Anti-alpha-fodrin antibodies in patients with Sjögren’s syndrome secondary to rheumatoid arthritis

Anticorpos antialfafodrina em pacientes com síndrome de Sjögren secundária a artrite reumatoide

Renato Nishihara1,2; Thelma L. Skare1; Elisa Cenci1; Denise Gabardo6; Flavia Nass1; Shirley R. R. Utiyama3

1. Faculdade Evangélica do Paraná (Fepar), Paraná, Brazil. 2. Universidade Positivo (UP), Paraná, Brazil. 3. Universidade Federal do Paraná (UFPR), Paraná, Brazil.

ABSTRACT

Introduction: The Sjögren’s syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration. The currently most researched antibodies for its diagnosis are anti-La and anti-Ro, which, however, have low specificity in the case of SS secondary to rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The antibodies against alpha-fodrin (AF) have been proposed to diagnose SS. Objective: In the present study, we investigated the anti-AF antibody in a group of RA patients with and without secondary SS (sSS). Methods: Were studied 90 consecutive patients with RA (48.8% of them with SS), and samples of 45 healthy volunteers. Anti-AF immunoglobulin class G (IgG) and anti-AF immunoglobulin class A (IgA) were investigated by enzyme-linked immunosorbent assay (ELISA) and were considered positive when ≥15 U/ml. Demographic, clinical, and serological data were obtained from chart reviews. Results: Anti-AF IgA was positive in 46/90 (51.1%) of the RA sample and 3/45 (6.7%) of controls (p < 0.001); anti-AF IgG was found in 21/90 (23.3%) of RA patients and none of controls (p = 0.037). Neither IgA nor IgG anti-AF antibodies showed significant difference in patients with and without sSS. Conclusion: In our study, anti-AF IgA and anti-AF IgG neither allowed diagnosis of sSS in RA patients, nor marked any special clinical or serological finding.

Key words: Sjögren’s syndrome; autoimmunity; autoantibodies; arthritis rheumatoid.

INTRODUCTION

Sjögren’s syndrome (SS) is an autoimmune disease in search of its autoantibody. Although anti-Ro and anti-La have been found in 60%-90% and 30%-60% of primary cases, respectively(2,3), they lack specificity(2,3), mainly in cases of secondary SS (sSS) associated with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Antibodies against alpha-fodrin (AF) have been proposed to fill this space. AF is an actin-binding protein found in the membrane skeleton that is involved in exocytosis and gland secretion(3). It is cleaved in apoptosis by caspase 3 and calpain, forming a 120-kDa protein that represents a neoantigen(4). AF cleavage during apoptosis is the main stimulus for induction of the disease in SS mouse models(4).

According to Watanabe et al. (1999)(2), anti-AF antibodies (immunoglobulin class G [IgG] and/or class A [IgA]) are found in 95.3% of patients with primary SS (pSS) and in 62.5% of secondary cases, have a sensitivity of 67% and a specificity of 93% in both situations. However, these authors studied only nine patients with primary disease and 15 patients with SS secondary to SLE, comparing them with 44 SLE patients. They used the European classification criteria for diagnosis. Similar numbers were found by Haneji et al. (1997)(5), with a 96% prevalence of anti-AF IgG in 43 SS patients classified by the Japanese criteria. These autoantibodies have been associated with the intensity of lymphocytic infiltration in minor salivary glands and appear to reflect the degree of disease activity(4).

Witte et al.(6) detected that anti-AF IgA provided higher sensitivity than anti-AF IgG, but they found lower prevalence of anti-AF IgA in pSS (64%) and sSS (47%) than the previous authors. Others are even less enthusiastic. Loch et al. (2008)(7), comparing sensitivity and specificity of anti-AF antibodies in 321 patients with pSS, could not confirm that they had a higher performance than anti-Ro/La. Furthermore, Zandbelt et al. (2004) (8), using the US/European classification criteria for SS diagnosis, stated...
that neither IgG nor IgA anti-AF antibodies added much to SS diagnosis. They searched for these autoantibodies in a cohort of 51 patients with rheumatic diseases that included 21 patients with pSS, six with sSS, 12 with RA, six with SLE and six with scleroderma. Turkçapar et al. (9), also looking for anti-AF IgG and IgA in patients with pSS and sSS (classified by the European criteria), reported low prevalence of these antibodies and found that anti-Ro and anti-La are more useful in the diagnosis of this syndrome.

A recent meta-analysis (10) encompassing 23 other studies concluded that the anti-AF antibody has moderate accuracy for the diagnosis of SS, with high specificity (83.1% in the IgG subtype and 82.8% in the IgA subtype) and relative low sensitivity (38% in the IgG subtype and 41.9% in the IgA subtype). These authors concluded that in order to avoid misdiagnosis of SS, anti-AF may be used in combination with anti-Ro/La antibodies.

Although common, SS disease does not have well-established classification criteria yet, so it is difficult to compare the existing data. SS patients classified according to the San Diego criteria, which are more stringent, seem to have higher prevalence of anti-AF IgG and anti-AF IgA than those classified by the European criteria (11). Also, patients' ethnic background and the sensitivity of the different methods used to measure anti-AF have been found to contribute to the diversity of results (10).

We analyzed the presence of anti-AF IgG and anti-AF IgA in RA patients. We aimed at establishing these antibodies' value to determine which patients had sSS in this context. Secondly, we looked for other RA clinical and serological associations with anti-AF.

RESULTS

Anti-AF IgA was positive in 46/90 (51.1%) of the RA sample and 3/16 (18.7%) of the controls (\(p = 0.027\)); anti-AF IgG was found in 21/90 (23.3%) of RA patients and none of the controls (\(p = 0.037\)), as shown in Figure 1.

Analyzing positivity of anti-AF IgG and anti-AF IgA according to demographic, clinical and serological profiles in RA patients, we found the data shown in Table 1. Neither IgA nor IgG anti-AF antibodies showed significant difference in patients with and without sSS. In Figure 2, the receiver operating characteristic (ROC) curve analysis shows the poor performance of anti-AF IgA and anti-AF IgG to distinguish RA patients with and without sSS.

Comparing titer values of anti-AF IgA and anti-AF IgG in RA patients with and without sSS, we found no statistical differences, as seen in Table 1.

The analysis of anti-AF IgA and anti-AF IgG regarding RA demographic, clinical and serological profiles is seen in Table 2.

METHODS

This study was approved by the local Research Ethics Committee, and all participants signed a consent. We included sera of 90 consecutive patients who filled at least four of the American College of Rheumatology classification criteria (1987) for RA (12) [48.8% of them with sSS according to the American-European criteria (13)] and sera of 45 healthy volunteers. Anti-AF IgG and anti-AF IgA were investigated by enzyme-linked immunosorbent assay (ELISA) (Orgentec Diagnostika, Mainz, Germany). In accordance with the manufacturer's instructions, values were considered positive when > 15 U/ml, for both anti-AF IgG and anti-AF IgA.

Demographic, clinical, serological and functional (13) data were obtained from chart review. In this sample, 20 (22%) were Afro-descendants (mulattos and Blacks), and 70/90 (78%) were Caucasians. None was Asian in origin.

Association studies were done by Fisher's and chi-square test for nominal data, and by unpaired \(t\)-test and Mann-Whitney test using the software Graph Pad Prism 4.0 for numerical data. The adopted significance was of 5%. Data showing \(p < 0.05\) were further analyzed through logistic regression to assess variable independence.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>RA without sSS</th>
<th>RA with sSS</th>
<th>(p) (Mann-Whitney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-AF IgA</td>
<td>6-35</td>
<td>8-40</td>
<td>0.97</td>
</tr>
<tr>
<td>(U/ml)</td>
<td>Median 15 (11.5-20.5)</td>
<td>Median 14 (12-19)</td>
<td></td>
</tr>
<tr>
<td>Anti-AF IgG</td>
<td>5-38</td>
<td>7-48</td>
<td>0.68</td>
</tr>
<tr>
<td>(U/ml)</td>
<td>Median 12 (8-15)</td>
<td>Median 11 (9-14)</td>
<td></td>
</tr>
</tbody>
</table>

IgG: immunoglobulin class G; IgA: immunoglobulin class A; AF: alpha-fodrin; RA: rheumatoid arthritis; sSS: secondary Sjögren syndrome.
Anti-alpha-fodrin antibodies in patients with Sjögren's syndrome secondary to rheumatoid arthritis

As anti-AF IgA showed positive association with lung fibrosis, antinuclear antibodies (ANA) and functional classification in univariated analysis, we further studied these variables in a logistic regression analysis where only functional class index showed association with the presence of anti-AF IgA (odds ratio [OR] = 1.8; 95% confidence interval [CI] of 1.02-3.16).

DISCUSSION

Both SS and non-Sjögren's syndrome can show the clinical symptoms of dry eyes and dry mouth, so sicca symptoms are poor discriminators of SS(14). Secondary SS was found to be present in 22% of RA patients in our geographical area(15). Its diagnosis, according to the American-European criteria, requires a minor salivary gland biopsy or an objective evidence of salivary gland involvement(12). Although salivary biopsy is a minor procedure, it is invasive and not always well accepted by patients. The objective measurement of salivary gland involvement by unstimulated salivary flow, parotid sialography and salivary scintilography is not accessible in daily practice. If a specific biomarker, such as an autoantibody, were found, this problem could be avoided.

The AF antigen has been postulated to be involved in the autoimmune responses leading to destruction of exocrine glands(16). It is associated with ion channels and pumps, and therefore it is possible that antibodies directed against AF could disturb their function(16). Thus, anti-AF seems a good candidate to mark this

**TABLE 2** — Association studies of demographic, clinical and serological data with IgA and IgG anti-AF antibodies in 90 RA patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Anti-AF IgA</th>
<th>Anti-AF IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n = 46</td>
<td>Negative n = 44</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>9/37 (24.3%)</td>
<td>4/40</td>
</tr>
<tr>
<td>Mean age at diagnosis (years)</td>
<td>40 ± 12.2</td>
<td>41.3 ± 13.23</td>
</tr>
<tr>
<td>Median disease duration (months)</td>
<td>90 (IQR 36-132)</td>
<td>84 (IQR 48-168)</td>
</tr>
<tr>
<td>Ethnic background (Caucasians/Afro-descendants)</td>
<td>37/9</td>
<td>33/11</td>
</tr>
<tr>
<td>Rheumatoid nodules</td>
<td>5/46 (21.2%)</td>
<td>3/44 (6.8%)</td>
</tr>
<tr>
<td>Lung fibrosis§</td>
<td>7/42 (17%)</td>
<td>0/42</td>
</tr>
<tr>
<td>Secondary Sjögren’s</td>
<td>20/43 (46.5%)</td>
<td>21/41 (51.2%)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>5/46 (10.8%)</td>
<td>6/40 (15%)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>26/45 (58%)</td>
<td>28/33 (65.1%)</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>32/38 (84.2%)</td>
<td>25/35 (71.4%)</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>9/44 (20.4%)</td>
<td>2/42 (4.7%)</td>
</tr>
<tr>
<td>Anti-Ro</td>
<td>4/44 (9.1%)</td>
<td>3/42 (7.1%)</td>
</tr>
<tr>
<td>Tobbaco exposure</td>
<td>16/42 (38.1%)</td>
<td>14/39 (35.8%)</td>
</tr>
</tbody>
</table>

IgA: immunoglobulin class A; IgG: immunoglobulin class G; AF: alpha-fodrin; RA: rheumatoid arthritis; IQR: interquartile range; CCP: cyclic citrullinated protein; *: Student's t-test; **: Mann-Whitney test; #: Fisher's exact test; f: chi-square test.
Introdução: A síndrome de Sjögren (SS) é uma doença autoimune caracterizada por inflamação linfocítica. Atualmente os anticorpos mais pesquisados para seu diagnóstico são anti-Ro e anti-La, que, no entanto, apresentam baixa especificidade nos casos de SS secundária a artrite reumatoide (AR) e lúpus eritematoso sistêmico (LES). Os anticorpos contra alfafodrina (AF) foram propostos para diagnosticar SS. A síndrome de Sjögren (SS) é uma doença autoimune caracterizada por infiltração linfocítica. Atualmente os anticorpos mais pesquisados para seu diagnóstico são anti-Ro e anti-La, que, no entanto, apresentam baixa especificidade nos casos de SS secundária a artrite reumatoide (AR) e lúpus eritematoso sistêmico (LES). Os anticorpos contra alfafodrina (AF) foram propostos para diagnosticar SS. Material e métodos: Foram estudados 90 pacientes consecutivos com AR (48,8% com SS) e amostras de 45 voluntários saudáveis. Imunoglobulina da classe G (IgG) e imunoglobulina da classe A (IgA) anti-AF foram investigadas por ensaio imunossorvente ligado à enzima (ELISA), sendo consideradas positivas quando acima de 15 U/ml. Dados demográficos, clínicos e sorológicos foram obtidos a partir de revisão de prontuários. Resultados: IgG anti-AF foi encontrada em 21/90 (23,3%) pacientes com AR e 3/45 (6,7%) das amostras-controle (p < 0,001); IgG anti-AF foi encontrada em 31/90 (34,4%) de pacientes com AR e nenhum dos controles (p = 0,037). IgM anti-AF e IgA anti-AF foram investigadas e não contribuíram especificamente para diferenciar pacientes com AR que sofrem ou não de SS, além de não conseguirem estabelecer o diagnóstico de SS em pacientes com AR. Conclusão: IgG anti-AF e IgA anti-AF foram investigadas e não contribuíram especificamente para diferenciar pacientes com AR que sofrem ou não de SS, além de não conseguirem estabelecer o diagnóstico de SS em pacientes com AR. RESUMO

Resultados: IgG anti-AF foi positiva em 46/90 (51,1%) das amostras com AR e 3/45 (6,7%) das amostras-controle (p < 0,001); IgG anti-AF foi encontrada em 31/90 (34,4%) de pacientes com AR e nenhum dos controles (p = 0,037). IgM anti-AF e IgA anti-AF foram investigadas e não contribuíram especificamente para diferenciar pacientes com AR que sofrem ou não de SS, além de não conseguirem estabelecer o diagnóstico de SS em pacientes com AR. Conclusão: IgG anti-AF e IgA anti-AF foram investigadas e não contribuíram especificamente para diferenciar pacientes com AR que sofrem ou não de SS, além de não conseguirem estabelecer o diagnóstico de SS em pacientes com AR.
REFERENCES


CORRESPONDING AUTHOR

Renato Nishiara
Rua João Azolin, 660; Santa Felicidade; CEP: 82015-040; Curitiba-PR, Brasil; Phone/fax: +55 (41) 9911-9572; e-mail: renatonishiara@up.edu.br.