RESEARCH ARTICLE

Anti-PGL-1 Positivity as a Risk Marker for the Development of Leprosy among Contacts of Leprosy Cases: Systematic Review and Meta-analysis

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Abstract

Background
There is no point of care diagnostic test for infection with M. Leprae or for leprosy, although ELISA anti PGL-1 has been considered and sometimes used as a means to identify infection.

Methods
A systematic review of all cohort studies, which classified healthy leprosy contacts, at entry, according to anti-PGL1 positivity, and had at least one year follow up. The outcome was clinical diagnosis of leprosy by an experienced physician. The meta-analysis used a fixed model to estimated OR for the association of PGL-1 positivity and clinical leprosy. A fixed model also estimated the sensibility of PGL-1 positivity and positive predictive value.

Results
Contacts who were anti PGL-1 positive at baseline were 3 times as likely to develop leprosy; the proportion of cases of leprosy that were PGL-1 positive at baseline varied but was always under 50%.

Conclusions
Although there is a clear and consistent association between positivity to anti PGL-1 and development of leprosy in healthy contacts, selection of contacts for prophylaxis based on anti PGL1 response would miss more than half future leprosy cases. Should chemoprophylaxis of controls be incorporated into leprosy control programmes, PGL1 appears not to be a useful test in the decision of which contacts should receive chemoprophylaxis.
Contacts of leprosy cases are more likely to be infected and develop leprosy. But not everyone infected with *M. Leprae* develops clinical leprosy. We examined and summarized all the eight studies that evaluated how well PGL-1 predicts which contacts of leprosy will become cases. PGL-1 positive contacts were 3 times more likely to develop leprosy; a variable proportion, but less than 30% of the cases were attributed to PGL-1 and less than 45% of the PGL-1 contacts developed leprosy. PGL1 would not be an appropriate test to decide which contacts of leprosy should receive preventive therapy if this was proposed in leprosy control programmes.
antigen other than PGL1 conjugated with bovine serum albumin (BSA) met the exclusion criteria. When more than one paper described the same cohort, we included the one with most information.

We searched PUBMED, EMBASE, LILACS, IMSEAR, WPRIM, WHOLIS, IMEMR and INDMED from 1983, when the technique for detection of anti-PGL-1 was published, to April 2015. The electronic search strategy on PUBMED was:


We decided to include papers written in English, French, Spanish or Portuguese. Endnote files kept all selected references and abstracts. Two authors (SN and PI) read the abstracts and selected the papers for inclusion in the review. When they disagreed, a third author (MLFP) reviewed it based on the paper’s full text. These three authors assessed the paper’s full text defining those to include in the systematic review.

One of the authors (MLFP) abstracted the data and another (SN) checked it. Our main measure of association was the odds ratio (OR) and its log transformation (LOR) based on the number of patients at the beginning of follow up in each category (anti_PGL1 positives and negatives) and the number of cases in each category.

We used the Tool to Assess Risk of Bias in Cohort Studies from Cochrane Bias Methods Group to classify each paper. We did not apply items 4 and 5 since these items were about the presence and control of other prognostic factors, which was not relevant for this review. We also abstracted data about the site of the study, proposed time of follow up, type of antigen used, technique of the test, dilution used and cut off point.

We estimated the summary LOR as the combined inverse-variance weighted LOR of the individual studies, i.e., used a fixed effect model.

As a measure of heterogeneity, we used Cochran’s Q (this has the same distribution as chi square with n -1 degrees of freedom, where n is the number of studies). The set of studies was considered heterogeneous if p<0.1. The inconsistency index was estimated (I^2) and if the index was 40% or less, we considered that the inconsistency was not important. A funnel plot evaluated publication bias Sensitivity analysis was based on the variation of the summary OR when one study was removed.

We present a ROC plane plot with the results of each study. Ulrich et al. was excluded from the plot because in this study included all contacts with negative reactions to M leprae and only a sample of those with positive reaction. The study sample is not balanced in respect of all possible immunological response among contacts, although it has internal validity.

**Results**

We retrieved abstracts of 462 papers and we selected 27 for full-text reading. From those, 9 were selected for the systematic review and 8 entered for the meta-analysis (Fig 1). We accepted the authors’ definition of household contacts and considered neighbourhood contact if the study selected their sample due to the presence of leprosy cases in an area.
Table 1 presents some characteristics of the selected studies [10,11,12,13,14,15,16,17,18]. Table 2 shows the extracted data for each study.

Table 3 shows the bias assessment of the papers. Brasil et al. paper [14] was excluded from the meta-analysis because the follow up procedures were not the same in those who were PGL-1 positive and those who were negative: the anti-PGL-1 positive group had annual medical consultation scheduled during the four years follow up period, but the anti-PGL-1 negative
group received the test result with information about leprosy signs and symptoms, and the PGL-1 results and interpretation, but there was no active follow up and leprosy diagnosis in this group depended on the individual demand for medical consultation. We considered this to be differential follow-up as the leprosy diagnosis strategy introduced severe ascertainment bias and thus excluded the study from the meta-analysis. We considered this differential follow-up.

Fi g 2 shows the forest plot of the included studies. The total number of contacts included in these studies was 18197, with 4140 anti PGL1 positives and 14057 anti PGL1 negatives. The summary ORs with Brasil et al. (14) study removed varied from 2.72 to 3.53, but all the 95% confidence interval included 3.11, the fixed model point estimate. The summary measure with

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### Table 1. Characteristics of the studies selected in the systematic review.

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<tr>
<th>FIRST AUTHOR</th>
<th>YEAR</th>
<th>PLACE</th>
<th>ANTIGEN</th>
<th>ASSAY</th>
<th>DILUITION</th>
<th>CUT POINT</th>
<th>TIME* (YEAR)</th>
<th>TYPE OF CONTACT</th>
<th>PREVALENCE PGL1 + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHANTEAU</td>
<td>1993</td>
<td>FRENCH POLINESIA</td>
<td>NTP-BSA</td>
<td>ELISA</td>
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<td>9</td>
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<td>YAL, ZAIRE</td>
<td>PGL1-BSA</td>
<td>ELISA</td>
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<td>KALO, PAPUA NEW GUINEAN</td>
<td>PGL1-BSA</td>
<td>ELISA</td>
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<td>ULRICH</td>
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<td>VENEZUELA</td>
<td>NATIVE PGL-PBS-BSA</td>
<td>ELISA</td>
<td>1:300</td>
<td>0.25</td>
<td>4</td>
<td>HOUSEHOLD</td>
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<td>BRASIL*</td>
<td>2003</td>
<td>ESTADO SÃO PAULO, BRAZIL</td>
<td>KIT ULTRAMICRO ELISA</td>
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<td>Missing</td>
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<td>INDIA</td>
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<td>ELISA</td>
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<td>PGL1-O-BSA</td>
<td>ELISA</td>
<td>Missing</td>
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<td>7</td>
<td>NEIGHBORHOOD</td>
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<tr>
<td>GOULART</td>
<td>2008</td>
<td>UBERLANDIA, BRAZIL</td>
<td>KIT ML FLOW</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>HOUSEHOLD</td>
<td>12.31</td>
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<tr>
<td>DUPPRE</td>
<td>2012</td>
<td>RIO DE JANEIRO, BRASIL</td>
<td>KIT ML FLOW</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22</td>
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* maximum duration of follow up proposed by the authors

& excluded from the final analysis

doi:10.1371/journal.pntd.0004703.t001

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### Table 2. Data extracted from the selected papers.

<table>
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<tr>
<th>Study name</th>
<th>anti-PGL1+</th>
<th>DISEASE</th>
<th>Total</th>
<th>%</th>
<th>anti-PGL1-</th>
<th>DISEASE</th>
<th>Total</th>
<th>%</th>
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<td>10</td>
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<td>BAGSHAWE 1990 [10]</td>
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<td>12</td>
<td>552</td>
<td>2.17</td>
<td>12</td>
<td></td>
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<tr>
<td>ULRICH 1991 [12]</td>
<td>14</td>
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<td>6</td>
<td>6349</td>
<td>0.09</td>
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<td>SINHA 2004 [16]</td>
<td>1</td>
<td>26</td>
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<td>4.29</td>
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<tr>
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<td>19</td>
<td>342</td>
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<td>41</td>
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<td>2.29</td>
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doi:10.1371/journal.pntd.0004703.t002
Table 3. Risk of bias asessment.

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<th>Q 1</th>
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<th>Q 7</th>
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<th>PRESENCE OF BIAS</th>
<th>OBS</th>
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<td>YES</td>
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<td>YES</td>
<td>YES</td>
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<td>PROB YES</td>
<td>YES</td>
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<td>PROB NO</td>
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<td>NO</td>
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<td>PROB NO</td>
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<tr>
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<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
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<td>YES</td>
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<td>YES</td>
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<td>YES</td>
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<td>YES</td>
<td>PROB YES</td>
<td>PROB NO</td>
<td></td>
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</table>

Q1. Was selection of exposed and non-exposed cohorts drawn from the same population?
Q 2. Can we be confident in the assessment of exposure?
Q 3. Can we be confident that the outcome of interest was not present at start of study?
Q 6. Can we be confident in the assessment of outcome?
Q 7. Was the follow up of cohorts adequate?
Q 8. Were co-interventions similar between groups?

Excluded questions:
Q4. Did the study match exposed and unexposed for all variables that are associated with the outcome of interest or did the statistical analysis adjust for these prognostic variables?
Q5. Can we be confident in the assessment of the presence or absence of prognostic factors?

Q=9.549 p= 0.219 df=26.693

Fig 2. Results of studies and summary OR for leprosy.
random effects estimate was 3.05 CI95% [1.99–4.67]. The point out of the confidence limit of the funnel plot (Fig 3) represents the excluded paper that had an OR of 10.18.

Fig 4 graphically represents the sensitivity and 1-specificity of each study. The sensitivity varied from 2% [16] to 39% [17] and the specificity from 83% [13] to 98% [16]. Table 4 presents these values and the positive predictive value (PPV) of each study, i.e., the proportion of positives results that developed clinical leprosy. Douglas 2004 is the study with higher PPV due to a high specificity and a moderate sensibility. Chanteau 1993 and Sinha 2004 had higher specificity but a very low sensibility.

Discussion

The meta-analysis shows that, among healthy contacts of leprosy cases, the risk of developing leprosy is roughly 3 times higher in those who are positive to anti PGL1 than in those who are negative. This was very homogenous. The sensibility of the PGL1 test as a predictor of clinical leprosy development was below 50% for all studies and its specificity was above 80%.

The main methodological limitation of the studies included in this review is the high percentage of losses to follow up in the individual studies. The probability that these losses were associated to the serological result is low, and therefore we consider it unlikely that this would have introduced selection bias. Another limitation is that most papers did not report person years of follow up, so that only the OR could be used as the measure of the association. If we ignore these losses, the summary relative risk would be 3.02 CI 95% [2.2–4.2] (S1 Fig), very close to our summary OR estimate. Given the rarity of leprosy in contacts, we are confident that the OR is a good estimation of the relative risk. Here, the OR is the odds of a positive test among those that will developed clinical relatively to those who will not. We also accept that the risk of developing leprosy changes with time since exposure, which would make analysis of person years by duration of follow up more precise; but in the absence of this necessary information, we suggest that the assumption that the association is constant in time since the first
exposure is robust, since duration of follow up was the same for positive and negative controls in all studies. The studies LOR have a very weak correlation with follow up duration (correlation coefficient = -0.0786).

Heterogeneity of the ORs between the studies was not high, with an I² = 26.7, even if different techniques were used for serology, including different antigens. No paper had a clear definition of household or neighbourhood contact—different criteria that would lead to potentially very different level of exposure to leprosy infection in each study. This difference does not bias the estimate of the OR, but is probably the reason for the variation in incidence of leprosy and of the proportion of anti PGL1 positivity between the studies. The sensitivity analysis produced estimates that are included in the 95% CI of the main summary estimates, pointing that the result of the main analysis is not very sensitive to the choice of studies.

Although the OR summarizes the accuracy of the test, as it is here the ratio of sensitivity and one minus specificity, it is difficult to be used for programme decisions. We could not

<table>
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<th>STUDY</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
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<td>1.96</td>
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<td>GROENEN 1990</td>
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<td>93.81</td>
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<td>14.29</td>
<td>84.72</td>
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<td>DOUGLAS 2004</td>
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<td>1.69</td>
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<td>GOULART 2008</td>
<td>39.29</td>
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</tr>
<tr>
<td>DUPPRE 2012</td>
<td>31.67</td>
<td>83.52</td>
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doi:10.1371/journal.pntd.0004703.t004
summarize the sensitivity and specificity of all studies because of great heterogeneity ($I^2 = 80.8\%$ for sensitivity and $98\%$ for 1-specificity). This is expected because of the variation of techniques, dilution and cut points used. However, our revision points that we cannot expect a sensitivity over $50\%$. The reduction of heterogeneity of OR occurs for sensitivity and specificity are correlated.

Routine leprosy control programmes include contact tracing and activities for early diagnosis and prevention of leprosy cases. In addition to clinical examination of contacts for leprosy diagnosis and health education, a few countries include BCG vaccination or revaccination as a prophylactic measure [19]. Another measure often discussed but not yet approved or adopted is chemoprophylaxis with a single dose of rifampicin to treat infection before it develops into leprosy [20]. Currently, the scientific community awaits the result of ongoing controlled trial testing BCG and chemoprophylaxis in the reduction of leprosy in contacts [21]. A collaborative group that includes Novartis Foundation is now implementing a “Leprosy Post-Exposure-Prophylaxis (LPEP)” project that includes pilot subprojects around the world to introduce LPEP in contacts of newly diagnosed cases. The possibility of selecting contacts at higher risk of leprosy for prophylaxis through anti-PGL 1 testing is included in the report of Novartis Foundation Expert Group [22]. We did not find any data supporting this practice, although some authors suggest it [14]. Our findings do not support this recommendation.

Our results show that among the studies included in this review the highest sensitivity is less than $40\%$. This suggests that selecting contacts positive for anti-PGL for prophylactic measures, would only prevent less than half of leprosy cases among contacts, assuming that the efficacy of chemoprophylaxis in preventing leprosy is $100\%$. This selection would also give chemoprophylaxis unnecessarily to more than $80\%$ (see positive predictive value).

There is an association between anti-PGL 1 positivity and development of leprosy, but we cannot state that anti-PGL 1 result reflects recent infection by *M. leprae*. The relationship is more complex and involves host immunity: patient with tuberculoid form (TT) of leprosy are not positive to PGL-1, and for sure, they are infected with *M. leprae*. Because of this, the test cannot be used to measure infection rate in communities, as suggested by some papers [9,23]. The fact that the immunological response can vary among leprosy cases allows a hypothesis that anti PGL 1 antibodies production is present when the immunological response of an infected individual is in the lepromatous end of the disease spectrum (LL). If this is the case, why is the proportion of positives contacts that develop the disease not higher? (Table 3).

A large number of serological test for tuberculosis diagnosis were developed and commercialized in many countries, with many claiming high accuracy, but the current evidence do not support these claims [24]. For leprosy there is only a few commercial tests: *Leprosy Detect ELISA Kit* from InBios, USA proposed for diagnosis [25] or screening [26] and *OL Hanseniase* from OrangeLife, Brazil. Is it possible that these are tests in search of an application?

We found few papers analysing the mechanisms and functions of humoral immunity in the interaction of *M. leprae* and humans. Studies had shown that antibodies produced by tuberculosis infection/disease target about $0.5\%$ of *M. tuberculosis* proteome and that the target antigen varies a lot among individuals [27]. Different *M. tuberculosis* lineages also produce different immunological response. Antibody-mediated immunity is often found to be irrelevant in the control of the infection of intracellular microorganisms, but the current literature points otherwise [28].

The antibody response in tuberculosis correlates positively with bacillary burden the same way anti PGL1 antibodies in leprosy patients do. This correlation could indicate that healthy contacts positive for anti-PGL1 have been exposed to *M. leprae* and have a high bacillary burden. This hypothesis is consistent with the fact that the test detects IgM antibody, an early response to infection and it is therefore interpreted as indicating recent infection. Fig 4.
graphically represents the sensitivity and 1-specificity of each study. nevertheless, animal models had shown that IgM antibody might last and participate in long-lasting protection against obligate intracellular bacterium [28]. The hypothesis that not all infected individuals produce anti PGL1 IgM antibodies and that presence can result from both recent and old infection with M leprae is plausible and consistent with the lack of ability of anti PGL1 to predict accurately who will and who will not develop leprosy.

Conclusion

Although there is a clear and consistent increase in risk of development of leprosy in anti PGL1 positive healthy contacts, selection of cases for prophylaxis intervention based on anti PGL1 response would reach less than half of future leprosy cases, and result in much unnecessary treatment. Leprosy research must explore the role of antibody production in leprosy and it is similar to that in tuberculosis.

Supporting Information

S1 Fig. Meta-analysis using relative risk as association measure. Results and forest plot. (PDF)

S1 Checklist. PRISMA checklist. (PDF)

Author Contributions

Conceived and designed the experiments: MLFP GOP LCR. Performed the experiments: MLFP PCI SN. Analyzed the data: MLFP LCR. Wrote the paper: MLFP LCR GOP PCI SN.

References