation between the degradation of functional capacity and the levels of anti-PGL-I, although both symptoms have been directly correlated with high bacilloscopic index (BI) and MB leprosy.\textsuperscript{25–28}

Serology is useful for the identification of those with an increased risk of developing leprosy among high-risk groups such as contacts.\textsuperscript{16,20,29} Studies of the seroprevalence among HC have reported seropositivity of approximately 1.9% to 18.4%,\textsuperscript{6} but our results are significantly higher than these published data. Such variance may be attributed to differences in the level of regional endemicity.\textsuperscript{30} The ELISA protocols are also different among studies, and disparities such as sera dilution, the type of antigen and the cut-off for positive results could partially explain the variability of the results. Of eight new cases detected among HC, four were clinically classified as PB, three of them seronegative for anti-PGL-I; and four were clinically classified as MB, three of them seropositive for anti-PGL-I. These findings are in accordance with previously published data.\textsuperscript{16}
The high seroprevalence and, particularly, the elevated prevalence of previously undiagnosed new cases among SC were the most surprising results of this study. These data clearly suggest the presence of several active foci of infection and that *M. leprae* is widely circulating among this population. If this finding is reproducible for the entire population of SC from the public schools of Castanhal, it is possible that approximately 1,884 undiagnosed cases exist among SC at this time (a rate of 479/10 000 students).

While 66.5% of the SC were anti-PGL-I positive, eight (88.9%) of nine SC newly detected as leprosy cases were seropositives, indicating a possible role for anti-PGL-I in functioning as a tool for detection of SC with a higher risk of developing leprosy. However, the present sample is not sufficient to evaluate this hypothesis, and new studies are necessary to elucidate this.

Five (55%) of the newly diagnosed students were younger than 15 years old, and two of their HC evaluated and also diagnosed with leprosy during visits to their homes were also children under 15 years old. Single skin-lesion paucibacillary leprosy was the most common clinical form found in these cases. Such clinical presentation is often not detected by less experienced clinicians, nor is it noticed by the patient. The total number of new cases detected among children during this cross-sectional study (nine, including HC + SC + contacts of SC) was almost two-fold higher than that detected during the entire year of 2009 in Castanhal (five, according to notifications registered at SINAN up to May 2011).

Another unexpected and important result was the significant association between seropositivity and BCG vaccination status. We assume that people who were vaccinated have a higher tendency to develop immunological surveillance against *Mycobacterium spp.* infections. Thus, once infected, they may be more likely to produce antibodies. Other studies have found a similar association, but without statistical significance. One group of researchers hypothesised that the high reactivity toward *M. leprae* lipoarabinomannan (LepLAM), a cell wall antigen, in healthy volunteers who did not have any known exposure to either tuberculosis (TB) or leprosy in a region where leprosy and TB are endemic could be the result of increased exposure to pathogenic or avirulent environmental mycobacteria (for example, in the soil and water) or to BCG vaccination.

It has been shown that infants produce and progressively increases detectable serum levels of anti-PPD IgM after a single BCG dose; that BCG coupled to homologous anti-BCG serum IgM lead to more rapid necrosis and resolution of experimental granulomas in rats and that melanoma GM2 protein coupled to BCG induces more IgM antibodies than GM2 or BCG alone. The high levels of anti-PGL-I IgM found in SC may be explained by the co-occurrence of early *M. leprae* exposure and BCG, inducing and maintaining high IgM levels, which may decrease after effective cellular immune response, as shall occur in the majority of the population.

We also analysed the seroepidemiological results using a receiver operating characteristic (ROC) curve. This analysis indicated a cut-off point of 0.411 (OD), with a sensitivity of 88.9% and a specificity of 56.4%. Even considering these data with a higher cut-off for positivity, the seroprevalence among SC remains extremely elevated (45.2%). These findings are substantially higher than those observed in other similar studies of SC.

We presume that people from hyperendemic areas are exposed to infection starting in early childhood and develop some degree of immunological response. Godal and Negassi reported that approximately 50% of subjects with household or occupational contact with leprosy for at least a year have immunological evidence of exposure to *M. leprae*, indicating that the subclinical infection rate is much higher than the prevalence of the disease.
Our data indicate that children may be infected very early, produce IgM antibodies for months or years, and then for genetic, immunological or less probable \textit{M. leprae} strain reasons, shift for a good Th1 cellular immune response, eliminating the bacteria. Some of them may not develop a good cellular immune response or may not develop at all, and will present the different clinical forms of leprosy.

It seems that the majority of people infected by \textit{M. leprae} will never present clinical signs and symptoms of leprosy, harbouring a subclinical infection for some time, but that a considerable number of people will be affected by the disease. The main challenge is to discover who will become ill and what can be done to prevent it.

Chemoprophylaxis has been associated with reduced leprosy incidence in the first years after implementation.\textsuperscript{38–42} The use of single-dose rifampicin given to contacts of newly diagnosed leprosy patients is a cost-effective intervention strategy, but further research should be conducted to evaluate the impact of this treatment strategy on endemicity of the Brazilian Amazon region.

The seroepidemiology of anti-PGL-I and the PPUL among both HC and SC suggest that there are many active foci of infection and that \textit{M. leprae} is circulating among this population. Therefore, it is necessary to identify and properly handle the hidden prevalence of leprosy in the Brazilian hyperendemic municipalities, otherwise, the targets of the leprosy elimination programme will not be achieved in the coming decades for these areas of the world.

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\section*{Authors’ Contributions}

Josafá Barreto designed the study, interviewed the participants, performed the laboratory assays, statistically analysed the data and wrote the manuscript. Layana Guimarães evaluated the functional status of the subjects and analysed these data. Márcia Leão interviewed and examined the participants. Denis Ferreira collected and processed samples. Rosanna Lima interviewed patients and participated in the local logistics. Claudio Salgado designed and coordinated the study; clinically examined, diagnosed and classified the subjects; supervised the data analysis and checked the manuscript. All authors participated in the interpretation of the data, read and approved the final manuscript.

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References


