Clinical Presentation and Serum Antibody Reactivity of Leprosy Patients Attending a Dermatology Clinic in Caracas, Venezuela

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Summary  Leprosy is a stigmatising disease that remains a public health problem in the Americas, Asia and Africa. To gain insight as to the extent of the problem in Venezuela, we conducted a cross sectional study of 57 leprosy patients who attended the specialised clinic at the Institute of Biomedicine Dr. Jacinto Convit, Caracas. The majority presented with five or more lesions at the time of diagnosis, with the most commonly observed clinical lesion being nodules (in 66.7% of the patients), followed by maculae (36.8%), papules and plaques (each 28.1%). Following World Health Organisation guidelines for clinical classification, 77.3% of the patients were classified as multibacillary (MB). By Ridley-Jopling classification, 47.4% were LL, 24.6% BL and 5.3% BB, with the rest presenting with paucibacillary forms: 12.3% BT, 8.8% indeterminate and 1.8% TT. Regarding bacteriological index, 42 patients (73.7%) were scored as 2 or more, while M. leprae were not observed in skin slit smears of the remaining 26.3%. Upon serological evaluation, enzyme linked immunosorbent assay (ELISA) detected antibodies in MB patients against ND-O-HSA, LID-1 and the conjugate of these antigens, ND-O-LID, at similar rates. Together, our data indicate that many patients attending the clinic have advanced M. leprae infection and disease presentation. Our results provide further evidence that LID-1, ND-O-HSA and the ND-O-LID conjugate represent important reagents in the management of leprosy, with the detection of circulating antibodies having the potential to facilitate referrals for clinical diagnosis of leprosy in Venezuela.

Keywords: Hansen’s disease; antibody; diagnosis; serology

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Introduction

Leprosy, also referred to as Hansen’s disease, is a chronic infectious disease caused by Mycobacterium leprae. Although M. leprae infection can become systemic, it mainly affects skin, peripheral nerves, eyes and mucous membranes. Leprosy remains a public health problem in many countries and is most often associated with poverty, lack of sanitation, overcrowding and malnutrition. Prolonged close contact with an index case and host genetics also strongly influence the risk of M. leprae infection and development of disease.

In most cases, the clinical diagnosis of leprosy is considered to occur late in the evolution of the disease, with patients likely contagious and able to propagate further cases until they are recognised and treatment is provided. Diagnosis is only currently possible once symptoms have manifested to the point of skin lesions and damage to the nerves. Relatively few doctors have had the opportunity to attend a leprosy patient, making the lack of clinical experience a significant factor confounding early diagnosis. The definitive diagnostic method for leprosy is achieved by direct observation of acid fast bacilli following histological analysis of a biopsy collected from a skin lesion. This invasive and complex assessment requires the input of several clinical and laboratory specialities, and it is therefore considered neither rapid nor easy to achieve under field conditions. The introduction of a simple, sensitive, specific and rapid test that can be used on a large scale to aid early diagnosis and case management would be a highly beneficial breakthrough for leprosy control programs.

A number of M. leprae antigens that are indicated as having effects on the host immune response have now been well characterised and purified. Phenolic glycolipid (PGL)-I is one of the major components of the cell wall of M. leprae and a target of the infection-induced antibody response. Although the diagnostic value of detecting circulating antibodies against PGL-I is generally limited to the MB clinical forms, synthetic mimetics of PGL-I have been used in diagnostic test platforms (natural octyl di- or tri-saccharide linked to either bovine or human serum albumin: ND-O; NT-P; BSA; HSA, respectively). Exploring other components, particularly mycobacterial proteins, has provided additional understanding of the disease by providing insight with regard to immune reactivity, progression of disease and risk of relapse or development of reversal reactions. Such proteins are also of great interest for the development of both vaccines and diagnostic tests. Among the lead diagnostic proteins is Leprosy IDRI Diagnostic (LID)-1, the fusion protein derived from the conjoined expression of the ml0405 and ml2331 genes. This protein can be used in conjunction with PGL-I to enhance the diagnostic sensitivity of tests and has also been adapted into rapid diagnostic test platforms. In this study, we contrasted the serological response to LID-1 and ND-O-HSA, as well as the conjugate of these individual components ND-O-LID, in Venezuelan leprosy patients.

Materials and Methods

STUDY POPULATION

A study of sera from patients diagnosed with leprosy (Hansen’s disease) was conducted. This study was approved by the bioethics committee of the Institute of Biomedicine Dr. Jacinto Convit to allow the use of medical records in compliance with ethical guidelines and confidentiality of information, in accordance with the Declaration of Helsinki. The study population consisted of patients who were diagnosed with leprosy at the Dermatology...
Institute of Biomedicine, Caracas, Venezuela. Patients were included only if full clinical, bacteriological and histological data were available to confirm the diagnosis of Hansen’s disease and if they were over 18 years of age, and were excluded if they were pregnant, had severe mental disorders, a history of chronic diseases or were HIV infected. A total of 57 sera were collected.

**SERUM ANTIBODY RESPONSES**

Serum antibody responses were determined by enzyme-linked immunosorbent assay (ELISA) using the recombinant protein LID-1, synthetic mimetic of PGL-1 ND-O-HSA and the conjugate LID-NDO, as previously described. Flat bottom 96-well plates (Dynatech, Immunolon 2) were coated with each antigen in a volume of 50 μl per well: 0·1 μg conjugate per mL, 1 μg LID-1 or ND-O-HSA per mL, respectively. After incubating overnight at 4°C, 100 μl blocking buffer was added to each well and then incubated for 1 hour at room temperature with gentle stirring. Plates were washed with phosphate buffered saline (PBS)/Tween 20 and then with PBS, then 50 μl sera added at a dilution of 1:200 in diluent solution. Positive and negative control samples were included on each plate. Secondary peroxidase-conjugated human anti-IgG/IgM (Fc specific, Sigma) diluted in PBS/ Tween 20/ BSA was added to all wells for 2 hours at room temperature with gentle agitation. After this incubation, the plates were washed in the washing solution and finally 50 μl of substrate, hydrogen peroxide, H2O2 (30%, Merck) with ortofenilenodiamino-2HCl chromogen (Sigma), was added. Plates were incubated in the dark at room temperature for 15 minutes prior to stopping the reaction. To stop the reaction, 25 μL 1N H2SO4 solution was added. Plates were read in an ELISA reader (Labsystems Multiskan EX, type 355, Finland) using a 492 nm filter. Values were expressed in optical density (OD), and a threshold value of 0·2 derived from the sum of the mean OD + 3 times the standard deviation of a pool of sera from relatively healthy people (i.e, dermatology clinic attendees with benign conditions) was used to establish negative and positive criteria.

**STATISTICAL ANALYSES**

Averages and standard deviations for continuous variables were calculated and comparisons of continuous variables as nominal variables were made with the nonparametric Mann-Whitney U test. In the case of nominal variables, frequencies and percentages were calculated and the chi-square test of Pearson was applied. Fisher’s exact test was used in the case of 2 × 2 tables. Data were analysed with JMP-SAS 11.0. Differences were considered significant when P-value < 0·05 were obtained.

**Results**

**CLINICAL PRESENTATION**

The 57 Hansens’ disease patients enrolled had an average age of 39 years old, and the majority were male (39; 68.4%). Over three quarters were enrolled as MB patients (77.3%), with distribution across the Ridley-Jopling scale, but some skewing toward the lepromatous extreme (47.4% LL (27), 24.6% BL (14), 5.3% BB (three)). The remaining patients were enrolled as PB (12.3% BT (seven) patients, 8.8% IL (five) and only 1.8% (one) TT) (See Figure 1A).
Consistent with the Ridley-Jopling classifications, 48 patients (84.2%) had five or more lesions at the time of diagnosis, while nine patients (15.8%) had less than five lesions. Regarding lesion appearance, nodules were the most frequent manifestation, being observed in two thirds of the study participants, followed by maculae, then papules and plaques, which were observed in just over one quarter of the patients (See Figure 1b).

When skin slit smears were examined, the majority of patients had a bacteriological index of 2+ or above (42; 73.7%), while 15 patients (26.3%) were assessed as negative (BI 0) (See Table 1).

Together, these data indicate that many patients attending the clinic have advanced *M. leprae* infection and disease presentation.

![Figure 1. Leprosy classification and lesion appearance at time of diagnosis. Data are shown as per cent of patients within each Ridey-Jopling classification (LL; lepromatous leprosy; BL, borderline lepromatous; BB, borderline borderline; BT, borderline tuberculoid; TT, true tuberculoid; IL, indeterminate leprosy).](image)

Table 1. Demographics of the study population. Bacteriological index was established by skin slit smear (1+, equivalent to 1–10 bacilli in 100 fields; 2+, 1–10 bacilli in 10 fields; 3+, 10–100 bacilli in a single field; 4+, 10–100 bacilli in a single field; 5+, 100–1000 bacilli in a single field; 6+, more than 1000 bacilli in a single field)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number (from 57 total)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 17</td>
<td></td>
</tr>
<tr>
<td>Duration of symptoms pre-diagnosis (months)</td>
<td>12 (2–108)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>68.4</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>31.6</td>
</tr>
<tr>
<td>Number of lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>9</td>
<td>15.8</td>
</tr>
<tr>
<td>&gt;5</td>
<td>48</td>
<td>84.2</td>
</tr>
<tr>
<td>Bacteriologic index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>26.3</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>0.0</td>
</tr>
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<td>8</td>
<td>14.0</td>
</tr>
<tr>
<td>4+</td>
<td>9</td>
<td>15.8</td>
</tr>
<tr>
<td>5+</td>
<td>22</td>
<td>38.4</td>
</tr>
</tbody>
</table>
Antigen-specific antibody responses were evaluated by ELISA using serum from each patient. As expected, when stratified by clinical form, antibody responses were readily detected in patients with high BI and the proportion of patients, as well as magnitude of positive responses, declined as BI became lower (See Table 2 and Figure 2). Using the WHO operational diagnostic criteria, detection of an IgM response to ND-O-HSA test was achieved for 75% MB (33 of 44; LL, BL and BB) and 7·7% for PB patients (1 of 13; BT, TT and indeterminate), whereas detection of an IgG response to LID-1 facilitated recognition of 79·5% MB patients but no (0%) PB patients. Accordingly, antibody responses were readily detected in LL patients and the proportion of patients with positive responses was lowest toward the tuberculoid pole (See Figure 2). IgM antibody responses to the synthetic mimetic of PGL-I, ND-O-HSA, were detected in 70·4% of patients (19 of 27) that had diagnosis of LL, followed by 85·7% BL (12 of 14), 66·7% BB (2 of 3) and 14·3% BT (1 of 7) (See Figure 2A). An IgG antibody response against the fusion protein LID-1 was detected in 74·1% (20) cases that had an LL diagnosis, 92·9% BL (13) and 66·7% BB (2) (See Figure 2B). Responses to conjugate ND-O-LID were detected in 63-0% (17) LL cases, 85-7% (12) BL and 66-7% (2) BB cases (See Figure 2C). The conjugate of ND-O-LID detected antibodies in the sera of 70-5% MB patients and no (0%) PB patients (See Figure 2D). These data verify previous reports that antigen-specific antibodies are readily detected in MB, but not PB, patients.

### Table 2. Serum antibody responses by bacterial index. Detection of antigen-specific antibodies of only IgG (IgG) or both IgM and IgG isotypes (IgM + IgG) was conducted by ELISA. An OD value ≥ 0.2, the threshold derived from the sum of the mean OD + 3 times the standard deviation of the sera of relatively healthy people, was considered positive. $c^2 = 4.022$, $p$-value = 0.478

<table>
<thead>
<tr>
<th>BI</th>
<th>Seropositive (n)</th>
<th>ND-O-HSA (IgM + IgG)</th>
<th>LID-1 (IgG)</th>
<th>ND-O-LID (IgM + IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%¹</td>
<td>$n$</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>2</td>
<td>13.3</td>
<td>0</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>3</td>
<td>2</td>
<td>66.7</td>
<td>2</td>
</tr>
<tr>
<td>3+</td>
<td>8</td>
<td>6</td>
<td>75.0</td>
<td>5</td>
</tr>
<tr>
<td>4+</td>
<td>9</td>
<td>7</td>
<td>77.8</td>
<td>8</td>
</tr>
<tr>
<td>5+</td>
<td>20</td>
<td>17</td>
<td>85.0</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>34</td>
<td>100</td>
<td>35</td>
</tr>
</tbody>
</table>

¹Per cent within each BI category was calculated.

### Antigen-specific Antibody Responses

Antigen-specific antibody responses were evaluated by ELISA using serum from each patient. As expected, when stratified by clinical form, antibody responses were readily detected in patients with high BI and the proportion of patients, as well as magnitude of positive responses, declined as BI became lower (See Table 2 and Figure 2).

Using the WHO operational diagnostic criteria, detection of an IgM response to ND-O-HSA test was achieved for 75% MB (33 of 44; LL, BL and BB) and 7·7% for PB patients (1 of 13; BT, TT and indeterminate), whereas detection of an IgG response to LID-1 facilitated recognition of 79·5% MB patients but no (0%) PB patients. Accordingly, antibody responses were readily detected in LL patients and the proportion of patients with positive responses was lowest toward the tuberculoid pole (See Figure 2). IgM antibody responses to the synthetic mimetic of PGL-I, ND-O-HSA, were detected in 70·4% of patients (19 of 27) that had diagnosis of LL, followed by 85·7% BL (12 of 14), 66·7% BB (2 of 3) and 14·3% BT (1 of 7) (See Figure 2A). An IgG antibody response against the fusion protein LID-1 was detected in 74·1% (20) cases that had an LL diagnosis, 92·9% BL (13) and 66·7% BB (2) (See Figure 2B). Responses to conjugate ND-O-LID were detected in 63-0% (17) LL cases, 85-7% (12) BL and 66-7% (2) BB cases (See Figure 2C). The conjugate of ND-O-LID detected antibodies in the sera of 70-5% MB patients and no (0%) PB patients (See Figure 2D). These data verify previous reports that antigen-specific antibodies are readily detected in MB, but not PB, patients.

### Relationship of Serological Antibody Responses

When comparing results obtained with the ND-O-LID conjugate against results obtained with its individual components, we observed a very good agreement with both ND-O-HSA and LID-1 (kappa = 0.822 and 0.714; Table 3).

Although there were some samples that recognised only either LID-1 or ND-O-HSA, the ND-O-LID conjugate detected antibodies in the majority of sera that demonstrated antigen-specific responses (30 of 39; Figure 3).
Not surprisingly, there was a statistically significant positive correlation in the magnitude of response to ND-O-LID and that was observed against either LID-1 (overall $r = 0.780$) and ND-O-HSA (overall $r = 0.777$) (Figure 4; both $P$-value $= 0.001$). These data suggest that both components are contributing to the response detected by the conjugate.

![Graphs A, B, C, D](image)

**Figure 2.** Serum antibody responses by clinical presentation. Antibodies binding (A) LID-1, (B) ND-O-HSA and (C) the ND-O-LID conjugate, were assessed. Detection of antigen-specific antibodies of only IgG (IgG) or both IgM and IgG isotypes (IgM + IgG) was conducted by ELISA. Each point shows the response of an individual serum sample, with values expressed as optical density (OD). The bar represents the mean of each classification. In (D), the proportion of patients in each clinical classification with a positive antibody responses is tabulated against bacteriological index (BI). An OD value $>0.2$, the threshold derived from the sum of the mean OD +3 times the standard deviation of the sera of relatively healthy people, was considered positive. $c^2 = 3.884$, $p$-value $= 0.574$.

1Ridey-Jopling classification (LL: lepromatous leprosy; BL, borderline lepromatous; BB, borderline borderline; BT, borderline tuberculoid; TT, true tuberculoid; IL, indeterminate leprosy).

2per cent within each clinical classification was calculated.

### Table 3. Agreement of antibody responses

The level of agreement of the conjugate ND-O-LID with respect to its individual components was evaluated. Detection of antigen-specific antibodies of only IgG (IgG) or both IgM and IgG isotypes (IgM + IgG) was achieved by ELISA. An OD value $>0.2$, the threshold derived from the sum of the mean OD +3 times the standard deviation of the sera of relatively healthy people, was considered positive. A kappa value $<0.20$: poor, 0.21 to 0.40: weak, 0.41 to 0.60: moderate, 0.61 to 0.80: good, 0.81 to 1.00: very good

![Table 3](image)

Not surprisingly, there was a statistically significant positive correlation in the magnitude of response to ND-O-LID and that was observed against either LID-1 (overall $r = 0.780$) and ND-O-HSA (overall $r = 0.777$) (Figure 4; both $P$-value $= 0.001$). These data suggest that both components are contributing to the response detected by the conjugate.
The clinical evidence was suggestive of a late diagnosis in most cases, and indeed, almost one third of the patients (29.8%; 17 of 57) estimated that more than 24 months had elapsed from the appearance of signs and symptoms before clinical diagnosis of leprosy was attained (See Table 4).

It has previously been reported that antibody responses can rise prior to clinical diagnosis and we therefore hypothesised that the proportion of seropositive patients would be increased among the most delayed cases. There was a slightly increased proportion of patients with circulating antibodies against ND-O-HSA and ND-O-LID among the group reporting a delayed diagnosis, but this was not observed for LID-1 (See Table 4). Taken together, these

![Figure 3](image3.png)

**Figure 3.** Relationship of antibody responses according to antigens recognised. Detection of antigen-specific antibodies against LID-1, ND-O-LID and ND-O-HSA was achieved by ELISA, with a positive response determined as providing an OD value > 0.2, the threshold derived from the sum of the mean OD + 3 times the standard deviation of the sera of relatively healthy people. The distribution of responses among the patient serum samples evaluated (n = 57) evaluated is shown.

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![Figure 4](image4.png)

**Figure 4.** Correlation of antibody responses according to WHO classification. Detection of antigen-specific antibodies of only IgG (IgG) or both IgM and IgG isotypes (IgM + IgG) was achieved by ELISA. Each point shows the response of an individual serum sample, with values expressed as optical density (OD). Responses against ND-O-LID are contrasted against those detected against (A) LID-1 or (B) ND-O-HSA. For ND-O-LID versus LID-1, coefficients of $r = 0.683$ (p-value = 0.000) and $r = 0.648$ (p = 0.000) were found for MB and PB patients, respectively. For ND-O-LID versus ND-O-HSA, coefficients of $r = 0.676$ (p-value = 0.000) and $r = 0.996$ (p = 0.000) were found for MB and PB patients, respectively.
results suggest that patients with elongated periods of time with symptoms are more likely to have circulating antibodies.

Discussion

Despite the declining prevalence of leprosy in the most countries, the annual number of new cases reported globally remains relatively high and indicates that transmission of *M. leprae* is still occurring.\(^{21}\) In recent years, a series of tools to help diagnosis in the early stages of the disease have emerged, and these could help to prevent the continued transmission of *M. leprae* from patients and facilitate the eradication of leprosy. Currently diagnosis is based mainly on identifying clinical symptoms and signs. Considering that skin lesions may go unnoticed or unattended for years, leprosy is often diagnosed only when irreversible neurological disorders or disabilities have developed. One factor that adversely affects the ability to make this diagnosis is the shortage of health care professionals with the training and ability to correctly identify leprosy lesions or to facilitate the referral to specialised centres for appropriate case management. When used by general health and medical personnel (e.g. community nurses) as a diagnostic tool in patients suspected of Hansen’s disease, simple serological testing could address these shortcomings and facilitate referrals to experts. In the work presented here we evaluated the serological response of patients diagnosed with leprosy to the conjugate ND-O-LID, a new diagnostic alternative that had not previously been evaluated in Venezuela.

We observed a predominance of males amongst the patients in the present study, an observation consistent with most reports.\(^ {22}\) When we evaluated the number of lesions patients were presenting with at the time of diagnosis, it was noticeable that most patients had greater than five and that there was a predominance of MB (LL, LB and BB) forms. These observations were consistent with the high bacteriological indices detected in a majority of the patients at the time of diagnosis. Each of these indicators is suggestive of advanced leprosy due to a delay in diagnosis for most patients. Indeed, approximately one third of the patients studied reported that they had had experienced signs or symptoms of disease for over 24 months before attaining an accurate diagnosis as a leprosy patient.

Several studies have argued that the presence of antigen-specific antibodies is an indicator of *M. leprae* infection and, as such, a risk factor for the development of leprosy. Antibody

### Table 4. Relationship of duration of symptoms prior to, and serological response at, clinical diagnosis. Individuals that has antigen-specific antibodies at the time of diagnosis reported their estimated duration of symptoms prior to obtaining a diagnosis as a leprosy patient. Detection of antigen-specific antibodies of only IgG (IgG) or both IgM and IgG isotypes (IgM + IgG) was achieved by ELISA. An OD value > 0.2, the threshold derived from the sum of the mean OD + 3 times the standard deviation of the sera of relatively healthy people, was considered positive. Results are presented in percentages and duration of disease refers to the elapsed time from the onset of symptoms until appropriate diagnosis. \( \chi^2 = 1.325, P\text{-value} = 0.268 \)

<table>
<thead>
<tr>
<th>Disease duration (months)</th>
<th>ND-O-HSA (IgM + IgG)</th>
<th>LID-1 (IgG)</th>
<th>ND-O-LID (IgM + IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>% Positive</td>
</tr>
<tr>
<td>&lt;24</td>
<td>18</td>
<td>21</td>
<td>53.8</td>
</tr>
<tr>
<td>&gt;24</td>
<td>5</td>
<td>13</td>
<td>72.2</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>34</td>
<td>59.6</td>
</tr>
</tbody>
</table>

The presence of antigen-specific antibodies is an indicator of *M. leprae* infection and, as such, a risk factor for the development of leprosy.
responses to PGL-I generally correlate well with the bacillary load, and PB leprosy patients who present with low bacterial loads typically have low or undetectable antibodies. Many contacts of patients never develop disease despite having circulating IgM anti-PGL-I antibodies and others do not present with antibodies against this antigen but do develop the disease. These data have led to much debate regarding the overall utility of detecting anti-PGL-I antibodies and the suggestion that tests using synthetic mimetics of PGL-I, where the terminal di- or tri-saccharide portion is coupled with a linker to an inert carrier protein molecule such as albumin to allow use in traditional antibody detection platforms, be used in a more limited, confirmatory setting to support the classification of leprosy. In an attempt to discover antigens that can enhance the diagnosis of leprosy over that achievable by PGL-I-based molecules alone, many protein antigens were screened against patient sera and the LID-1 fusion protein developed. LID-1 has the ability to specifically detect the antibodies in sera from leprosy patients (most MB and a subset of PB) from different geographical areas (Brazil, China, Japan, Philippines, etc.). While complementing the ability of PGL-I to detect patients, LID-1 is also indicated as a useful prognostic indicator able to detect leprosy several months before the onset of clinical symptoms in some patients. We previously reported the performance of LID-1, as well as the components ML0405 and ML2331, in sera of Venezuelan patients and also noted that after completion of treatment (2 years) patients did not have circulating anti-LID-1 antibodies. In the present study we found that most MB patients were positive for ND-O-HSA and LID-1, with similar results for the ND-O-LID conjugate of these antigens. These data are somewhat in contrast with findings in Brazil, Philippines and China that observed a higher sensitivity and specificity of LID-1 compared to PGL-I, but this discrepancy may be due to the relatively small number of samples evaluated in our study. In addition, when assessed individually the very similar performance of the ND-O and LID-1 components suggests that the potential for complementation was limited in our study.

Despite the persistence of leprosy in many regions, clinical expertise continues to wane and there appears to be an increasing need for simple tests that could facilitate either diagnosis or referral to a specialist for clinical confirmation and management. Through this study, we expand the geographic range of the data to Venezuela and provide further evidence that LID-1, ND-O-HSA and the ND-O-LID conjugate are important reagents in the management of leprosy.

Acknowledgements

This work was conducted with financial support from National Fund for Science and Technology, Caracas Venezuela. Antigens were produced at IDRI with support from American Leprosy Missions. We confirm the independence of the researchers from the funding sources. We are extremely thankful to the patients and health care personnel, especially those in the clinical section, who attended the Central Dermatology Institute of Biomedicine Dr Jacinto Convit.

Conflict of Interest

Malcolm Duthie has provided antigen to companies for fabrication of rapid diagnostic tests. All other authors have declared that they have no conflict of interest. We certify that this
article contains the original data from our research activities and is for the first time submitted for publication.

**Ethical approval**

This article does not contain any studies with animals. All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

**References**

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