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LETTERS OF BIOMEDICAL AND CLINICAL RESEARCH

# Negative specificity for DSF70 in ANA positive leprosy sera

Sandra Lúcia Euzébio Ribeiro[1] · Gabriel Pacifico Seabra Nunes[1] · Henrique Euzébio Guedes de Freitas[1] · Cristóvão Mangueira[2] · Morton Aaron Scheinberg[2,3]

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## Introduction

Leprosy is a chronic infectious granulomatous disease in humans that is caused by the acid fast bacillus, *Mycobacterium leprae*. In Brazil, it is an endemic disease and occupies the second place in the world in the number of cases behind India. The detection rate is around 12.02 million inhabitants, and still is a public health challenge due to its potential physical deformities when there is a delay in diagnosis. In the Amazon State, from where the patients were evaluated, the disease is also endemic and has a prevalence of 18.08, the third highest in the country [1–4].

Clinical manifestations of leprosy are a spectral of signs and symptoms with two distinct polar forms in one side of the spectrum, tuberculoid leprosy with single or few skin lesions and strong immune resistance against *M. leprae*, and on the other pole of the spectrum lepromatous leprosy with widely disseminated skin nodules and little immunity to *M. leprae*. Autoantibodies in leprosy have been demonstrated, but a controversy exists about the significance and prevalence of such antibodies. The presence of antinuclear antibodies (ANA) in patients with leprosy has been reported to vary from 5 to 30%. Our own previously published experience finds around one-third of the patients with positive antinuclear antibodies with low and high titers and no prevalence of a specific pattern on immunofluorescence. In a previous study on the same topic about specificity of the ANA in leprosy patients, we did not find autoantibodies with specificities to double-stranded ANA, Ro/SSA, La/SSB, Sm, RNP, and P proteins [1–4]. Recently, the presence of ANA with specificity for the dense fine speckled 70 (DFS70) pattern have attracted interest because of its relative common occurrence among patients with no clinical evidence of systemic rheumatic autoimmune disease, in addition, the disease was suggested to be a marker of benign or no emergence systemic autoimmune disease [5, 6]. We investigated the presence of the specific antigen DFS70 as responsible for the positive ANA antibodies in leprosy sera. Our results showed that the positive ANA antibodies in our patient population are not DSF70 as initially thought taking in consideration that the concomitant presence of lupus, Sjogren, vasculitis, or rheumatoid arthritis is uncommon in leprosy patients.

## Material and methods

A total of 60 sera from leprosy patients attending the Tropical Dermatology and Venereal Clinic of Alfredo the Matta Foundation in Manaus capital on the state of Amazon were tested for ANA with conventional indirect immunofluorescence. Specific demographics were 34 males and 26 females, mean age 37 years (range 18–77), and mean disease duration going from zero day of diagnosis to 24 years. In ANA detection, we used Hep-2 cells as a substrate and looked for the apple green fluorescence on the nucleus or cytoplasm where autoantibodies are bound defining the patterns of fluorescence. In addition, we analyzed the presence of positive or negative DSF70 using a specific assay for in vitro determinations of autoantibodies against dense fine speckles in a human serum. Anti-DFS70 antibodies of the IgG class were tested by ELISA, using the DFS70 IgG kit (Euroimmun, Lübeck, Germany), following strictly the manufacturer's recommended protocol, in which the samples, controls, and calibrators are incubated in the wells of the ELISA microplate for 30 min at room temperature (+ 18 °C to + 25 °C), followed by a triple wash step, to remove unbound material; then the anti-IgG enzyme conjugate is pipetted into each well of the microplate and incubated for 30 min at room temperature, followed by

✉ Morton Aaron Scheinberg

[1] Federal University of Amazonas, Manaus, State of Amazonas, Brazil

[2] Hospital Israelita Albert Einstein, São Paulo, Brazil

[3] Hospital Israelita Albert Einstein and Research Institute, São Paulo, Brazil

further washing step to remove the unbound conjugate. Subsequently, the enzyme substrate was pipetted into each well of the microplate and incubated for 15 min at room temperature, protected from direct light. The reaction was stopped with the stop solution and the plate was read photometrically at 450 nm. The results were calculated by dividing the absorbance value of the control or patients by the absorbance of the calibrator, as instructed by the manufacturer. The study was approved by the ethics committee of Fundaçao Alfredo Matta (009-04) and all participants signed free consent.

## Results

Thirteen sera were positive for ANA with titers ranging from 1/80 to 1/320 with patterns distributed between fine speckled, nuclear dots cytoplasmic, and nucleolar. Only two samples were positive for DSF70 and ANA, but the immunofluorescence pattern was cytoplasmic rather than dense fine speckled in one of the sera; the other was dense fine speckled (Table 1). On descriptive analysis, 21% of leprosy sera were positive for ANA in our cohort, 84% of leprosy sera were negative for DFS70, and only 16% was positive. There is a slightly higher presence of ANA positivity in the lepromatous form, but not statistically significant which was randomly distributed among the clinical spectrum.

## Discussion

Autoantibodies in leprosy have been demonstrating predominantly rheumatoid factor and antinuclear antibodies. The presence of ANA has been reported by us and other several authors; however, titers and specificities were mentioned little and in most of the studies are reported as negative or positive. However, the presence of concomitant autoimmune disease with clinical forms of the disease is scanty suggesting that the specificity of ANA maybe due to the recent described protective effect of positive DSF70 ANA for the development of systemic autoimmune disease. Our results did not find that this antigen specificity is responsible for the presence of ANA positivity in our patient population, and only two patients had DSF70 positivity and a specific immunofluorescence pattern described with DSF-70 in a patient with LL (lepromatous leprosy). A recent paper suggested that improvements were made in a substrate used for the detection of DSF70 that may help in the interpretation of mixed immunofluorescence patterns using a mixture of engineered HEp-2 devoid of the DFS70 autoantigen and conventional HEp-2 cells. We could not exclude at this moment that our results would be changed using refinements on the current techniques as suggested by the authors. In addition, our findings are somewhat restricted for LL clinical subgroups since the patient population had very few tuberculoid or indeterminate clinical subgroups. In summary, our findings are sufficient to conclude that positive ANA in leprosy is not related to the nuclear antigen DSF70 resembling what have been described with rheumatoid factor, where we find negative association with anti-CCP3 [7].

Rheumatic manifestations are relatively common during the course of the disease sometimes in the initial manifestation; and due to the clinical heterogeneity confusion with autoimmune disease, rheumatic diseases can occur delaying the proper diagnosis. Additional details on the significance of positive ANA in such patients was evidenced in our study and may help in the proper management of such patients. Our results also point out the importance of performing ANA and DSF70 simultaneously as illustrated in the current paper [8, 9].

**Table 1** Antinuclear antibodies in leprosy patients

| Clinical stage | ANA positive | ANA negative | DFS70 positive | DFS70 negative | ANA positive and DFS70 positive | ANA positive and DFS70 negative | DFS70 positive and ANA negative |
|---|---|---|---|---|---|---|---|
| Indeterminate | 1 | 1 | 2 | 0 | 1 | 0 | 1 |
| Tuberculoid | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Lepromatous | 6 | 11 | 2 | 15 | 1 | 5 | 1 |
| Borderline tuberculoid | 2 | 11 | 1 | 12 | 0 | 2 | 1 |
| Borderline borderline | 2 | 7 | 1 | 8 | 0 | 2 | 1 |
| Borderline lepromatous | 2 | 6 | 0 | 8 | 0 | 2 | 0 |

**Compliance with ethical standards** The study was approved by the ethics committee of Fundaçao Alfredo Matta (009-04) and all participants signed free consent forms.

**Disclosures** None.

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