Immunological, histopathological and clinical considerations in Sjögren’s syndrome – a retrospective study

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Summary

The aim of this study was to evaluate associations between the serologic profiles and clinical manifestations in a series of patients with Sjögren’s syndrome.

This retrospective observational study was conducted using clinical data and laboratory findings of patients diagnosed with Sjögren’s syndrome. Laboratory assessments included routine tests and special immunology determinations: erythrocyte sedimentation rate, C reactive protein, inflammatory markers, anti Ro/SS-A antibodies, anti La/SS-B antibodies, antinuclear antibodies, anti-cardiolipin antibodies, and rheumatoid factor. Minor labial salivary glands biopsies were graded according to the Chisholm-Mason scale.

A number of 47 patients were included in the study, all women aged 56.57±9.91 years. At least one focus of inflammatory cells was identified in all 11 cases with biopsies.

The presence of anti-SS-A antibodies was significantly associated with systemic manifestations, ocular, oral, neurological, osteoarticular, and immunological manifestations, as well as with the presence of antinuclear antibodies and high erythrocyte sedimentation rate. An increased titre of anti-SS-B antibodies was significantly associated with liver disease and an increased titre of rheumatoid factor. Corroborating data regarding cellular changes, antibody levels and their impact on the organism are essential in the diagnosis and monitoring of this disease.

Introduction

Sjögren's syndrome (SjS), also known as sicca syndrome, is one of the most common systemic autoimmune diseases, second in frequency after rheumatoid arthritis. The prevalence is about 1%, and it mostly affects women, with onset between 45 and 55 years (Patel & Shahane, 2014).

The aetiology is plurifactorial and includes genetic elements, environmental factors (e.g. viral infections), and hormonal factors (e.g. oestrogen deficiency). These are responsible for the altering of cellular and humoral immunity, with massive production of cytokines and chemokines. The direct consequence on glandular epithelial cells is the formation of germinal centres of ectopic lymphoid tissue, with the possibility of malignant degeneration and development of lymphomas (P. C. Fox; R. I. Fox, 2012; Mackay et al., 2007). SjS is a slowly progressive systemic autoimmune disease characterized by the destruction and dysfunction of exocrine glands, particularly the salivary and lacrimal glands. Lymphocytic infiltrates are arranged around the excretory ducts, replacing functional epithelium, and causing a decrease in exocrine secretion as
well as the production of auto-antibodies (anti-Ro/ SS-A and anti-La/ SS-B), as a result of B-cell hyperactivity (P. C. Fox; Mackay et al., 2007; Mariette et al., 2009; Miller & Diamond, 2015). Extra-glandular manifestations are the result of extension of lymphoid infiltrates into the epithelial tissues of the lungs, kidneys and liver; these appear at the beginning of the disease and usually show a benign evolution. In contrast, vasculitis, glomerulonephritis and peripheral neuropathy are extraepithelial manifestations that associate increased morbidity and a high risk for the development of lymphoma (Patel & Shahane, 2014). SjS can be either primary (due to the autoimmune dysfunction of the exocrine glands) or secondary. The latter form appears in the presence of other systemic autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematos (SLE), systemic sclerosis (SSc), or inflammatory myopathies (R. I. Fox, 2012; Ramos-Casals et al., 2007).

The aim of this study was to evaluate associations between the serologic profiles and clinical manifestations in a series of patients with SjS.

Materials and methods

This retrospective observational study was conducted on patients hospitalised between September 2013 and September 2014 in the Clinic of Rheumatology of Tîrgu Mureș. The study included patients with a diagnose of primary or secondary SjS based on the 2002 diagnostic criteria of the American-European Consensus Group, whom have had clinical, serologic and histopathologic assessments. Clinical data and laboratory findings were retrieved from the observation sheets, discharge notes and medical reports. Information about clinical manifestations, form of the disease (primary/ secondary), and associated immune-inflammatory diseases were all recorded.

Laboratory assessments included routine tests and special immunology determinations: erythrocyte sedimentation rate (ESR), C reactive protein (CRP), inflammatory markers, anti Ro/ SS-A antibodies, anti La/ SS-B antibodies, antinuclear antibodies (ANA), anti-cardiolipin antibodies (ACA), and rheumatoid factor (RF). In case of minor labial salivary glands biopsies, we’ve taken into consideration the ones with results graded according to the Chisholm-Mason scale. This grading system evaluates the presence of focal inflammatory infiltrates (number of lymphocytes and plasma cells) per 4 mm² of glandular tissue. Grade 0 corresponds to the absence of inflammation, grade 1 denotes a slight infiltrate, and grade 2 represents a moderate infiltrate (less than 50 lymphocytes/ 4 mm². Grades 3 and 4 correspond to the presence of one or multiple foci in 4 mm² of tissue, a focus being defined as the accumulation of over 50 lymphocytes and plasma cells. Thus grade 3 corresponds to a single focus/ 4 mm² of tissue and grade 4 is the presence of more than one focus in the same amount of glandular tissue.

Statistical analysis was performed using MedCalc Software, version 12.5.0.0. Quantitative variables were described using mean and standard deviation (SD) as centrality and dispersion measures; qualitative variables were presented using frequencies. Significant differences between frequencies of nominal variables were assessed using the Chi-square test. The level of statistical significance was set to 0.05.

Results and discussions

Based on the inclusion criteria, a number of 47 patients were identified, all women with a mean age of 56.57±9.91 years – data regarding type of SjS and immunologic characteristics are presented in table 1.

Minor salivary gland biopsies were harvested in 11 cases from apparently normal glands, under local anaesthesia, from the inferior labial mucosa on the midline. In each case 4-6 salivary lobules were taken, obtaining 1.5-2 cm samples. Assessment was performed on slides with standard hematoxylin and eosin staining, from 4 levels of sectioning. The presence of inflammatory infiltrates was graded using the Chisholm-Mason scale.
Table 1. Immunologic characteristics and type of disease of patients included in the study

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary SjS</td>
<td>3</td>
</tr>
<tr>
<td>Secondary SjS</td>
<td>44</td>
</tr>
<tr>
<td>Anti-SS-A antibodies present</td>
<td>26</td>
</tr>
<tr>
<td>Anti-SS-B antibodies present</td>
<td>17</td>
</tr>
<tr>
<td>RF present</td>
<td>10</td>
</tr>
<tr>
<td>ANA present</td>
<td>13</td>
</tr>
<tr>
<td>ACA present</td>
<td>2</td>
</tr>
<tr>
<td>ESR ≥ 28 mm/h</td>
<td>14</td>
</tr>
</tbody>
</table>

Glandular and systemic manifestations of the disease identified in the study populations are presented in figure 1. SjS is often accompanied or is associated with other autoimmune diseases – figure 2 shows the presence of these disorders in the studied patients.

The presence of anti-SS-A antibodies was significantly associated with systemic manifestations, ocular, oral, neurological, osteoarticular, and immunological manifestations, as well as with the presence of ANA and high ESR. An increased titre of anti-SS-B antibodies was significantly associated with liver disease and an increased titre of RF (table 2).

Table 2. Associations of clinical manifestations with the presence of anti-SS-A and anti-SS-B antibodies in the study group.

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>Anti-SS-A positive patients</th>
<th>Anti-SS-A negative patients</th>
<th>P</th>
<th>Anti-SS-B positive patients</th>
<th>Anti-SS-B negative patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xerostomia</td>
<td>28</td>
<td>12</td>
<td>0.013</td>
<td>15</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>Xerophthalmia</td>
<td>22</td>
<td>10</td>
<td>0.016</td>
<td>15</td>
<td>17</td>
<td>0.05</td>
</tr>
<tr>
<td>Fatigue</td>
<td>21</td>
<td>8</td>
<td>0.002</td>
<td>7</td>
<td>10</td>
<td>0.82</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>15</td>
<td>2</td>
<td>0.001</td>
<td>11</td>
<td>16</td>
<td>0.65</td>
</tr>
<tr>
<td>Renal disorders</td>
<td>3</td>
<td>2</td>
<td>0.82</td>
<td>1</td>
<td>4</td>
<td>0.76</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>15</td>
<td>6</td>
<td>0.08</td>
<td>10</td>
<td>11</td>
<td>0.2</td>
</tr>
<tr>
<td>Hepatic involvement</td>
<td>9</td>
<td>3</td>
<td>0.21</td>
<td>9</td>
<td>3</td>
<td>0.003</td>
</tr>
<tr>
<td>Myalgia</td>
<td>6</td>
<td>3</td>
<td>0.69</td>
<td>2</td>
<td>7</td>
<td>0.56</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>3</td>
<td>3</td>
<td>0.77</td>
<td>4</td>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>9</td>
<td>2</td>
<td>0.09</td>
<td>9</td>
<td>2</td>
<td>0.10</td>
</tr>
<tr>
<td>Keratitis sicca</td>
<td>20</td>
<td>8</td>
<td>0.016</td>
<td>11</td>
<td>16</td>
<td>0.65</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>9</td>
<td>1</td>
<td>0.03</td>
<td>5</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>Cranial neuropathy</td>
<td>3</td>
<td>4</td>
<td>0.75</td>
<td>1</td>
<td>6</td>
<td>0.30</td>
</tr>
<tr>
<td>Anemia</td>
<td>8</td>
<td>2</td>
<td>0.15</td>
<td>5</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>1</td>
<td>1</td>
<td>0.87</td>
<td>1</td>
<td>1</td>
<td>0.67</td>
</tr>
</tbody>
</table>

In 3 patients with negative serology for anti-SS-A/SS-B antibodies, glandular biopsy was necessary to establish the SjS – the histopathologic assessment showed...
characteristic histological changes. At least one focus of inflammatory cells was identified in all 11 cases with biopsies; the foci predominantly contained small, mature lymphocytes and a variable number of plasma cells. These foci were mostly located in the periductal space, usually not affecting the salivary acini. No granulomas were noted in any of the examined cases, and no lymphoepithelial lesions or lymphoid aggregates with germinal centres were identified.

Two patients diagnosed with anti-phospholipid syndrome, with negative results for SjS-specific antibodies but severe clinical manifestations (keratitis sicca, peripheral neuropathy and vasculitis), presented grade 3 histologic changes on biopsy (figures 3 and 4). In addition, one patient with negative SjS serology, but severe renal histological changes, showed grade 4 histopathological changes of the salivary gland biopsy – in this case the bioptic examination was required to clarify the diagnosis (figures 5-7).

systemic lupus erythematosus, APS = anti-phospholipid syndrome, SSc = systemic sclerosis, CTD = connective tissue disorders, IM = inflammatory myositis.

Fig. 1. Clinical manifestation of Sjögren’s syndrome present in the studied patients.

Fig. 3. Grade 3 focal lymphocytic sialadenitis – one focus with over 50 lymphocytes. Hematoxylin and eosin, magnification x2.

Fig. 4. Grade 3 focal lymphocytic sialadenitis – a focus with lymphocytes in a salivary lobule with normal acinar structure. Hematoxylin and eosin, magnification x4.

Fig. 5. Grade 4 focal lymphocytic sialadenitis – salivary lobule containing multiple foci of > 50 lymphocytes. Hematoxylin and eosin, magnification x2.
lymphocytes/ 4 mm². Hematoxylin and eosin, magnification x2

While grade 1 and 2 histopathological changes correlated with the presence of specific antibodies, grade 3 and 4 changes correlated with intensely positive antibody titres and visceral and systemic clinical manifestations.

![Image](image1.png)

**Fig. 6.** Grade 4 focal lymphocytic sialadenitis – multiple lymphocyte foci are separated by normal acini. Hematoxylin and eosin, magnification x4.

![Image](image2.png)

**Fig. 7.** Grade 4 focal lymphocytic sialadenitis – the inflammatory infiltrate from the focus contains mature lymphocytes, some of them infiltrating the ductal and acinar epithelium. Hematoxylin and eosin, magnification x10.

Active forms of rheumatoid disease and systemic lupus erythematosus with increased titre of ANA, RF and high ESR significantly correlated with the presence of anti-SS-A and anti-SS-B antibodies, as well as with grade 2-4 histopathological changes. Secondary SjS was present in patients with rheumatoid arthritis (36.17% of all patients) and systemic lupus erythematosus (21.27% of patients), and was significantly correlated with severe visceral manifestations.

**Discussion**

Despite its frequency, SjS is often an underdiagnosed immune system disorder that affects the entire body (Carsons, 2009). Its characteristic feature is the chronic lymphocytic infiltration of exocrine secretory organs. Disease evolution is usually silent for a long time, but the involved immune cells will eventually destroy exocrine glands, particularly the salivary and lacrimal glands (Jonsson et al., 2011). In some patients vascular complications might be present, and an increased incidence of non-Hodgkin lymphoma, leading to increased mortality. A series of clinical and laboratory markers have been suggested as main predictors of disease progression towards lymphoma. These are: persistent parotid swelling, small vessel vasculitis, hypocomplementemia, and cryoglobulinaemia. Identifying these markers associated with poor prognosis could play an important role in selecting patients who require closer monitoring (Delaleu et al., 2008).

Most patients develop SjS in the fourth and fifth decade of life. In our study the mean age was 56.57±9.91 years; this is explained by the fact that the disease is diagnosed relatively late, when sicca-type symptoms are already evident. The female to male ratio in SjS can be as high as 20: 1 (Patel & Shahane, 2014). Our study group included only female patients because all patients hospitalised with SjS in the study’s time frame were female.

Both forms of SjS (primary and secondary) were represented in our study. Primary SjS occurs in the absence of other auto-immune or rheumatic disorders, while the second type has a more complex clinical picture in which SjS accompanies diseases such as scleroderma, SLE, or RA. In our study group 17 (36.17%) patients had an associated diagnosis of RA, 10 (21.27%) patients had SLE and 5 (10.63%) patients had been diagnosed with SSc.

Studies report that approximately 60-80% and 50-80% of SjS cases are associated with the presence of RF and ANA, respectively (Miller & Diamond, 2015). Our results also
demonstrated significant correlations between the presence of anti-SS-A antibodies and ANA, as well as between anti-SS-B antibodies and RF.

Al-Hashimi reported that xerostomia is a common symptom in the elderly population (Al-Hashimi, 2005), and studies have shown that 40% of xerostomia in the elderly is associated with SjS (Mathews et al., 2008; Moerman et al., 2013; Villa et al., 2015). Inflammation and lymphocytic infiltrates in exocrine glands are classical characteristics of SjS, during the course of which acinar cells of the exocrine gland are replaced by fibrotic tissue, rendering them non-functional. This explains why SjS remains one of the most underdiagnosed conditions, especially in the elderly, as sicca symptoms (which are the hallmark of the disease) are often attributed to aging and/or medication use, thus delaying proper diagnosis (Al-Hashimi, 2007; Moerman et al., 2013; Villa et al., 2015).

Systemic manifestations of the disease occur frequently, and include deterioration of the patient’s general condition, fatigue, fever, weight loss, arthralgia, myalgia, and organ manifestations (Kassan & Moutsopoulos, 2004). Fatigue is a very common symptom, as our own results have also demonstrated. Although most of the time fatigue is not associated with fever, night-time sweating or weight loss, the association of these symptoms with fatigue might be a warning sign of malignant lymphoma (Ramos-Casals et al., 2005). Some authors associated fatigue with the degree of autoimmune disease activity, which mostly correlates with the inflammatory syndrome (Ostuni et al., 2002). However, this finding is not always true in case of SjS, because the increase in acute phase proteins is not consistently correlated the inflammatory changes in glands. The results of the present study support this idea, as no significant correlation between auto-antibodies and markers of inflammation was identified.

Studies have shown that peripheral sensory neuropathy is common in SjS, with a prevalence of 10-50%, and it is usually associated with the presence of anti-Ro and anti-La antibodies (R. I. Fox, 2005; Lopate et al., 2006). Similar results were obtained in the current study, with 9 of the patients (19.14%) experiencing symptoms of peripheral sensory or sensorimotor neuropathy, significantly associated with the presence of anti-SS-A antibodies.

According to literature, the most commonly encountered musculoskeletal manifestations of SjS are inflammatory symmetrical arthralgias, while frank arthritis is rare. Generally these are short in duration and resolve spontaneously. In patients with primary or secondary SjS not associated with RA, the articular involvement is non-erosive and non-deforming (R. I. Fox, 2005; Ramos-Casals et al., 2006). From the patients included in the study, arthralgia was present in 27 cases (57.44%) – 11 anti-SS-B antibody positive and 16 anti-SS-B antibody negative patients. Patients with associated RA had erosive arthritis with arthralgia.

The presence of focal lymphocytic sialadenitis is an accepted diagnostic criterion for SjS, thus minor salivary gland biopsies are useful in the clinical diagnosis of patients with SJS, particularly in serologically negative cases. From the studied patients, in 3 cases biopsy was necessary to confirm diagnosis. Similar specific changes appear in both large and minor salivary glands, and studies have demonstrated that histopathology results are similar for biopsies obtained from either large or minor glands (Colella et al., 2010; Pijpe et al., 2007). Minor gland biopsy is recommended because of the higher risk of complications associated with parotid biopsies: paralysis of the facial nerve, or the development of sialocele or fistulas.

Focal lymphocytic sialadenitis is usually graded using the system developed by Chisholm and Mason in 1968. The system introduced a threshold associated with SjS called the focus, defined as a focal accumulation of 50 or more lymphocytes/4 mm², where the adjacent acini are normal. Although the grading system has proven its diagnostic utility, it had some limitations, particularly when assessing samples with more than 1 focus. Thus an extension was developed, called the focus score (Greenspan et al., 1974). This system has 12 levels of
grading based on the number of inflammatory foci/4 mm\(^2\) of glandular tissue. All cases with at least one focus are positive for SjS. Still, it must be kept in mind that other disorders can also present with more than 1 focus of focal lymphocytic sialedenitis: e.g. RA or SLE (Bodeutsch et al., 1992; Lindahl & Hedfors, 1989). Also approximately 5-10% of normal individuals can show this type of changes, which decreases the grading system’s specificity for SjS (Lindahl et al., 1989). It’s also important to note that grading sensitivity is decreased in smokers and those undergoing corticosteroids treatment (Manthorpe et al., 2000; Zandbelt et al., 2001).

The most characteristic histopathological changes are related to the presence of inflammatory infiltrates arranged in foci in the lobules. In the initial stages, these infiltrates are found mostly around the excretory ducts and perivascularly, while the acini still retains their normal structure. These initial infiltrate consists predominantly of small mature lymphocytes, but as the lesions progress and the infiltrate becomes more abundant, foci tend to confluence and reduce the acinar system, which completely disappears in the advanced stages of the disease. Lymphoid aggregates may have germinal centres; when present in great numbers, these changes are suggestive of lymphoma development. Alongside the predominant population of lymphocytes, smaller numbers of plasma cells are also present – their number increases in the late stages of the disease. Sometimes rare non-caseous granulomas can also be identified, but this occurrence should not be linked to a possible sarcoidosis. Unlike the changes present in large salivary glands, the minor glands never develop lymphoepithelial islands.

Conclusions

Sjögren's syndrome is an autoimmune exocrinopathy, often associated with SLE and RA, in which the ocular involvement-arthritis-fatigue symptom-triad is the most frequently encountered form in practice. This is associated with anti-SS-A/SS-B antibody titre despite therapeutic interventions and with specific histopathological changes related to the presence of an inflammatory infiltrate. The presence of anti-SS-A antibodies was significantly associated with systemic manifestations and immunological manifestations, and with the presence of ANA and high ESR. Anti-SS-B antibodies were significantly associated with liver disease and an increased titre of RF. Grade 1 and 2 histopathological changes correlated with the presence of specific antibodies; grade 3 and 4 changes correlated with intensely positive antibody titres and visceral and systemic manifestations. Corroborating data regarding cellular changes, antibody levels and their impact on the organism are essential in the diagnosis and monitoring of this disease.

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