QUANTITATIVE ASSESSMENT OF SALIVARY GLAND INFLAMMATORY INFILTRATION IN PRIMARY SJÖGREN’S SYNDROME: ITS RELATIONSHIP TO DIFFERENT DEMOGRAPHIC, CLINICAL AND SEROLOGICAL FEATURES OF THE DISORDER

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SUMMARY

The aim of this study was to investigate the possible relationship between the degree of inflammatory infiltration of salivary glands in Sjögren’s syndrome (SS) and the different demographic, clinical and serological features of the disease. A quantitative assessment of the extension of the infiltrates was performed on histology samples from the labial salivary glands (LSG) of 82 patients with primary SS, by calculating the ratio of the infiltrated area to the total area of glandular tissue in the samples. The correlations between the amount of inflammatory infiltrate and the main features of the disorder were then analysed. A significant negative correlation between the degree of LSG infiltration and the patient’s age at disease onset was observed ($P < 0.05$). In contrast, the percentage of infiltrate did not correlate with the disease duration. A significant correlation was found between the degree of infiltration of the salivary tissue and (i) the total number of extraglandular features ($P < 0.01$) and (ii) the presence of specific extraglandular features such as Raynaud’s phenomenon ($P < 0.05$), vasculitis ($P < 0.0001$), lymph node or spleen enlargement ($P < 0.05$) and leucopenia ($P < 0.02$). Finally, patients with antinuclear antibodies, anti-SSA/Ro antibodies, or anti-SSA/Ro plus anti-SSB/La antibodies showed a more widespread inflammatory infiltration in the LSG tissue than patients without these autoantibodies ($P < 0.01$). The degree of infiltration in the salivary tissue was significantly greater in those patients with anti-SSA/Ro plus anti-SSB/La antibodies in their sera than in patients with anti-SSA/Ro antibodies alone ($P < 0.05$). In conclusion, patients with SS and active inflammatory infiltration of the salivary glands usually experience an earlier disease onset and a larger number of systemic extraglandular manifestations. In addition, the antibodies directed against certain nuclear/cytoplasmic specificities, and particularly those which react with the SSB/La antigen, seem to play a key role in enhancing the autoimmune process in the salivary glands.

KEY WORDS: Sjögren’s syndrome, Anti-Ro(SSA) antibodies, Anti-La(SSB) antibodies, Salivary glands, Inflammatory infiltrates.

Sjögren’s syndrome (SS) is a disorder characterized by diffuse chronic inflammation of the exocrine glands, particularly of the salivary and lacrimal glands. Secretion impairment leads to the appearance of sicca complaints, usually characterized by the subjective feeling of dry eyes and dry mouth. These manifestations may occur alone or in association with some non-specific systemic features (primary SS) or, alternatively, the sicca syndrome may develop during the course of a well-defined systemic connective tissue disease (secondary SS) [1].

Lip salivary gland (LSG) biopsy (to assess the inflammatory infiltration of the exocrine glands) is generally regarded as the most reliable objective criterion for defining salivary gland involvement [2]. Its accuracy in the diagnosis of SS, however, is less clear. Some SS patients do not show significant LSG lymphocytic infiltration [3–5]. On the other hand, focal sialoadenitis has been described in apparently healthy elderly subjects and in patients with other disorders [6, 7].

There are also conflicting reports on the correlation between the presence and degree of LSG infiltrates and other demographic, clinical and serological features of SS. For instance, salivary gland function does not seem to be dependent on the amount of lymphocytic infiltrates in the LSG [8]. On the other hand, a correlation has been found between focal sialoadenitis and the presence of (i) extraglandular involvement [9] or (ii) serological abnormalities such as rheumatoid factor (RF), antinuclear antibodies (ANA), anti-SSA/Ro and anti-SSB antibodies (Abs) [10], which are commonly held to be the serological markers of SS. Finally, very little information is available on how the level of lymphocytic infiltrates in LSG may vary with the age of the patient and the disease duration.

Different hypotheses could explain this lack of correlation between the presence and degree of LSG infiltrates and certain features of SS. According to the opinion of some authors, the level of lymphocytic infiltrates could be dynamic and thus vary over time [11]. It is also possible that major and minor salivary gland involvement cannot evolve simultan-
eously [3–5]. These discrepancies could also be due to the scoring methods employed, which provide only a semiquantitative assessment of the degree of infiltration. An inflammatory focus is commonly defined as an aggregate of at least 50 lymphocytes, plasma cells or macrophages [2]. However, when the cell density greatly exceeds 50, the foci may become confluent, thus making this scoring technique impractical. Finally, it has been shown that the extent of infiltrates in LSG drawn using the same biopptic procedure may vary greatly from gland to gland in a single patient, which can make the inter-observer and intra-observer reliability quite poor.

In the present study, we assessed LSG infiltration using a quantitative system, i.e. by evaluating the ratio of the infiltrated area to the total tissue specimen. We then correlated this ratio with various demographic, clinical and serological features in a large series of patients with primary SS.

PATIENTS AND METHODS

Patients

Patients who had been referred to the Rheumatology/Clinical Immunology Units of the University of Perugia and the University of Pisa, and to the Clinical Immunology Unit of the University of Ancona for symptoms suggesting sicca syndrome were evaluated using the recently proposed European classification criteria for SS [12]. Patients who had been treated during the last 12 months with either immunosuppressive agents or other drugs known potentially to cause a reduction in salivary and lacrimal secretions were not included in the study. Only those patients in whom a LSG biopsy was available for inclusion in the present study. Informed consent was obtained from each subject before the diagnostic tests were performed.

Histopathological study

Salivary gland samples were obtained from the lower lip. Biopsy was performed only through normal-appearing mucosa to avoid tissue with non-specific inflammatory features. Only those specimens containing at least four salivary gland lobules were used for study. Paraffin-embedded sections stained with haematoxylin and eosin were studied by the same pathologist (MG), using a microscope equipped with an ocular micrometer. He was blind to both the clinical and serological profile of the patients.

Minor salivary gland sections were first evaluated using a scoring system based on the number of foci/4 mm² of glandular tissue, where a focus was defined as an agglomerate of at least 50 mononuclear cells [2]. The confluence of foci to a diffuse infiltrate was graded as 12. We also used the simplified scoring method of Chisholm and Mason [13], where grade 0 = absence of any infiltrate; grade 1 = slight infiltrate; grade 2 = moderate infiltrate or less than one focus score (i.e. <1 focus/4 mm² of salivary tissue); grade 3 = one focus score; grade 4 = more than one focus score.

Finally, the degree of infiltration in each sample was quantified by the following ratio: % infiltration = extent of infiltrated glandular tissue (mm²) × 100/total extent of the histologically observed glandular tissue (mm²).

Clinical evaluation

A complete clinical work-up was performed in all the selected patients to assess the presence and number of extraglandular manifestations. The following extraglandular features (as defined in [14]) were examined: Raynaud’s phenomenon, vasculitis, hand arthropalgia or non-erosive arthritis, lymph node or spleen enlargement, interstitial lung involvement, leucopoenia or trombocytopenia, myositis and peripheral neuropathy.

Serological evaluation

An indirect immunofluorescence procedure using Hep-2 cell substrates was employed to detect the presence and titre of ANA. The serum levels of RF were evaluated by laser nephelometry. ANA titres $\geq 1:160$ and RF levels $>40$ IU/ml in at least two consecutive determinations were considered positive. Anti-SSA/Ro and anti-SSB/La Abs were detected by counterimmunoelectrophoresis (CIE) using human spleen and calf thymus extracts as antigen substrate, respectively [15].

Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the differences between continuous variables. Linear regression analysis or Spearman’s rank correlation was employed when indicated to study the correlation between some demographic and clinical features of the patients and the extent of glandular inflammatory infiltrate. Differences in the amount of glandular infiltration in patients with or without particular clinical and serological features were analysed using the unpaired t-test and Mann–Whitney U-test.

RESULTS

Eighty-two patients who met the European classification criteria for primary SS were enrolled in this study [12]. Patients with the secondary variant of the syndrome were excluded in order to have a uniform study population. The main demographic, clinical and serological features of this population are reported in Table I. It is worth noting that no patient exhibited anti-SSB/La and/or anti-SSA/Ro positivity in the absence of ANA, and none had circulating anti-SSB/La without anti-SSA/Ro antibodies.

At the histopathological examination, most of the glands showed a focal lymphocytic sialoadenitis surrounding the vascular endothelium or glandular acini and ducts. Signs of diffuse acinar atrophy were never seen. The mean percentage of glandular infiltration in the 82 biopsies was $6.4 \pm 12$ (s.d.) %, ranging from the complete absence to the almost complete infiltration of the tissue specimen.
quantitative scoring system was applied, 67 patients had a LSG focus score \( \geq 1 \) (grade 3 plus grade 4 according to Chisholm and Mason), while 15 patients had a focus score < 1 (grade from 0 to 2 following the Chisholm and Mason scoring system). The distribution of focus scores in this population of SS patients is reported in Fig. 1, where it was plotted against the percentage of infiltrated tissue. As expected, highly significant correlations were present when the three scoring systems used to assess the degree of glandular infiltration were compared to each other (Spearman’s \( r > 0.70 \) and \( P < 0.0001 \) in any case).

With regard to the relationship between the extent of glandular infiltrate and the demographic features of the patients, a significant negative correlation was found between the percentage of LSG infiltration and the age at disease onset (see Fig. 2), while there was no correlation between the percentage of infiltration and disease duration.

When the relationships between salivary gland infiltrate and the extraglandular manifestations of the disease were examined, a significant correlation was found between the degree of infiltration in the salivary tissue and the number of extraglandular features (Spearman’s \( r = 0.35, P < 0.01 \)). In particular, a significantly greater extent of the salivary gland infiltration was found in patients with Raynaud’s phenomenon (\( P < 0.05 \)), vasculitis (\( P < 0.0001 \)), lymph node or spleen enlargement (\( P < 0.05 \)), or leucopenia (\( P < 0.02 \)), with respect to those patients without these clinical manifestations (see Table II).

With regard to serological abnormalities, those patients with ANA and anti-SSA/Ro and/or anti-SSB/La antibodies presented a higher degree of inflammatory infiltration than patients without these autoantibodies (\( P < 0.005 \)). This was not seen in the case of RF (Fig. 3). Figure 4 shows the relationship between the presence of anti-SSA/Ro and anti-SSB/La, and the degree of LSG infiltration and the age at disease onset. Patients with both anti-SSA/Ro and anti-SSB/La antibodies showed significantly greater LSG infiltration than patients without these autoantibodies (\( P < 0.01 \)) or patients with antibodies to SSA(Ro) alone (\( P < 0.05 \)). Furthermore, in patients without serological evidence of either autoantibody, the disorder began significantly later than in patients with anti-SSA/Ro alone or those with anti-SSA/Ro plus anti-SSB/La antibodies (Fig. 4).

When the demographic, clinical and serological features of this study population were correlated with the two semiquantitative scoring systems for glandular infiltration, somewhat different results were obtained. The correlation between a high degree of inflammatory infiltration and a low age at disease onset was confirmed for both scoring systems (Spearman’s \( r = -0.35, P < 0.002 \) and Spearman’s \( r = -0.31, P < 0.01 \) for the focus score and Chisholm and Mason’s grading systems, respectively).

For the clinical features, a correlation was found between the two scoring systems and the number of

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**TABLE I**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>82</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>7/75</td>
</tr>
<tr>
<td>Mean age (range) at the time of observation</td>
<td>48.8 (21–84) yr</td>
</tr>
<tr>
<td>Mean age (range) at disease onset</td>
<td>44.6 (17–81) yr</td>
</tr>
<tr>
<td>Mean disease duration (range)</td>
<td>4.2 (0.5–20) yr</td>
</tr>
<tr>
<td>Positivity (%) for antinuclear antibodies (titre ( \geq 1:160 ))</td>
<td>50/82 (61%)</td>
</tr>
<tr>
<td>Positivity (%) for rheumatoid factor (titre ( \geq 1:80 ))</td>
<td>50/82 (61%)</td>
</tr>
<tr>
<td>Positivity (%) for anti-Ro and anti-La antibodies</td>
<td>33/82 (40.2%)</td>
</tr>
<tr>
<td>Positivity (%) for anti-Ro antibodies alone</td>
<td>16/82 (19.5%)</td>
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</tbody>
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**Fig. 1.**—Linear regression analysis between the focus score, i.e. the number of inflammatory foci/4 mm\(^2\) of glandular tissue, and the percentage of infiltrated tissue in LSG of patients with primary SS.

**Fig. 2.**—Linear regression analysis between the percentage of infiltration in LSG and the age at disease onset of patients with primary SS.

**Fig. 3.**—Linear regression analysis between the presence of anti-SSA/Ro and anti-SSB/La antibodies and the age at disease onset of patients with primary SS.
extraglandular manifestations (Spearman’s $r = 0.32$, $P < 0.005$ and Spearman’s $r = 0.28$, $P < 0.02$ for the focus score and Chisholm and Mason’s grading systems, respectively). With regard to the single manifestations, only articular involvement and vasculitis were associated with significantly higher histopathological scores, the significance of this association being lower for Chisholm and Mason’s grading system ($P < 0.02$ and $P < 0.05$ for articular manifestations and vasculitis, respectively) than for the focus scoring method ($P < 0.005$ for both articular involvement and vasculitis).

Significantly higher histopathological scores, using both Chisholm and Mason’s grading system and the focus scoring method, were observed in those patients with ANA, and in patients with anti-SSA/Ro and anti-SSB/La antibodies in particular, than in those without autoantibodies ($P < 0.0001$ for all the comparisons). However, the histopathological score for patients with both anti-SSA/Ro and anti-SSB/La antibodies did not differ significantly from the score for patients with anti-SSA/Ro alone, whichever scoring system was used.

**DISCUSSION**

In this study, a quantitative assessment of the inflammatory infiltrates in the minor salivary glands of a large series of patients with primary SS was performed. The amount of the inflammatory infiltration (expressed as the ratio of infiltrated glandular

<table>
<thead>
<tr>
<th>Extraglandular feature (%)</th>
<th>Prevalence (%)</th>
<th>% infiltration (mean ± s.d.) in patients with</th>
<th>% infiltration (mean ± s.d.) in patients without</th>
<th>Student’s $t$ value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular manifestation*</td>
<td>45/82 (54.9%)</td>
<td>8.4 ± 15.5</td>
<td>4.1 ± 5.0</td>
<td>1.606</td>
<td>0.112</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>15/82 (18.3%)</td>
<td>12.2 ± 25.0</td>
<td>5.2 ± 6.2</td>
<td>2.074</td>
<td>0.0413</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>9/82 (11.0%)</td>
<td>20.8 ± 30.3</td>
<td>4.7 ± 5.9</td>
<td>4.112</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymph node/spleen enlargement</td>
<td>15/82 (18.3%)</td>
<td>12.2 ± 24.5</td>
<td>5.1 ± 6.6</td>
<td>2.081</td>
<td>0.0407</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>21/82 (25.6%)</td>
<td>12.2 ± 21.8</td>
<td>4.4 ± 5.0</td>
<td>2.620</td>
<td>0.0105</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>4/82 (4.87%)</td>
<td>3.10 ± 2.57</td>
<td>6.6 ± 12.4</td>
<td>–0.56</td>
<td>0.575</td>
</tr>
</tbody>
</table>

Myositis and lung interstitial involvement are not reported in the table since their prevalence in the studied population was 0%.

*Hand arthralgia or non-erosive arthritis.

![Fig. 3.—Degree of LGS infiltration in patients with primary SS, subdivided according to their serological features. RF+/RF−, patients positive/negative for rheumatoid factor in their sera; ANA+/ANA−, patients positive/negative for antinuclear antibodies in their sera; Ro+/Ro−, patients positive/negative for anti-Ro antibodies in their sera.](image)

![Fig. 4.—Age at disease onset and degree of LGS infiltration in patients with primary SS, subdivided according to the presence/absence of anti-SSA/Ro (Ro+/Ro−) and anti-SSB/La (La+/La−) antibodies.](image)
tissue to the total extent of histologically analysed tissue) was correlated with the main demographic, clinical and serological features of this population of patients.

The results of our study indicate that the degree of salivary gland inflammation in SS is greater in patients with an earlier disease onset, but does not depend on the disease duration. More widespread glandular inflammatory changes were observed in the presence of specific or multiple systemic extraglandular manifestations, and in the presence of disease-specific autoantibodies such as anti-SSA(Ro) and anti-SSB(La) antibodies. In particular, anti-SSB antibodies seemed to be the serological abnormality most highly correlated to the level of inflammatory infiltrates in the salivary glands.

The association between the extent of glandular infiltrates and specific clinical and serological features of the disease was not as clearly demonstrated when the most common semiquantitative histopathological scoring systems were used to evaluate the degree of glandular infiltration. Therefore, the quantitative measurement of the inflammatory infiltrates in the LSG of patients with SS is probably the most reliable method to assess the histopathological changes caused by the disorder in target tissues. It is, in fact, likely that the amount of infiltration might be related to the disease activity and to the different phases of the autoimmune process in the salivary glands. The commonly used semiquantitative methods probably introduce a simplification of the histological assessment, and although this kind of histological approach may be sufficient for diagnostic purposes, it can result in a loss of information when the relationships between the pathological process in the salivary glands and the other features of the disorder are studied.

On the whole, this study suggests that when the disease begins in young patients, it is commonly characterized by: (i) more aggressive immune-mediated inflammatory changes in the target organs; (ii) systemic extraglandular manifestations; and (iii) the expression of specific autoantibodies. In contrast, when the disorder develops in older patients, the inflammatory damage to the salivary glands is less severe, and the clinical and serological features of the disease are less extensive.

However, it is possible that a certain number of patients with sicca syndrome rather than true SS were included in the present series, thus influencing the results. These could have included older patients with involutive fibrosis of the lacrimal and salivary glands, and consequently without evidence of focal sialoadenitis (i.e. with a focus score < 1) and probably without autoantibodies in their sera. However, the 15 patients in the present study who showed no evidence of focal sialoadenitis (although slightly older (mean age 48.9 ± 11 yr vs 43.7 ± 15 yr, P > 0.05), did seem to be similar to the rest of the study population. In fact, at least one autoantibody [either RF, ANA, anti-SSA(Ro) or anti-SSB(La)] was found in nine patients from this group, and at least one of the extraglandular systemic manifestations included in the clinical record was found in 8/15 individuals. Finally, involutive fibrosis with acinar atrophy was never observed in the salivary gland specimens collected in this subgroup of patients. Therefore, we concluded that this subset most probably also consisted of patients with SS and, in fact, all of them met the European classification criteria for this disorder [12].

Most of our findings are in agreement with the results reported by others. After studying a series of 48 patients with primary SS, Pease et al. [16] concluded that two subsets can be distinguished. The first is characterized by extraglandular involvement, antibodies to SSA(Ro) and SSB(La) antigens, and a frequent association with the HLA B8, DR3 and DRw52 haplotypes. Patients in the second subset often do not exhibit extraglandular features or the typical immunogenetic markers, and are significantly older. An association between the HLA B8, DR3 and DRw52 haplotypes, specific autoantibodies, and the presence of extraglandular involvement has also been reported in other studies performed in different countries, including Italy [17–22].

Although immunogenetic markers were not determined in our patients, our study adds another element to the profile of SS. Those patients with autoantibodies, particular extraglandular features or more extensive extraglandular involvement, also have a larger amount of inflammatory infiltrates in their glandular tissue. This confirms that disease susceptibility and disease expression, including autoimmune inflammatory aggression and damage to target organs, may vary widely in the patient population and are probably genetically determined.

On the contrary, the absence of any correlation between the amount of inflammatory infiltrates and disease duration found in our study is not in accordance with previous reports, which indicated a time-related progression of sialoadenitis measured as the focus score [8]. This could have been the result of the quantitative approach used by us to assess the inflammatory infiltrates in the salivary glands.

In the complex immunogenetic mechanisms which underlie the development and progression of SS [23], it is likely that a key role is played by the SSB(La) antigen and production of the specific autoantibodies. The quantitative analysis performed here indicates that patients with antibodies against the SSB/La specificity exhibit a higher degree of LSG infiltration than subjects with anti-SSA/Ro alone or those negative for both autoantibodies. This observation supports the hypothesis that inflammatory infiltration of the exocrine glands is closely related to the development of immunoreactivity to the SSB/La rather than to the SSA/Ro antigen. In this context, it is important to remember that anti-SSA/Ro antibodies are not entirely specific for primary SS, since they have been associated with various subsets of systemic lupus erythematosus (SLE), including subacute...
cutaneous lupus and the SLE-like disease of homozygous C2 and C4 deficiency [24]. On the contrary, anti-SSB/La antibodies seem to be characteristic of primary and (to a lesser extent) secondary SS, particularly of SS associated with SLE [25].

There are contrasting reports on the relationship between the anti-SSA/Ro and anti-SSB/La antibodies and the degree of glandular infiltration. In contrast with our findings, some data suggest the absence of any such association [26]. This discrepancy could be explained in various ways: different studies may have included patients with both primary and secondary SS, and different methods may have been used to assess LSG inflammatory changes or to detect autoantibodies. In this respect, the quantitative analysis of the extent of LSG infiltrates used in the present work is probably the most reliable histopathological approach to investigate the factors influencing the inflammatory process in this tissue. Recently, a high correlation between anti-SSB/La serum levels (detected by ELISA) and the degree of LSG inflammation has been reported in patients with primary SS [10]. Therefore, it has been suggested that the serum level of anti-SSB/La antibodies, which reflects the fluctuations in the degree of glandular infiltration, could represent a reliable marker of disease activity [10].

The close correlation between anti-SSB(La) antibodies and the degree of salivary gland infiltration shown by the present study also supports the hypothesis that immunoreactivity against the SSB/La antigen may play an important role in sustaining immune-mediated glandular damage in SS. This is further sustained by the fact that the presence of serum anti-SSB/La antibodies identifies a subset of patients with primary SS characterized by a particular pattern of peripheral and salivary gland T-cell dysfunction [27]. In addition, the nuclear SSB(La) antigen has been observed at the surface of the conjunctival epithelial cells in SS patients [28] and the surface overexpression of an SSB(La) epitope has been shown on inflamed salivary gland tissue from SS subjects [29]. Moreover, in vitro experiments have demonstrated that the viral infection of human Hep-2 cell lines can lead to membrane translocation of the SSB/La antigen from the nucleus [30]. Thus, one may speculate that the appearance of SSB/La on the membrane of glandular epithelial cells in predisposed subjects may be induced by a virus and may trigger a T-cell-dependent activation, with consequent anti-SSB/La antibody production. This, in turn, may favour widespread and persisting lymphoid cell infiltration in the exocrine glands.

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REFERENCES


