The synergistic value of focus score and IgA% score of sublabial salivary gland biopsy for the accuracy of the diagnosis of Sjögren’s syndrome: a 10-year comparison

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Abstract

Objective. Increasing the accuracy of the diagnosis of Sjögren’s syndrome (SS) by placing emphasis on objective findings such as the presence of anti-Ro and anti-La autoantibodies and abnormal salivary gland tissue (SGT) histology is a current issue. In order to obtain optimal disease sensitivity and specificity of SGT findings, histological and immunohistological SGT examinations were compared. The first describes the extent of the lymphocytic infiltrate as a focus score (LFS), whereas the latter describes the composition of the infiltrate as a percentage of IgA-containing plasma cells (IgA%).

Methods. Both the LFS and IgA% score were assessed in 279 SGT biopsies taken from patients with symptoms suggestive of SS. In case histological conclusions did not match immunohistological conclusions patients were assigned to so-called mismatch groups. Patients in the mismatch groups were further classified using objective, serological parameters [rheumatoid factor (RF), anti-Ro, anti-La, anti-nuclear antibodies, gammaglobulin level].

Results. In 249 samples (89%), LFS and IgA% resulted in the same conclusion. Within this group a total of 63 SGT samples (25%) were characteristic for SS showing LFS > 1.0 and IgA% < 70. In the mismatch groups after serological classification, both false positive as well as false negative scores were observed less frequently for IgA% as compared with LFS (50 vs 75% and 25 vs 50%, respectively).

Conclusions. Additional immunohistological SGT examination provides greater disease sensitivity and specificity than histological SGT examination alone, thereby increasing accuracy of SS diagnosis.

KEY WORDS: Sjögren’s syndrome, Salivary gland biopsy, Focus score, IgA%, Immunohistological, Diagnosis, Sampling error, Serological, Classification.
the prevalence of SS in European countries is expected to decrease from 1 to 3% to ~0.5%. The prevalence of SS in Europe would then be similar to the proportion diagnosed with San Diego or San Francisco criteria applied in the US [5]. However, when a greater value is attributed to Ro and La autoantibodies and SGT biopsy, the disease sensitivity and disease specificity of each of these objective parameters becomes more important and should be reconsidered.

The LFS, based on the extent of the lymphocytic infiltrate in SGT biopsy, is the most used objective target-organ specific sign in the diagnosis of SS. It is also the only histological parameter named in the current classification criteria. It is generally considered that a LFS >1 is strongly associated with SS. However, several reports have shown that a LFS >1 can also be found in other systemic diseases [e.g. rheumatoid arthritis (RA), lupus erythematosides disseminatus, primary biliary cirrhosis, AIDS, myasthenia gravis, graft-versus-host disease] as well as in 5–10% of normal healthy subjects, thereby reducing its disease specificity for SS [6–8]. More recently it was shown that a potential negative influence of external factors like smoking and use of medication on the number of foci cannot be ruled out, thereby reducing the sensitivity of the LFS [9, 10].

Therefore, additional examination of the SGT might help to differentiate between true and false positive and negative LFSs. One of the methods used to do so is quantitative immunohistological (QIH) examination of the SGT biopsy. Here the composition of the lymphocytic infiltrate is described in terms of immunoglobulin subtypes (IgG, IgM and IgA). In the QIH criterion introduced in 1982 by Bodeutsch et al. [6], a percentage IgA-containing plasma cells (IgA%) <70 was very specific for SS. With the QIH criterion, some patients previously not fulfilling ESG criteria for SS because of a LFS <1, could be identified as having SS. Thus, when shifting towards more objective and target-organ specific signs, examining SGT biopsies immunohistologically instead of or next to histologically appears favourable. The objective of this study was to compare disease sensitivity and specificity of histological (LFS) results with immunohistological (IgA%) results from examination of SGT biopsies in order to evaluate both methods, to which have been part of a routine examination of SS in our department for over 10 yr now.

Methods

From 1988 to 1998 a total of 279 SGT biopsies were taken from patients with symptoms suggestive for SS. Both the LFS and IgA% score were assessed in the biopsies. Samples were assigned to one of four groups: a group with LFS >1.0 and IgA% <70 (i.e. histological diagnosis ‘SS’), a group with LFS <1.0 and IgA% >70 (i.e. histological diagnosis ‘no SS’), and two mismatch groups A and B. Group A consisted of samples with LFS >1.0 and IgA% >70, and group B of samples with LFS <1.0 and IgA% <70. The mismatch groups A and B were classified further using the following major and minor objective serological criteria: elevated gammaglobulin level and the presence of Ro and La autoantibodies were considered major objective parameters suggesting SS, whereas the presence of rheumatoid factor (RF) or anti-nuclear antibodies (ANA) were considered minor objective parameters suggesting SS. If both major parameters were present, or if one major plus one minor parameter were present, the mismatch patient was considered as having SS. Instead, if patients had only minor criteria, they were considered as having (secondary) sialadenitis but not SS.

In the double positive or double negative concordant biopsies groups, the number of false positive and false negative LFSs and IgA% scores was estimated based on the oldest half (first 5 yr) of the data (n = 149). As these biopsies showed concordant immunohistological results, not only the serological classification criteria used in the mismatch groups, but also the current ESG criteria could be used in these groups, enabling comparison of both definitions.

Results

Of the 279 biopsies, 63 showed a LFS >1 and an IgA% <70, which immunohistologically classified these patients as having SS. A total of 186 biopsies showed an LFS <1 and an IgA% >70 which led to the immunohistological diagnosis ‘no SS’. Furthermore, 16 biopsies (group A) showed a LFS >1 and an IgA% >70, and 14 biopsies (group B) showed a LFS <1 and an IgA% <70. Thus, the diagnosis based on the two different histological criteria did not match in 30 of 279 biopsies (11%). Additional serological analysis (using the major and minor objective criteria as described in the Methods section) of these two ‘mismatch’ groups classified 12 out of 16 positive LFSs (75%) and seven out of 14 (50%) positive IgA% scores as not having SS. The converse also applies: based on the serological classification criteria, four out of 16 (25%) negative IgA% scores and seven out of 14 negative LFSs (50%) could still be classified as having SS (Tables 1 and 2).

In the further analysed double positive concordant group (n = 31), 71% could be classified as having SS when applying the current ESG classification criteria. The remaining 29%, strictly speaking, does not have SS but has a (immuno-)histological picture which is strongly suggestive for SS. Of this latter group (n = 9), 44% has Ro/La presence and therefore are positive for two objective and most disease-specific parameters.

In the double negative group (n = 90), which as a consequence already lacks one of six items (no LFS >1), 31% fulfilled three out of six items (oral and ocular sicca symptoms, ocular sicca signs) of the ESG classification criteria, but only one patient fulfilled four out of six items, leading to diagnosis of SS (Tables 2 and 3). When applying the serological criteria that were used for classification of the mismatch groups, in the double positive group (n = 31) 61%
could be classified as having SS, whilst in the double negative group (n = 90) 13% could be classified as having SS.

### Discussion

In this analysis of collected SGT biopsy data the additional value of immunohistological examination of SGT was confirmed. When both LFS and IgA% are assessed, an objective, target-organ-specific diagnosis could be established in the majority (89%) of cases. However, an unambiguous histological diagnosis is not necessarily equivalent to an unambiguous clinical diagnosis, as can be concluded from the additional analysis of concordant biopsies. Although this analysis showed that SGT biopsy contributes considerably in distinguishing between SS and non-SS patients, biopsy results therefore still need further confirmation by, for example, serological parameters, unless a more disease specific and sensitive tissue marker is available.

In 11% of cases the conclusion of histological examination of SGT biopsies (LFS) did not match the immunohistological result (IgA% score). In these so-called 'mismatch' cases the IgA% appears superior to the LFS when objective, serological findings are taken into account. After this serological classification, both the numbers of false positive and false negative IgA% scores were considerably lower than the numbers of false positive and false negative LFSs.

In our model chosen for the classification of the two mismatch groups, serological parameters were used as objective decisive data, since a gold standard to compare the SGT biopsy results with is lacking. Furthermore, none of the existing classification criteria could be used for categorization of patients within the mismatch groups because the LFS itself is part of these criteria.
Table 3: Characteristics of patients in concordant groups

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<th>Oral sicca (%)</th>
<th>Ocular sicca (%)</th>
<th>Ocular test (%)</th>
<th>Ro/La (%)</th>
<th>ANA (%)</th>
<th>RF (%)</th>
<th>Gamma (%)</th>
<th>SS (%)</th>
<th>SS (4+) (%)</th>
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</thead>
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<tr>
<td>LFS &lt;1, IgA% &gt; 70</td>
<td>90</td>
<td>64</td>
<td>72</td>
<td>56</td>
<td>9</td>
<td>47</td>
<td>30</td>
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ANA, the presence of anti-nuclear antibodies; RF, the presence of rheumatoid factor; Gamma, elevated gammaglobulin level (>15.5 g/l); SS, Sjögren’s syndrome, classified according serological criteria; SS (4+), diagnosis of Sjögren’s syndrome based on four positive items of ESG classification criteria.

Oral sicca: xerostomia symptoms as described in ESG classification criteria; ocular sicca: xerophthalmia symptoms as described in ESG classification criteria; ocular test: Schirmer-I test positive (<5 mm in 5 min).

Not only should the model contain objective decisive parameters, it should also enable distinguishing (secondary) focal sialadenitis and secondary SS. The latter term should be used with caution, i.e. if next to a LFS > 1 additional objective signs of SS are present in a patient with connective tissue disease. Thus, a patient suffering from RA, with a LFS > 1 but no other objective signs of SS should be classified as having secondary focal sialadenitis, not SS. Previous findings of different immunohistochemical characteristics in SGT support the need to distinguish between sialadenitis and (secondary) SS. The combination of a LFS > 1, combined with an IgA% > 70 and a normal La expression pattern is typically observed in sialadenitis, as seen in RA patients, whereas in primary SS and secondary SS (e.g. next to RA) a LFS > 1, combined with an IgA% < 70 and an abnormal La expression pattern was found, suggesting a different pathogenesis [11]. Furthermore, auto-immune disease (such as RA) patients with a LFS > 1 (i.e. sialadenitis) but without any signs or symptoms of SS have been described previously [6, 7]. Therefore, in this model differentiation between sialadenitis secondary to RA vs SS was enhanced by attributing more value to an elevated gammaglobulin level and the presence of Ro and La autoantibodies. These parameters appear to be more SS-specific objective parameters than RF and ANA, which are also frequently present in, for example, RA patients. This is further supported by the measured percentages of serological markers present in histologically positive patients as compared with histologically negative patients (Table 3). However, the presence of Ro or La antibodies is almost inevitably associated with positive ANA testing (but not vice versa). Therefore, applying more strict disease-specific criteria in the model for categorization of the mismatch groups was also considered by comparing SGT results with the presence of Ro or La antibodies only. In SS the presence of these antibodies is by far the most disease-specific serological sign to date. When the presence of Ro or La autoantibodies was considered as decisive for the diagnosis of SS, the results showed the same pattern of false negative and false positive scores (data not shown).

The conclusion remained that the IgA% score in these cases is superior over the LFS. However, the latter model leaves no place for seronegative SS patients with Ro and La autoantibodies, which are generally believed to form ~20% of the SS population [12–15].

Should the LFS be considered as obsolete? Our data suggest that the combination of both the LFS and IgA% score can have a synergistic value for the accuracy of diagnosis. In cases of doubt, the other parameter can direct towards the right diagnosis. In this study the number of false negative IgA% scores was remarkably low. An IgA% > 70 in combination with a LFS > 1 should therefore be interpreted as a false positive LFS. The interpretation of a LFS < 1 in combination with an IgA% < 70 is less clear, since both false negative LFSs and false positive IgA% scores appear to occur about equally in this mismatch group. However, in the majority of cases assessment of both the LFS and IgA% score leads to an unambiguous histological conclusion. Nevertheless, whether histological and immunohistological findings match or not, neither of them have been shown to be 100% sensitive and specific, as was also depicted in the analysis of concordant biopsies. Therefore, these SGT parameters should still be related to other available objective data such as the serological profile. An additional advantage of immunohistological examination of SGT is that immunohistological changes, such as IgA%, are not necessarily associated with lymphocytic foci and, in contrast to histological findings, are much more diffusely located throughout the minor salivary glands tissue [6]. As a consequence, the sampling error with regard to the IgA%, or other yet to be developed immunohistological disease-specific markers, is less than that of the LFS.

In conclusion, immunohistological examination of SGT provides valuable additional information and reduces sampling error, thereby increasing the accuracy of diagnosis. Thus, in order to acquire a maximum of relevant information out of the target organs in SS, histological examination of SGT only is not sufficient and should be accompanied by immunohistological examination as well. In other words, two sub-optimal tissue markers in terms of disease specificity and sensitivity together provide more sensitivity and specificity than applying only one of them.
References


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