Autoantibodies in leprosy patients, with and without joint involvement, in the state of Amazonas

S.L.E. Ribeiro1,2, H.L.A. Pereira1,2, N.P. Silva2, E.I. Sato2

ABSTRACT

Objective: Determine the frequency of rheumatoid factor (IgM-RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP), antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), anticardiolipin antibodies (aCL), and anti-β2 glycoprotein I antibodies (anti-β2GPI) in leprosy patients, with and without joint involvement, and to evaluate the possible association among those antibodies and articular manifestations, clinical type, reactional episodes, polychemotherapeutic treatment (PCT), and discharge from PCT. Patients and methods: One hundred and fifty-eight leprosy patients were divided in two groups of 73 patients (Group I) and 82 patients (Group II). Group III was composed of 129 healthy individuals. Methods: Semi-quantitative latex agglutination test for IgM-RF, indirect immunofluorescence for ANA and ANCA, and ELISA for anti-CCP, aCL, and anti-β2GPI. Results: Fifty-six (35.4%) of 158 leprosy patients had lepromatous leprosy (LL). The frequency of anti-CCP, RF, and ANA antibodies in Groups I and II was similar to that of Group III. Antineutrophil cytoplasmic antibodies were not detected in any patient. Anticardiolipin antibodies were more frequent in leprosy patients (Groups I and II) than in control group (15.8% vs. 3.1%; \(P < 0.001\)), and differences between Groups I and II \((P = 0.67)\) were not observed. Anti-β2GPI antibodies were also more common in leprosy patients than in control group (46.2% vs. 9.4%; \(P < 0.001\)), without differences between Groups I and II. A predominance of IgM isotype over IgG isotype was observed both for aCL (88% vs. 16%; \(P = 0.001\)) and anti-β2GPI (97.3% vs. 12.3%; \(P < 0.001\)). Patients did not present manifestations suggestive of vascular thrombosis. Conclusion: The frequency of aCL and anti-β2GPI antibodies was significantly increased in leprosy patients than in healthy individuals. However, positivity to other autoantibodies was similar to that observed in the control group. An association between autoantibodies and joint involvement, reactional episodes, polychemotherapeutic treatment, discharge, and clinical type of leprosy was not observed, except for aCL antibodies, which were more frequent in lepromatous leprosy.

Keywords: leprosy, arthritis, autoantibodies, anticardiolipin antibodies, anti-β2 glycoprotein I antibodies.

INTRODUCTION

Leprosy is an infectious disease caused by Mycobacterium leprae, which is responsible for a wide spectrum of clinical manifestations that depend on the immune response of the host.1 The classification of this disease is based on the immunological, histological, and microbiological parameters described by Ridley & Jopling.2 In tuberculoid leprosy, the host has an efficient cell-mediated immune response, while lepromatous leprosy is characterized by humoral immunity. Borderline types that reflect the gradual variation in resistance to the bacillus are between those extremes.2 Joint involvement is considered the third most common manifestation in leprosy, after dermatologic and neurological manifestations. The frequency of articular manifestations reported ranges from 1% to 78%.3-5 This wide variability can, occasionally, be attributed to patient selection. Joint involvement can manifest as symmetrical polyarthritis of small and large joints, simulating rheumatoid arthritis.4,7

Besides the wide variability in the prevalence of the different autoantibodies in patients with leprosy reported in the literature, there are few studies correlating the presence of autoantibodies and joint manifestations of this disease.4,9,11,12
In leprosy, the presence of autoantibodies associated with systemic manifestations similar to those of rheumatic diseases can hinder its diagnosis.

Considering that leprosy is among the differential diagnosis of rheumatic diseases, this study was designed to determine the frequency of rheumatoid factor (IgM-RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP), antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), antiphospholipid antibody syndrome (aPL), and anti-β₂-glycoprotein I antibodies (anti-β₂-GPI) in patients with leprosy, with or without joint involvement, with similar age, gender, and disease-related characteristics, and to study the possible association among those autoantibodies and joint involvement, clinical type, reactive episodes, polychemotherapeutic treatment (PCT), and discharge from PCT.

PATIENTS AND METHODS

A transversal descriptive study with 158 patients with the diagnosis of leprosy seen regularly at the Dermatology and STDs Outpatient Clinic of the Fundação Alfredo da Mata (FUAM) in Manaus, Brazil, from June 2004 to October 2006 was undertaken. All patients had diagnosis of leprosy according to the classification of Ridley & Jopling (1966). Leprosy was classified as: undetermined (U), tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and lepromatous (LL).

During routine follow-up, patients who complained of articular pain were referred for evaluation with two rheumatologists (SLER and HLAP) who did a detailed history and physical exam to characterize the extension of the joint involvement and determine the presence of any other clinical manifestation. Patients with rheumatic diseases or neuropathic arthropathy were excluded from the study. Complementary exams were requested for patients with chronic arthropathy to exclude rheumatoid arthritis and seronegative spondyloarthopathies. Joint involvement was classified as: arthritis (presence of swelling and tenderness on palpation and movement) or arthralgia (pain on motion, but no other signs of inflammation).

Initially, 76 leprosy patients with joint involvement were identified, forming Group I. Afterwards, 82 leprosy patients without joint involvement (Group II), matched for age, gender, and characteristics of leprosy to Group I, were selected. Group III (control group) was formed by 129 healthy individuals, matched to Groups I and II in age and gender, recruited among health professionals, professors, students, and workers at FUAM in Manaus (AM, Brazil).

Data on leprosy, gathered on the interview and review of the medical records, as well as clinical findings, were recorded on a standardized form and included in a computer data base (Excel, 2003, Microsoft®). Patients and controls were asked specifically about any history of vascular thrombosis (arterial or venous).

This study was approved by the Ethics Committees from Fundação Pedro Matta (FUAM) – number 009/04 and from Universidade Federal de São Paulo (UNIFESP) – number 1282/07, and all study subjects signed an informed consent.

LABORATORY EXAMS

To determine the presence of autoantibodies, 20 mL of peripheral venous blood were collected from all subjects. After being centrifuged, the serum was divided in aliquots and stored at -70°C at the FUAM laboratory for posterior analysis at the rheumatology laboratory of UNIFESP.

The presence of IgM-RF was evaluated by semi-quantitative latex agglutination using the commercially available RapiTex RF kit (Dade Behring, Marburg, Germany), according to the instructions of the manufacturer. Titers above 20 IU/mL were considered positive.

To detect anti-CCP, the commercially available Immunoscan RA Anti-CCP2 kit (RA-96RT- Eurodiagnostics, Malmö, SE) was used according to the recommendations of the manufacturer, and titers ≥ 25 U/mL were considered positive.

Antinuclear antibodies were detected by indirect immunofluorescence (IIF) using a commercially available kit (BION, Enterprises, Ltda., USA) that uses Hep-2 cells, as the substrate, and anti-human immunoglobulin antibody conjugated with fluorescein, according to the specifications of the manufacturer. The exam was read by two experienced observers who did not know the origin of the sera.

Sera that tested positive for ANA on IIF were also tested by double immunodiffusion to identify the specificity. In summary, double diffusion in agarose plates was performed using extract of veal spleen as antigen, to detect anti-U1-RNP, anti-SS, anti-SS-A/Ro and anti-SS-B/La antibodies, and extract of rabbit thymus, to detect anti-Jo-1 and anti-Scl-70 antibodies. Standard sera with known specificity were used to compare precipitation lines.

The presence of anti-dsDNA antibodies was also evaluated in sera positive for ANA using IIF with Crithidia luciliae as substrate. Slides were prepared from a culture of C. luciliae, according to the standard technique of the laboratory. Sera were tested at the initial dilution of 1:5 and read on a fluorescence microscope (Olympus BX50) with a 400x power.
The presence of ANCA was determined by IIF using peripheral blood neutrophils, fixed and permeated according to the standard in house technique. All sera were tested at an initial dilution of 1:20.

The presence of aCL antibodies was determined by ELISA, in plaques prepared according to the routine of the laboratory, using international standards (Louisville APL Diagnostics, Inc, Doraville, USA, Prod#LAPL-GM100 IgG/IgM Calibrators). Levels above 20 GPL and 10 MPL for IgG and IgM aCL antibodies, respectively, were considered positive. Those results were obtained by calculating the 95% percentile of 200 blood donor samples analyzed in the same laboratory.

The presence of anti-β₂GPI IgG and IgM antibodies was detected by ELISA, using commercial kits (BINDAZYME Human Anti-β₂GPI IgG and Anti-β₂GPI IgM, The Binding Site, Birmingham, UK) according to the instructions of the manufacturer. Results above 20 U/mL, for anti-β₂GPI IgG antibodies, and 10 U/mL, for anti-β₂GPI IgM antibodies, were considered positive.

STATISTICAL ANALYSIS

The SPSS 15.0.1 software (Chicago, USA) was used to analyze the data. Mean, median, and standard deviation (SD) were used for the descriptive analysis of quantitative data, minimal and maximal values for continuous parameters, and proportions for categorical parameters. Student t test was used to compare continuous parameters with normal distribution, according Kolmogorov-Smirnov test, among the study groups; and for parameters that did not show normal distribution, the non-parametric Mann-Whitney and Kruskal-Wallis tests were used. Pearson’s Chi-square test, Fisher’s Exact test, and the binominal test were used for categorical parameters. P-values < 0.05 were considered statistically significant.

RESULTS

Demographic and Clinical Data

Leprosy patients had a mean age of 39.9 ± 15.2 years; 113 (71.5%) were males men and 45 (28.5%) females women (P = 0.001). Regarding polychemotherapy, 40.5% were being treated and 59.5% had been discharged from PCT. Reactional episodes were observed in 36.7% of the patients at the moment of the study, and the LL type, seen in 56 (35.4%) patients, was more common.

Demographic data and clinical characteristics of leprosy were similar in Groups I and II. Erythema nodosum leprosum was the most common reaction observed in both groups (Table 1).

The distribution of clinical types between Groups I and II (P = 0.938) did not show significant differences, and the LL type was the most frequent in both (Figure 1).

Sixty-one (80.3%) of 76 patients with joint involvement (Group I) had arthritis and 15 (19.7%) arthralgia. Most common joint involvement patterns included polyarthralgia (13/15) and polyarthritis (46/61). Among the 61 patients with arthritis, 65.6% did not present reactional episodes at the time of the study.

Table 1

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>Group I (n = 76)</th>
<th>Group II (n = 82)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51 (67.1)</td>
<td>62 (75.6)</td>
<td>0.237</td>
</tr>
<tr>
<td>Female</td>
<td>25 (32.9)</td>
<td>20 (24.4)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>39.8 (15.77)</td>
<td>40.06 (14.81)</td>
<td>0.716*</td>
</tr>
<tr>
<td>Length of disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) in months</td>
<td>39.4 (32.4)</td>
<td>32.2 (33.0)</td>
<td>0.124*</td>
</tr>
<tr>
<td>PCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discharged</td>
<td>50 (65.8)</td>
<td>44 (53.7)</td>
<td>0.121</td>
</tr>
<tr>
<td>Being treated</td>
<td>26 (34.2)</td>
<td>38 (46.3)</td>
<td></td>
</tr>
<tr>
<td>Reactional Episodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>24 (31.6)</td>
<td>34 (41.5)</td>
<td>0.198</td>
</tr>
<tr>
<td>Absent</td>
<td>52 (68.4)</td>
<td>48 (58.5)</td>
<td></td>
</tr>
<tr>
<td>Type of Reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>7 (29.2)</td>
<td>8 (23.5)</td>
<td>0.570</td>
</tr>
<tr>
<td>Type 2</td>
<td>16 (66.7)</td>
<td>22 (64.7)</td>
<td></td>
</tr>
<tr>
<td>Isolated Neuritis</td>
<td>1 (4.2)</td>
<td>4 (11.8)</td>
<td></td>
</tr>
</tbody>
</table>

Pearson’s Chi-square test. Mann-Whitney test*
In the control group, out of 129 patients, 59.7% were men and 40.3% women, with a mean age of 40.9 ± 14.1 years. The age and gender distribution of this group was similar to that of groups I and II (P = 0.611 and P = 0.057).

LABORATORY EXAMS

IgM-RF was positive in only two (1.2%) out of 158 leprosy patients, with titers of 80 and 160 IU/mL. Both patients with positive IgM-RF were in Group I, males, and had polyarthritis. In the control group, three of 129 (2.3%) healthy individuals had positive IgM-RF, two with low titers (80 IU/mL) and one with a high titer (1280 IU/mL). The frequency of IgM-RF did not show significant differences among the three groups (P = 0.357).

Antic-CCP antibodies were positive in only 4 patients (2.5%), whose titers ranged from 27 to 34 U/mL and none of them had joint involvement. All four patients were males, one had LL and the other three, borderline leprosy (BL, BB, BT). At the time of the study, two of those patients presented reactionary episodes, being treated with prednisone, and three had been discharged from PCT. For anti-CCP antibodies, 89 sera were tested in Group III, of which three were positive, two with low to moderate titers (44 and 53 U/mL) and one with a high titer (1827 U/mL). Anti-nuclear antibodies were positive in six (3.8%) of 158 patients with leprosy (2.6% in Group I and 4.9% in Group II) and in eight of 129 (6.2%) in the control group, 90% of anti-β2GPI were IgM and 12.3% IgG (P < 0.001). In the control group, 90% of anti-β2GPI antibodies were IgM and 10% IgG (P < 0.001).

The presence of aCL antibodies and anti-β2GPI did not differ among patients with or without reactionary episodes, both for aCL (19.0% vs. 14.0%; P = 0.41) and anti-β2GPI (51.7% vs. 43%; P = 0.28). However, considering the 58 patients with reactionary episodes, a significant difference in the proportion of the types of reactions among patients who were positive and negative for aCL antibodies (P = 0.028) was observed. All patients aCL positive presented erythema nodosum leprosum, while this reaction was present in 57.4% of those who were aCL negative. In patients with anti-β2GPI this difference was not observed (P = 0.17).

The presence of aCL antibodies and anti-β2GPI did not differ among patients in PCT and those who had been discharged from it (18.8% vs. 13.8%; P = 0.40 and 37.5% vs. 52.1%, respectively).

Titers above 40 U/mL were observed in 80% (20/25) of the patients with aCL antibodies and in 35.6% (26/73) of patients with anti-β2GPI antibodies, but not in healthy individuals. Mean aFL antibody levels in leprosy patients (Groups I and II) were 57 GPL and 56 MPL, for aCL antibodies, and 112 GPL, for IgG, and 94 U/L, for IgM, anti-β2GPI antibodies.

As for aFL antibodies (aCL and anti-β2GPI), the IgM isotype was more common both in leprosy patients and healthy controls. Out of 25 patients aCL positive, 88% presented the IgM isotype and 16% IgG (P < 0.001). The four healthy individuals who tested positive for aCL had IgM isotype antibodies. Out of 73 patients positive for anti-β2GPI, 97.3% presented the IgM isotype and 12.3% IgG (P < 0.001). In the control group, 90% of anti-β2GPI antibodies were IgM and 10% IgG (P < 0.001).

Table 2 shows the frequency of the different autoantibodies in Groups I, II, and III.

Both patients and healthy individuals had a negative history for arterial and venous thrombosis.

DISCUSSION

Brazil has the second higher prevalence of leprosy in the world. Leprosy is one of the differential diagnoses of...
rheumatic diseases, since autoantibodies and articular and systemic manifestations can be seen in both situations. The present study included patients seen in the outpatient clinic of a reference center for the treatment of leprosy in the state of Amazonas, in northern Brazil, including a large number with and without joint involvement, different clinical types, and different stages of the disease. The choice of the control group, composed of healthy individuals living in the same region, was oriented by the possibility of inhabitants of areas more exposed to infections presenting greater positivity of one of the autoantibodies.15

A study with similar design was not found in the literature. Few studies that evaluated rheumatic complaints included manifestations like enthesitis and erythema nodosum leprosum, besides arthritis and arthralgia,14,8,11,12 and only two studies evaluated a set of autoantibodies, but without evaluating the correlation with joint involvement.16,17

In the present study, joint involvement was predominantly polyarticular and most patients did not have active reaction, similar to the findings of other authors.4,10,12

In this study, 80 out of 158 (50.6%) leprosy patients were positive for at least one of the autoantibodies investigated. The frequency of IgM-RF, anti-CCP, ANA, and ANCA in leprosy patients was low and similar to that of the control groups. However, the prevalence of aCL antibodies and anti-β2GPI antibodies was significantly higher in leprosy patients than in the control group. An association between the presence of any antibody and articular involvement in leprosy was not observed.

Several studies indicate an increased frequency of autoantibodies in leprosy; however, the percentage reported varies considerably.4,12,16-24

This variability can be attributed to different selection criteria, genetic background of the study population, and techniques used to detect autoantibodies. One should also consider the possible presence of associated infectious diseases in some of those series.15

The presence of autoantibodies has been reported especially in multibacillary LL, which has marked humoral immune response. In paucibacillary tuberculoid leprosy, the cellular immune response is effective and the presence of autoantibodies is less common.25 However, the origin of autoantibodies in leprosy and in other chronic infectious diseases has not been established; it might be due to the polyclonal activation of B cells by bacterial components,26,27 presence of cross reaction between bacterial antigens and autoantigens,4,21 or to chronic tissue injury and exposure of antigens that are usually occult.

In the present study, the low prevalence of IgM-RF in leprosy patients did not allow the analysis of associations with reactional state and clinical type. In the literature, the prevalence of IgM-RF in leprosy patients varies considerably. Frequencies between 28 and 58%, especially in the LL type, have been reported by several authors;9,10,12,18 however, frequencies lower than 6%, similar to our results, have also been reported.12,20,21 Some authors found an association between IgM-RF and arthritis,9,28 which has not been corroborated by other studies.4,10-12 including ours.

### Table 2

<table>
<thead>
<tr>
<th>AUTOANTIBODIES</th>
<th>Group I (n = 76)</th>
<th>Group II (n = 82)</th>
<th>Group III (n = 129)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>IgM-RF</td>
<td>2 (2.6)</td>
<td>0 (0.0)</td>
<td>3 (2.3)</td>
<td>0.357</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>0 (0.0)</td>
<td>4 (4.9)</td>
<td>3 (3.4) *</td>
<td>0.169</td>
</tr>
<tr>
<td>ANA</td>
<td>2 (2.6)</td>
<td>4 (4.9)</td>
<td>8 (6.2)</td>
<td>0.519</td>
</tr>
<tr>
<td>ANCA</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>aCL-GPI</td>
<td>1 (1.3)</td>
<td>3 (3.7)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>aCL-MPL</td>
<td>12 (15.8)</td>
<td>10 (12.2)</td>
<td>4 (3.1)</td>
<td></td>
</tr>
<tr>
<td>aCL</td>
<td>13 (17.1)</td>
<td>12 (14.6)</td>
<td>4 (3.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>anti-β2GPI IgG</td>
<td>4 (5.3)</td>
<td>5 (6.1)</td>
<td>1 (0.9)**</td>
<td></td>
</tr>
<tr>
<td>anti-β2GPI IgM</td>
<td>33 (43.4)</td>
<td>38 (46.3)</td>
<td>9 (8.5)**</td>
<td></td>
</tr>
<tr>
<td>anti-β2GPI GMP</td>
<td>33 (43.4)</td>
<td>40 (48.8)</td>
<td>10 (9.4)**</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Anti-CCP = anti-cyclic citrullinated antibodies; IgM-RF = rheumatoid factor; ANA = antinuclear antibodies; ANCA = antineutrophil cytoplasmic antibodies; aCL = anticardiolipin antibodies; anti-β2GPI = anti-β2-glicoprotein I antibodies. n=89* n=106**
Anti-CCP antibodies are considered highly specific for the diagnosis of rheumatoid arthritis; however, very few studies evaluating the presence of anti-CCP antibodies in infectious diseases have been reported. Anti-CCP antibodies were found in up to 8.8% of the patients with hepatitis C. In two independent studies, relatively high frequencies, 32 and 37%, of anti-CCP antibodies were found in patients with active tuberculosis without associated joint involvement.

In the present study, we found a low frequency of anti-CCP antibodies in leprosy patients with or without joint involvement. Those data corroborate the results of Guedes-Barbosa et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.

In this study, we observed a low frequency of ANA in leprosy patients, similar to that of the control group, and without an association with joint involvement. Although the results of the present study do not agree with the high positivity reported by some authors, it is similar to the results reported in several studies. We emphasize that, with the exception of the study of Dacas et al., who also found low frequencies of anti-CCP antibodies in leprosy patients. However, those authors evaluated only 64 patients, without referring whether they had joint involvement and without a control group with healthy individuals.
However, Arvieux et al.,45 studying patients with multibacillary LL, did not observe a predominance of one isotype. Similar to other antibodies, we did not observe an association among the presence of anti-β2GPI and joint involvement, reactional episodes, or type of reaction.

Although the literature has studies referring a higher incidence of aCL antibodies than anti-β2GPI antibodies in leprosy,19,45 in our study, the frequency of anti-β2GPI was higher, which is in agreement with the results of Loizou et al.19 Those authors reported a predominance of the IgA isotype, in the tests for aCL antibodies, and IgM isotype, for anti-β2GPI. In the present study, the IgA isotype was not evaluated and the IgM isotype was the most frequent in both tests.

In our study, 84% of the leprosy patients positive for aCL antibodies also presented anti-β2GPI antibodies and, in 71% of those patients, those antibodies, of the IgM isotype, were present in titers higher than 40 U/m. Despite the high frequency of aFL, corroborating the data in the literature, we did not find an association between those antibodies and thrombotic manifestations.23,39,46 Although there are reports of cases associating IgM aCL antibodies with thrombotic phenomena in leprosy patients,49,50 it is possible that those cases represent a coincidence, since the clinical meaning of those antibodies is not clear in this disease.

To conclude, the frequency of RF, ANCA, ANA, and anti-CCP antibodies in leprosy patients was low and did not show an association with joint involvement, clinical type, or the presence of reactional episodes. We found a high prevalence of aCL antibodies and, especially, anti-β2GPI antibodies in leprosy patients, with a predominance of the IgM isotype; however, they were not associated with thrombotic manifestations. It is possible that the presence of aFL in leprosy constitutes just another marker of autoimmunity. It would be interesting to undertake a study with longitudinal follow-up of leprosy patients to evaluate the persistence of those autoantibodies, as well as the study of the fine specificity of those antibodies in comparison to those observed in the antiphospholipid syndrome.

**REFERENCES**

**REFERENCES**

17. Padhan V, Badakere SS, Shankar Kumar U. Increased incidence of cytoplasmic ANCA (c-ANCA) and other autoantibodies in leprosy patients from western India. Lepr Rev 2004; 75(1):50-6.