Association between anti-PGL-I IgM and clinical and demographic parameters in leprosy

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Summary

Objective To determine the risk factors and clinical significance of anti-PGL-I seropositivity.

Design A large-scale sero-epidemiological study (COLEP) was carried out in northwest Bangladesh. Blood on filter paper from 1025 newly diagnosed patients was collected before treatment was started and tested with an anti-PGL-I ELISA; the relation between patient determinants and seropositivity was calculated using logistic regression.

Results The median age was 30 years and the male:female ratio 1.9. Overall, 342 patients (33.4%) were seropositive. The following determinants showed a significant correlation with seropositivity ($P < 0.05$) in multivariate analysis: sex, age, disability grade, bacterial index and classification according to the World Health Organization (WHO) system. The number and extent of clinical signs correlated with seropositivity, except for the presence of satellite lesions. People with or without a BCG vaccination scar had a similar risk to be seropositive.

Conclusion Serology is a marker for a higher systemic bacterial load and may identify potential infectious sources among patients with few clinical signs. The size of skin lesions was positively correlated with seropositivity. We did not find different levels of seropositivity among patients with one or two skin lesions, neither did we find different levels among patients with or without satellite lesions.

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Introduction

Leprosy is a chronic infectious disease, which is still a major public health problem, mainly in Africa, Asia and Latin America.\(^1\) When left untreated, infection with *Mycobacterium leprae* may eventually lead to severe disabilities. Differences in the cellular immune response of the host determine the clinical features, which form a spectrum and vary from one or a few hypopigmented anaesthetic skin lesions to extensive skin involvement and irreversible damage to the peripheral nerve system.

Accurate diagnosis and classification of leprosy patients is important for treatment purposes as correct treatment may prevent disabilities, relapse and continued transmission. Currently, there are two classification systems in use, which are at least partially complementary:

1. The classification according to Ridley and Jopling is based on immunological and histopathological features and makes a distinction between tuberculoid (TT) and lepromatous (LL) leprosy. Between these poles there are three borderline groups (BT, BB and BL) and a separate indeterminate group (I).\(^2\)

2. The World Health Organization (WHO) designed a less demanding classification system for treatment purposes. The WHO classification system is based on clinical (and when available bacteriological) features and divides leprosy patients into multibacillary patients (MB, 6 or more skin lesions/satellite lesions and/or a positive bacterial index [BI as determined by microscopy]) and paucibacillary patients (PB, up to 5 skin lesions/ satellite lesions and a negative BI).\(^3\) In some control programmes, PB patients with a single lesion (SLPB) are recorded separately.

In the WHO classification system, satellite lesions, small (secondary) lesions in the vicinity of a larger (primary) lesion, are counted as separate lesions. The WHO system does not take into account the large variation in the size of lesions. However, there are theoretical arguments for a relation between lesion size and the proliferation of bacteria.\(^4\) Moreover, the authors learned from discussion with leprosy control staff in various countries that lesion size may influence the classification decision made by doctors and field workers (unpublished observations). This would decrease the power of any statistical analysis based on classification data.

There are currently two tools available for routine control programmes to aid the correct classification of leprosy patients:

1. The BI is a logarithmic scale ranking from zero to six, which defines the bacterial load found by microscopy after acid-fast staining of skin smears or biopsies.\(^5\)

2. With the development of rapid tools, serology has become an easily applicable method in the field.\(^6\) The presence of antibodies to the *M. leprae*-specific phenolic glycolipid-I (PGL-I) correlates with the bacterial load of a leprosy patient and its detection can aid the classification of confirmed leprosy patients as MB or PB for treatment purposes.\(^7\)

In this study, we relate the PGL-I-based serology results of 1,025 newly diagnosed, well characterized leprosy patients from Bangladesh to their detailed clinical and demographic characteristics. Factors determining seropositivity are established as well as the clinical relevance of serology results. This study is part of a prospective (sero-) epidemiological study on contact transmission and chemoprophylaxis in leprosy (COLEP).\(^8\)
Materials and methods

PATIENTS AND SAMPLES

This serological study is part of the COLEP study. The patients were from northwest Bangladesh and were detected through passive case detection. They were diagnosed at Danish Bangladesh Leprosy Mission (DBLM) clinics between May 2002 and October 2003. The districts of Nilphamari and Rangpur in northwest Bangladesh have a total population of approximately 4.3 million with 1505 new leprosy cases detected by the DBLM staff in 2001 (case detection rate 3.5/10,000 population). Patients were classified based on the WHO classification system. A medical doctor confirmed the diagnosis for every patient and treatment was given according to the WHO/DBLM guidelines. Group sizes were set at a maximum of 400 for SLPB and 400 for PB and a minimum of 200 for MB patients; patients with the pure neural form of leprosy were excluded. Eleven patients with a positive disability grade were reclassified from SLPB into PB. Four patients who were initially classified as SLPB (1) or PB (3) were reclassified as MB based on a positive BI. Ridley & Jopling classification is not performed at DBLM.

A single blood sample was obtained from 1025 of the 1037 patients enrolled in the COLEP study, consisting of 383 SLPB, 348 PB and 294 MB patients.

From each patient demographic and clinical data were collected. Finger prick blood was collected on 0.37 mm blotting paper (GB002 Schleicher and Schuell,’s Hertogenbosch, the Netherlands), air-dried and stored in plastic zip bags with silica gel at -20°C until use.

The study abides by the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences, CIOMS, Geneva, 1993). Ethical clearance was obtained from the Ethical Review Committee of the Bangladesh Medical Research Council and written informed consent was obtained from each patient before inclusion in the study.

COATING OF ELISA PLATES

Serology for the detection of IgM antibodies against *M. leprae* was performed using the ELISA technique previously described with natural trisaccharide linked to bovine serum albumin via a phenolic ring (NT-P-BSA) as a semi-synthetic analogue of PGL-I. Round-bottomed microtitrator plates (NUNC 96 U Invitrogen/Life Technologies, Taastrup, Denmark) were coated with 50 μl/well NT-P-BSA (0.01 μg carbohydrate/ml dilution in 0.1 mol/l ammonium hydrogen carbonate buffer, pH 8.0). Wells to control for non-specific binding were coated with 50 μl of a solution containing 0.082 μg/ml BSA of the same batch that was used for the preparation of NT-P-BSA. Plates were air dried for 2 days at room temperature and stored in sealed plastic bags with silica gel in the dark at room temperature until use (within 6 months).

ELISA

On the day before testing, a 3-17 mm diameter disc was punched from the blood impregnated filter paper card into a polystyrene tube and incubated overnight at 4°C in 25 μl phosphate buffered saline (pH 7.2) containing 0.1% (v/v) Tween 20 (PBST). The next day 183 μl of PBST + 10% (v/v) normal goat serum (Gibco Invitrogen/Life Technologies, Auckland, New Zealand; PBST + NGS) was added and incubated for 1 h. This corresponds to an approximately 1:167 dilution of serum.
Before adding the eluted samples, the pre-coated plates were washed with PBST (two times short and two times 2–5 min), followed by a blocking step with 100 μl/well PBS + 1% (w/v) BSA (Boehringer, Mannheim, Germany) at 37°C for 1 h. Next, 50 μl of the sample dilution was added to each well followed by incubation at 37°C for 1 h. Plates were washed as described above and 50 μl/well conjugate (1:10,000 dilution in PBST + NGS of a peroxidase-conjugated goat IgG fraction to human IgM 5FC μ; Capple/Organon Teknika, Turnhout, Belgium) was added and incubated at 37°C for 1 h. After another washing procedure 50 μl/well TMB substrate solution (0.4% (w/v) 3,3',5,5'-tetramethylbenzidine + 0.4% (w/v) urea hydrogen peroxide in DMSO [all three from Sigma-Aldrich, Steinheim, Germany], diluted 1:10 in 0.1 mol/l sodium acetate citrate buffer pH 4.0) was added to initiate a colouring reaction. The reaction was stopped by adding 50 μl/well 0.5 N H₂SO₄ when a standard serum reached a net optical density at 450 nm (OD) of 0.600. The status seropositive was given if the net OD was above 0.199. When the OD of the standard serum was either too low (OD < 0.55) or too high (OD > 0.75) the samples were retested. The ELISA performance was monitored using this standard plus a positive and negative control serum sample on each plate.

**BACTERIAL INDEX**

The BI was determined by microscopy on Ziehl–Neelsen stained slit skin smears taken from the earlobe, forehead and a skin lesion; the highest BI was recorded.

**CLINICAL SIGNS**

The clinical signs of the patient were recorded as number of skin lesions (hypopigmented and/or anaesthetic skin patches), number of nerves involved (nerves: facial, ulnar, radial cutaneous, median, lateral popliteal and posterior tibial; involved: enlarged, tender or painful) and as number of body areas affected (with a skin lesion and/or nerve involvement) according to the system described by Van Brakel et al., dividing the body into seven body parts, namely head, torso, back and the four extremities. Satellite lesions are recorded separately from the determinant “number of skin lesions”. The size of the largest skin lesion was estimated as being small (<10 cm diameter), medium (10–15 cm diameter) or large (>15 cm diameter). The clinical data set was based on body charts drawn by the DBLM staff.

**DATA ANALYSIS**

Patient and serological data were stored in Microsoft Access and Excel, respectively. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 11.0.1, SPSS Inc., Chicago, IL, USA; 2001). Logistic regression was used to identify independent determinants influencing the odds ratio for seropositivity. Determinants associated with seropositivity in univariate analysis (P < 0.10) were selected for multivariate analysis. In multivariate analysis, we tested for statistically significant (P < 0.05) interactions between determinants in the final model and for confounding.
Results

**PATIENT CHARACTERISTICS**

Blood samples were collected from 1025 newly diagnosed leprosy patients. The median age was 30 years (range 5–84) and the male:female ratio was 1.9. The distribution of the patients’ characteristics is presented in Table 1. The distribution of sex, age, BCG vaccination rate and disabilities differed among the three WHO classification groups. The MB group contained more male and older patients in comparison with the PB and SLPB groups ($P < 0.0001$). The MB group contained fewer BCG vaccinated patients ($P = 0.009$) and more patients with disabilities ($P < 0.0001$). The median age of the BCG vaccinated patients was lower, 25 years (interquartile range (IQR) 15–35) compared with non-vaccinated patients, who had a median age of 32 years (IQR 20–45, $P < 0.0001$). After age and sex adjustment there is an odds ratio of 1.54 for non-vaccinated patients to be classified as MB [exact binomial 95% confidence interval (95% CI) 1.09–2.18].

**FACTORS DETERMINING SEROPOSITIVITY**

Out of the 1025 patients, 342 (33.4%; 95% CI 30.5–36.3) were given the status seropositive based on ELISA testing (Table 2).

<table>
<thead>
<tr>
<th>Determinants</th>
<th>MB</th>
<th>PB</th>
<th>SLPB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determinants</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>28.7</td>
<td>348</td>
<td>34.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>230</td>
<td>78.2</td>
<td>221</td>
<td>63.5</td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
<td>21.8</td>
<td>127</td>
<td>36.5</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>3.59</td>
<td>1.74</td>
<td>1.38</td>
<td>1.91</td>
</tr>
<tr>
<td>Age (years)b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–14</td>
<td>28</td>
<td>9.5</td>
<td>63</td>
<td>18.2</td>
</tr>
<tr>
<td>15–29</td>
<td>78</td>
<td>26.5</td>
<td>118</td>
<td>34.1</td>
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<tr>
<td>30–44</td>
<td>98</td>
<td>33.3</td>
<td>99</td>
<td>28.6</td>
</tr>
<tr>
<td>45–59</td>
<td>65</td>
<td>22.1</td>
<td>49</td>
<td>14.2</td>
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<td>60 and above</td>
<td>25</td>
<td>8.5</td>
<td>17</td>
<td>4.9</td>
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<tr>
<td>Median age</td>
<td>39</td>
<td>12.9</td>
<td>29</td>
<td>8.2</td>
</tr>
<tr>
<td>BCG vaccinationc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58</td>
<td>19.9</td>
<td>100</td>
<td>29.2</td>
</tr>
<tr>
<td>No</td>
<td>233</td>
<td>80.1</td>
<td>243</td>
<td>70.8</td>
</tr>
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<td>Disability grade14</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>199</td>
<td>67.7</td>
<td>300</td>
<td>89.1</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>20.4</td>
<td>20</td>
<td>5.7</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>17.9</td>
<td>19</td>
<td>5.2</td>
</tr>
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</table>

*P*-value calculated with Pearson Chi-square.

b For two patients no age information was available.

c For eight patients no information on the BCG vaccination status was available.
A strong relation with seropositivity was shown for the determinants WHO classification and BI in both univariate and multivariate analyses (Table 2). In the multivariate analysis, the odds ratio (OR) for seropositivity was adjusted for differences in sex, age, BCG vaccination and disability distribution. Compared to SLPB patients, MB patients were more likely to be seropositive (adjusted odds ratio (aOR) MB 11.8; 95% CI 7.83–17.8) while PB patients had no difference in risk for seropositivity (aOR PB 1.27; 95% CI 0.86–1.86). Seropositivity increased with the BI: the aOR for patients with a BI 1 or 2 was 6.33 (95% CI 2.42–16.5) and the aOR for patients with a BI higher than 2 was 59.0 (95% CI 25.0–139) compared with BI negative patients.

### Table 2. Logistic regression analysis to determine risk factors for seropositivity among leprosy patients

<table>
<thead>
<tr>
<th>Determinants</th>
<th>No.</th>
<th>% Seropositive</th>
<th>Unadjusted OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted aOR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>95% CI&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
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<td></td>
<td></td>
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<tr>
<td>SLPB</td>
<td>383</td>
<td>16.7</td>
<td>1</td>
<td>0.93–1.95</td>
<td>1.27</td>
<td>0.86–1.86</td>
</tr>
<tr>
<td>PB</td>
<td>348</td>
<td>21.3</td>
<td>1.35</td>
<td>7.84–16.3</td>
<td>11.8</td>
<td>7.83–17.8</td>
</tr>
<tr>
<td>MB</td>
<td>294</td>
<td>69.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial Index&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>883</td>
<td>25.1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>23</td>
<td>69.6</td>
<td>6.81</td>
<td>2.76–16.8</td>
<td>6.33</td>
<td>2.42–16.5</td>
</tr>
<tr>
<td>&gt;2</td>
<td>104</td>
<td>94.2</td>
<td>48.6</td>
<td>21.0–112</td>
<td>59.0</td>
<td>25.0–139</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>673</td>
<td>34.3</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>352</td>
<td>31.5</td>
<td>0.88</td>
<td>0.67–1.16</td>
<td>1.46</td>
<td>1.05–2.04</td>
</tr>
<tr>
<td>Age (years)&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>5–14</td>
<td>142</td>
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<td>0.90</td>
<td>0.92</td>
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<tr>
<td>15–29</td>
<td>346</td>
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<tr>
<td>30–44</td>
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<td>33.4</td>
<td>0.97</td>
<td>0.70–1.34</td>
<td>0.67</td>
<td>0.45–0.98</td>
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<tr>
<td>45–59</td>
<td>167</td>
<td>34.1</td>
<td>1.00</td>
<td>0.68–1.48</td>
<td>0.53</td>
<td>0.33–0.86</td>
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<tr>
<td>60 or above</td>
<td>63</td>
<td>30.2</td>
<td>0.83</td>
<td>0.47–1.49</td>
<td>0.42</td>
<td>0.21–0.86</td>
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<tr>
<td>BCG vaccination&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
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<tr>
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<td>271</td>
<td>28.4</td>
<td></td>
<td>1</td>
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<tr>
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<td>1.36</td>
<td>1.00–1.84</td>
<td>1.20</td>
<td>0.84–1.72</td>
</tr>
<tr>
<td>Disability grade&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
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<td>29.2</td>
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<td>1</td>
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<td>4.27</td>
<td>2.65–6.89</td>
<td>1.79</td>
<td>1.01–3.16</td>
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<tr>
<td>2</td>
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<td>57.4</td>
<td>3.27</td>
<td>1.87–5.72</td>
<td>1.66</td>
<td>0.85–3.26</td>
</tr>
<tr>
<td>1–2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>134</td>
<td>61.2</td>
<td>3.83</td>
<td>2.63–5.58</td>
<td>1.73</td>
<td>1.09–2.76</td>
</tr>
</tbody>
</table>

<sup>a</sup> OR = odds ratio, 95% CI = 95% confidence interval.

<sup>b</sup> Adjusted OR; Determinants in the final model: WHO classification, sex, age, BCG vaccination and disability.

<sup>c</sup> aOR for Bacterial Index was calculated without the WHO classification determinant.

<sup>d</sup> For two patients the age was not recorded.

<sup>e</sup> For eight patients BCG data were not available.

<sup>f</sup> Analyses were performed with a recoded determinant disability grade (grade 1 and 2 were coded as 1–2).
Sex and age

In univariate analysis, the determinants sex and age were not significantly related with seropositivity. However, after adjustment for the other determinants in the multivariate analyses (model contains determinants: WHO classification, sex, age, BCG vaccination and disability grade) they appeared to be significantly related. Females were more likely to be seropositive than males (aOR 1.46; 95% CI 1.05–2.04) and the positivity prevalence decreased significantly with age (Table 2).

BCG vaccination

In univariate and multivariate analyses with the determinant BCG vaccination, no difference in seropositivity was found between BCG non-vaccinated patients and vaccinated patients (aOR 1.20; 95% CI 0.84–1.72). In addition, no correlation was found between BCG vaccination and BI positivity (OR BCG non-vaccinated patients 1.24; 95% CI 0.80–1.93).

Disability grade

In univariate analysis, the determinant disability grade had a significant relation with seropositivity; in the multivariate analysis this relation was reduced. Patients with a disability grade 1 and 2 were more likely to be seropositive compared to disability grade 0 patients, but only disability grade 1 showed a significant difference with disability grade 0 patients (aOR 1.79; 95% CI 1.01–3.16). Grouping the disability grade 1 and 2 together resulted in a significant aOR of 1.73 (95% CI 1.09–2.76).

Clinical signs

Detailed clinical data from 996 patients were available for analysis (Table 3).

Satellite lesions

Comparison between patients with satellite lesions and patients without satellite lesions showed no differences in sex, age or BI distribution and no correlation with serology was found in multivariate analysis (model contains: skin lesion (size), skin lesion (number), nerve, body area, sex and age). The correlation of satellite lesions in the univariate analysis was altered from significant to non-significant after adjustment with the determinant skin lesion (number) or with the determinant body area.

Skin lesion size

The size of a skin lesion was found to be a determining factor for seropositivity. The aORs of ‘medium’ and ‘large’ skin lesions were 1.45 (95% CI 0.94–2.23) and 2.37 (95% CI 1.47–3.83), respectively, compared with ‘small’ skin lesions.

Number of skin lesions

The determinants skin lesion (number), nerve and body area showed a positive correlation with seropositivity, in both univariate and multivariate analyses. The seropositivity rate...
increased significantly with the number of skin lesions: patients with three to five skin lesions had a significantly increased aOR of 2·54 (95% CI 1·42–4·54), while patients with two lesions did not have a significantly different aOR (aOR, 0·90; 95% CI 0·54–1·49) compared with patients with one lesion.

Number of nerves and body areas involved

Patients with more than two nerves involved and patients with more than five body areas affected were more likely to be seropositive. Having more than two nerves involved resulted in a significant aOR of 2·01 (95% CI 1·08–3·72) compared with no nerve involvement. When six or seven body areas were affected the aOR for seropositivity was 2·91 (95% CI 0·99–8·52) compared with one and two body areas.

None of the determinants showed any significant interaction. Analyses were repeated with the ELISA cut-off values 0·149, 0·249 and 0·299 to confirm the conclusions based on the cut-off value 0·199. No significant differences were found (data not shown).
Discussion

Leprosy serology has been studied frequently and many of the factors determining seropositivity are well known, as has been reviewed by Oskam et al.\textsuperscript{15} However, these studies were often performed on a limited number of patients. Here we describe a study on more than one thousand patients in which serology is compared to a large variety of clinical and demographic data and in which factors determining seropositivity are established.

The ELISA used was based on the detection of specific antibodies in peripheral blood eluted from blood spots on filter paper. Blood on filter paper was chosen for practical reasons: it is cheap, easy to collect in the field and requires no centrifugation or cold chain. However, blood eluted from blood spots is known to give a slightly lower signal in the ELISA compared with serum.\textsuperscript{16}

Patient Characteristics

The median age (30) and the male:female ratio (1.9) are in agreement with other reports from this area,\textsuperscript{17,18} taking into account that, due to the group size criteria described in the Materials and methods section, our study population included a higher percentage of MB patients (28.7%) than the actual situation (18.4% in 2002 and 21.9% in 2003).\textsuperscript{17}

The MB patient group comprised more males, older patients and more patients with a disability grade \( \geq 0 \) than the PB or SLPB groups. These differences between MB and PB patients are reported frequently\textsuperscript{18–20} and are thus in line with expectations.

In previous studies, BCG vaccination was held to be protective against leprosy and was highly associated with the development of tuberculoid leprosy instead of lepromatous leprosy, suggesting that BCG vaccination would protect against lepromatous leprosy.\textsuperscript{21,22} We see a similar, but less strong effect in our patient population with regard to the development of PB or MB leprosy: MB patients were less frequently BCG vaccinated than PB and SLPB patients (BCG coverage MB approximately 20%; PB and SLPB, 29%). After age and sex adjustment there is an aOR of 1.54 (95% CI, 1.09–2.18) for non-vaccinated patients to be classified as MB, compared to PB-SLPB patients. A lower BI and/or seropositivity among BCG vaccinated patients would have been a supplementary argument for this protective role of BCG and would confirm the hypothesis that BCG vaccination is responsible for a shift in the immune response towards the tuberculoid pole of the spectrum. However, the determinant BCG vaccination did not have any influence on either seropositivity or BI.

Factors Determining Seropositivity

As expected, a strong correlation was found between serology and the determinants WHO classification and BI.\textsuperscript{20,23–25} For the majority of patients who were MB, and particularly those who were skin smear positive, elevated levels of \( M. \text{leprae} \) specific IgM antibodies were found (Table 2).

The prevalence of seropositivity in this study population showed similar age and sex patterns as demonstrated in other studies.\textsuperscript{19,25–27} The decline in seropositivity prevalence with increasing age is consistent with the decrease of overall IgM levels with age. It has been suggested that females have higher innate IgM levels than males, which may be the explanation for the higher seropositivity rate found among females.\textsuperscript{28} The alteration in significance for the variable sex on seropositivity in multivariate analysis compared to univariate analysis can be explained by the high number of male MB patients (male:female...
The variable WHO classification confounded the correlation between sex and seropositivity in univariate analysis.

A correlation between disability grade and serology (aOR disability 1 and 2 1.73; 95% CI 1.09–2.76, \( P = 0.020 \)) was found, corresponding with the general trend reviewed by Oskam et al.\(^{15} \): PB patients with a disability generally had higher seropositivity rates than PB patients without a disability.

The seroprevalence among MB patients (69%) was rather low compared with other studies in which it varied between 75 and 100%.\(^ {15} \) Comparison between studies is difficult since the classification criteria have changed over the years and our data collection was done using filter paper blood which gives slightly lower titers than serum.\(^ {16} \) Another possible explanation for the relatively low seropositivity in the MB group may be the short detection delay: the DBLM leprosy control programme has been well established in the area since 1977. DBLM\(^ {17} \) and Richardus et al.\(^ {29} \) have reported gradually decreasing percentages of MB cases and disabilities in our study area, which may be caused by a reduction in detection delay due to intensive control efforts. Since the numbers of skin lesions, nerves involved and body areas affected are correlated with both detection delay and seropositivity, a lower number of clinical signs among the MB classified group due to a short detection delay would lead to a lower seropositivity in the MB group. Further study may explore this possibility.

**Clinical Signs Determining Seropositivity**

For a better understanding of the clinical significance of seropositivity, specific clinical determinants were related to serology results. Based on our results we can make a number of remarks with regard to the WHO classification system as it is currently used:

1. There was no serological difference between patients with and without satellite lesions (Table 3). Since there was also no serological difference between patients with one or two skin lesions it can be concluded that there is no serological evidence to distinguish between SLPB and PB with 2 lesions, with and without satellite lesions. This implies that the presence of satellite lesions may be ignored for quantification of skin lesions and that a distinction may be made between PB 1 and 2 lesions on the one hand and PB 3–5 lesions on the other hand, with PB 1 and 2 being equivalent with the current SLPB category.

2. The size of a lesion, here subjectively recorded from the largest lesion drawn on the patient information card, may also be a relevant factor for classification. There is a strong indication that the lesion size is a determining factor for seropositivity (Table 3).

The presence of not more than two lesions (regardless of the presence of satellite lesions) and no lesion larger than 10 cm diameter (small sized) may be new criteria for SLPB classification. If a separate SLPB treatment, such as ROM (rifampicin, ofloxacin, minocycline\(^ {3} \)), were used, this insight could have a large impact on the economic aspects of leprosy control. We realize that at the moment this is solely based on serological evidence and more detailed clinical information about response to treatment and risk of impairment will be needed to support our arguments for such an adjustment of WHO classification.

At an individual level seropositive PB patients may have disease that is behaving more like MB disease. It would be interesting to study if seropositive patients would benefit
from a longer treatment with regard to relapse and the development of reactions and nerve damage.

The exact number of lesions is less crucial for seropositivity among MB patients, although a difference was seen between patients with up to 15 lesions and patients with more than 15 lesions. The number of nerves and the number of body areas affected seem to be both independent factors for seropositivity.

It may be stated that seropositivity is highly correlated with clinical signs: numbers of skin lesions, nerves involved and body areas affected. All these clinical signs signify the dissemination of the bacterium in the body of the patient, indicating that seropositivity can be used as a marker for a higher systemic bacterial load, and therefore can be used to identify more infectious patients.30–33

CONCLUSIONS

In conclusion, we have shown that the presence of elevated anti-PGL-I antibody levels is highly correlated with the MB status, BI and the dissemination of clinical signs in a patient. It is clear that serology results reflect the overall systemic bacterial load of a patient. From a serological point of view, it seems reasonable to stop counting satellite lesions as whole lesions, to take skin lesion size into account for clinical decision-making, and consider the possibility to include patients with two skin lesions into the SLPB group. For individual patient management serological testing may give clinicians a better idea about the systemic bacterial load of a patient. The availability of simple serological tests makes this option feasible.

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