

Diagnostic utility of major salivary gland ultrasonography in primary Sjögren's syndrome

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Abstract

Objective

To investigate major salivary gland ultrasonography (US) in relation to symptoms and findings of oral and ocular dryness, and autoimmune disease, for potential use in diagnosis and follow-up of patients with primary Sjögren's syndrome (pSS).

Methods

Patients with pSS were recruited from the Department of Rheumatology, Haukeland University Hospital. The parotid and submandibular salivary glands were examined by US using a simplified scoring system for glandular homogeneity and hypoechogenic areas. Scans were graded on a scale 0–3, grades 0–1 considered corresponding to normal/non-specific changes and grades 2–3 to pathological changes. Sicca symptoms of the mouth and eyes, salivary gland capacity, tear secretion, minor salivary gland inflammation, serum autoantibodies, and fatigue were also investigated.

Results

US was performed in 97 patients. Oral and ocular sicca symptoms correlated with US score and decreased saliva levels. Fatigue VAS correlated with oral sicca symptoms but was inversely correlated with age. Patients with normal/non-specific US findings tended to be older than patients with pathological US findings. US score correlated with unstimulated and stimulated salivary secretion and tear secretion. Minor salivary gland inflammation correlated with major salivary gland US findings, and lymphoid organisation, germinal centre (GC)-like structures, in the minor salivary gland tissue biopsies was seemingly related to US pathology. Serum autoantibodies against Ro/SSA and/or La/SSB were associated with US pathology.

Conclusion

US findings in major salivary glands correlate with subjective and objective oral and ocular items as well as systemic autoimmune features of pSS. US represents a useful imaging tool for diagnostics and follow-up of pSS.

Key words

primary Sjögren's syndrome, ultrasonography, diagnosis, follow-up, antibodies, salivary glands

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Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune condition typically affecting the exocrine glands. Patients with pSS experience symptoms such as oral and ocular dryness (xerostomia and keratoconjunctivitis sicca, respectively) and extraglandular manifestations such as fatigue, arthralgia and arthritis (1). In addition to subjective and objective findings of salivary and/or lachrymal gland involvement, the pSS diagnosis is based on either the presence of autoantibodies against Ro/SSA and/or La/SSB and/or focal mononuclear cell inflammation corresponding to a focus score of 1 or more in a minor labial salivary gland biopsy (2). Serum autoantibodies have been recently presented as a very early marker of pSS (3). Minor salivary gland biopsy is part of the diagnostic process for pSS, but is not regarded as suitable for repeated follow-up.

Interest regarding the use of ultrasonography (US) examination of the major salivary glands (4) as a potential diagnostic tool for pSS has increased over the last 5–10 years (5–9), representing a non-invasive imaging method of the major salivary glands that may serve as a supplement to the minor salivary gland biopsy (7). Studies indicate that US findings have a high specificity for pSS (98.7%) (10). It has also been suggested that it may be an alternative to sialoscintigraphy (sSC) and sialography in the American-European classification criteria for pSS (11, 12) and a first-line diagnostic imaging tool (8, 13–16). Milic *et al.* showed that US was highly accurate to establish the pSS diagnosis and comparable to sSC and biopsy (6, 11).

The aim of this study was to investigate if parotid and submandibular gland US imaging findings correlate with subjective oral and ocular dryness symptoms and fatigue as well as objective findings such as secretory function of salivary and lacrimal glands and serum autoantibodies, and thus determine whether US examination using a simplified scoring system is suitable for assessment of glandular involvement in pSS.

Materials and methods

Patients

Patients with pSS were recruited from

the Department of Rheumatology, Haukeland University Hospital (n=97) and were either previously diagnosed with pSS (n=82) and part of a well characterised pSS cohort, or newly diagnosed (n=15) and included in the cohort. All patients met the criteria set by the American-European Consensus Group (AECG) in 2002 (2). Following oral and written information of the study all patients signed a consent form. Patient data collection and studies on minor salivary gland tissue, serum, saliva, and tear fluid were approved by the Regional Medical and Health Research Ethics Committees (145/96-44.96, 242.06 and 2009/686) and reported to the Data Inspectorate (NSD number 9646).

Ultrasonography of the major salivary glands

The parotid and submandibular glands were examined by US using a GE LogiqE9 with a linear high-frequency transducer (6–15MHz). The parotid glands were evaluated in a longitudinal and cross-sectional plane and the submandibular glands in a longitudinal plane. The US examination was performed by one out of two clinical investigators (DH and MVJ). Representative US images were stored digitally as jpg images and evaluated at a later time point, in a darkened room and on a standard high-quality computer screen. All images were evaluated separately by two investigators (DH and MVJ) who were both blinded for clinical findings such as subjective sicca symptoms, sialometry, serum autoantibodies and minor salivary gland inflammatory focus score.

Glandular homogeneity and presence of hypoechogenic areas were evaluated and graded (0–3) using a simplified scoring system, as previously described according to Hocevar *et al.* 2005 (17) and recently applied in a clinical study (18). Grades 0–1 were considered to correspond to normal/non-specific changes and grades 2–3 to correspond to pathological changes (Fig. 1). The number and size of hypoechogenic and/or anechoic areas as well as the degree of inhomogeneity presented in both submandibular and parotid glands were evaluated and graded by both investigators (DH and MVJ). A final overall

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score was then determined for all glands combined. In cases where hypo-/anechoic areas were not detected, the score was set to 0. If US showed a few minor focal hypo-/anechoic areas that were considered within normal, the score was set to 1. When at least one of the glands was more severely affected with multiple focal hypo-/anechoic areas but some homogenous and normal appearing salivary gland tissue remained, the score was set to 2 and changes considered as pathological. In cases of severe generalised affection of at least two of the glands with minimal normal appearing glandular tissue remaining, as well as at least a grade 2 affection of the remaining gland(s), the score was set to 3.

Sialometry

Salivary gland functional capacity was evaluated by unstimulated and stimulated sialometry of whole saliva, in ml/15 minutes and ml/5 minutes, respectively. The clinical investigations were performed in a consecutive setting, starting with the unstimulated whole saliva (UWS) with the patients fasting at least 90 min before examination. Stimulated whole saliva (SWS) was obtained by letting patients chew on paraffin for five minutes (19). Both unstimulated and stimulated saliva was collected in pre-weighed containers and the volume of secreted saliva determined by weighing, with 1 gram of saliva corresponding to 1 ml. Levels ≤ 1.5 ml/15 minutes of unstimulated saliva and ≤ 3.5 ml/5 minutes of stimulated saliva were considered pathologically reduced.

Tear secretion

Tear secretion in both eyes was evaluated by the Schirmer I-test. Levels of 5 mm or less wetting of the paper strip were considered pathologically reduced.

Minor salivary gland inflammation

Minor salivary gland tissue biopsies with determination of focus score had previously been performed for the majority of patients (n=82) (Table I). The minor salivary gland focus score denominates the number of focal mononuclear cell infiltrates with ≥ 50 cells per 4 mm² of otherwise normal appearing minor salivary gland tissue (1). In

Table I. Patient characteristics.

	US score 0–1 (n=46)	US score 2–3 (n=51)	p-value
Women (%)	43 (93.5)	48 (94.1)	0.896 [#]
Age, mean (SD)*	63.5 (9.0)	59.4 (13.6)	0.082 [§]
Fatigue VAS, mean (SD)	57.9 (19.3)	51.3 (25.9)	0.164 [§]
Dry eyes VAS, mean (SD)	5.5 (2.4)	6.2 (3.1)	0.194 [§]
Dry mouth VAS, mean (SD)	5.6 (2.4)	7.4 (2.3)	0.001 [§]
Anti-Ro/SSA and/or anti-La/SSB (%)	24 (52.2)	44 (88.0)	<0.001 [#]
Anti-SSA (%)	23 (50.0)	41 (82.0)	0.001 [#]
Anti-SSB (%)	8 (17.4)	29 (58.0)	<0.001 [#]
Focus score, mean (SD) (n)	1.6 (1.2) (n=42)	2.3 (2.0) (n=39)	0.069 [§]
Tear flow right eye [†] , mean (SD)	9.8 (9.9)	4.2 (5.9)	0.002 [§]
Tear flow left eye [†] , mean (SD)	10.4 (11.2)	4.1 (7.1)	0.002 [§]
Pathological Schirmer (%)	24 (53.3)	42 (84.0)	0.001 [#]
UWS [‡] , mean (SD)	1.9 (2.0)	0.8 (1.5)	0.002 [§]
Pathological UWS (%)	22 (50.0)	44 (88.0)	<0.001 [#]
SWS [‡] , mean (SD)	6.7 (3.7)	3.4 (3.6)	<0.001 [§]
Pathological SWS (%)	91 (20.5)	32 (64.0)	<0.001 [#]

*SD: standard deviation; [†]Schirmers I-test mm/5 min, normal >5 mm; [‡]unstimulated whole salivary flow ml/15 min, normal >1.5 ml; [§]stimulated whole salivary flow ml/5 min, normal >3.5 ml; [§]t-test with Welch correction; [#]Pearson Chi-square.

72/82 patients, data regarding lymphoid neogenesis/germinal centre (GC)-like structures was available and added to data analyses as a patient characteristic.

Subjective sicca symptoms and fatigue

Severity of sicca symptoms of the mouth and eyes were reported by patients on a visual analogue scale (VAS) ranging from 0–10 and the level of fatigue on a VAS ranging from 0–100, with a higher number representing more severe symptoms. Recordings of fatigue were measured using a graded ruler and recordings of sicca symptoms on a graded scale from 0–10.

Statistical analyses

Data were frequently not normally distributed. The Student's *t*-test with Welch's correction was used to study differences between groups and Spearman correlation for the linear relationship between two variables. For categorical data Chi-square analysis was employed. *p*-values <0.05 were considered significant. All analyses were performed using IBM SPSS Statistics version 19.0 (Armonk, NY, IBM Corp).

Results

Patient characteristics

US examinations were performed in 97 patients with pSS. The stored US images were separately analysed by

two examiners. In twenty cases images were initially scored different; three differed between score 0 and 1, ten between score 1 and 2, and seven between score 2 and 3. The measure of agreement (kappa) was 0.697. In the cases where the grading diverged, the images were reviewed and discussed by the two examiners until consensus was established. This score was then used in the following analyses.

US scores (Fig. 1) ranged from 0–1 (n=46) and 2–3 (n=51), a sensitivity of 52.6%, which is in accordance to earlier studies with a more detailed scoring system (17). In general, pathological changes were more frequent in the submandibular glands than the parotid glands, and were often accompanied by changes in the parotid glands; only one patient had normal appearing findings (score 0–1) in the submandibular glands and pathological findings (score 2–3) in the parotid glands. Mean age of patients with normal/non-specific US findings was 63 years compared to 59 years in patients with pathological US findings (*p*>0.05). Detailed results regarding patient demographics and differences between the groups with normal and pathological US scores are summarised in Table I.

Sicca symptoms and fatigue

The patients' experience of dry mouth,

dry eyes and fatigue were recorded using visual analogue scales (VAS). Mean dry mouth VAS was 7.4 in patients with pathological US compared to 5.6 in patients with normal US ($p<0.001$), and mean dry eyes VAS was 6.2 in patients with pathological US compared to 5.5 in patients with normal US ($p=0.194$) (Fig. 2). Although the difference in sicca symptoms of the eyes was not statistically significant in patients with normal and pathological US findings, they correlated with oral sicca symptoms ($p<0.001$, $r=0.549$, $n=96$). Oral dryness in turn correlated with US score ($p<0.001$, $r=0.392$, $n=96$), and saliva levels ($p<0.001$, $r=-0.439$, $n=93$) and ($p<0.001$, $r=-0.508$, $n=93$), unstimulated and stimulated saliva, respectively. Interestingly, VAS fatigue scores correlated with oral sicca symptoms ($p<0.05$, $r=0.214$, $n=93$) but were inversely correlated with age ($p=0.01$, $r=-0.265$, $n=93$).

Secretory function of salivary and lacrimal glands

Secretory capacity was investigated by unstimulated and stimulated whole sialometry. In 26/53 patients with stimulated saliva, levels within normal ($>3.5\text{ ml}/5\text{ min}$), the unstimulated saliva levels were also within normal range ($>1.5\text{ ml}/15\text{ min}$), whereas 39/41 patients with a reduction in stimulated saliva levels ($\leq 3.5\text{ ml}/5\text{ min}$) also had pathological levels of unstimulated saliva ($\leq 1.5\text{ ml}/15\text{ min}$) ($p<0.001$). Levels of unstimulated and stimulated saliva correlated ($p<0.001$, $r=0.679$, $n=94$).

In patients with normal/non-specific and pathological US, mean unstimulated saliva was 1.9 ml/15 min and 0.8 ml/15 min ($p<0.01$), respectively and the stimulated saliva levels were 6.7 ml/5 min and 3.4 ml/5 min ($p<0.001$) (Fig. 3). US scores correlated with unstimulated ($p<0.001$, $r=-0.424$, $n=94$) and stimulated saliva ($p<0.001$, $r=-0.503$, $n=94$). Forty-four out of 66 patients with unstimulated saliva $\leq 1.5\text{ ml}/15\text{ min}$ had pathological US changes compared to 6/28 with normal unstimulated saliva ($p<0.001$). Thirty-two out of 41 patients with stimulated saliva $\leq 3.5\text{ ml}/5\text{ min}$ had pathological US compared to 18/53 with normal stimulated saliva ($p<0.001$).

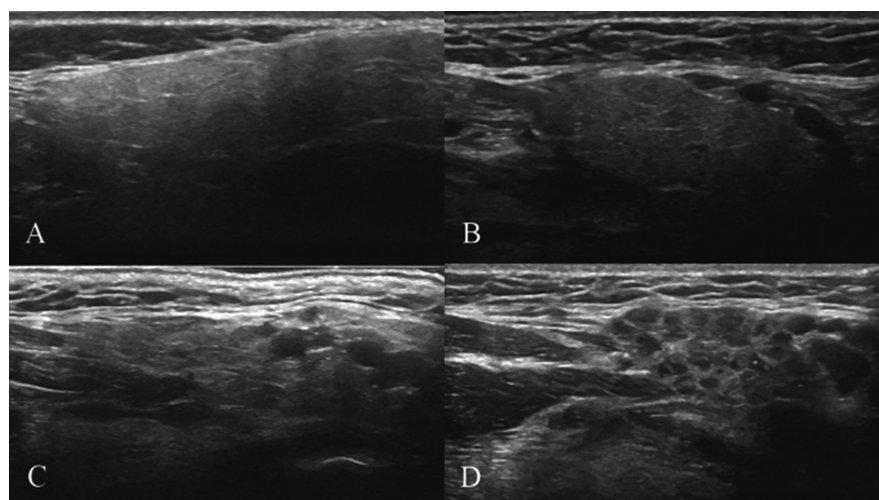


Fig. 1. Ultrasonographic images of four left submandibular glands illustrating varying grades of normal/non-specific to pathological changes. Grades 0–3 were used when evaluating US examinations of each patients submandibular and parotid glands. (A) represents grade 0, (B) grade 1, (C) grade 2 and (D) grade 3. Grades 0–1 were considered to correspond to normal morphology/non-specific changes, whereas grades 2–3 were considered to correspond to pathological changes, possibly related to pSS.

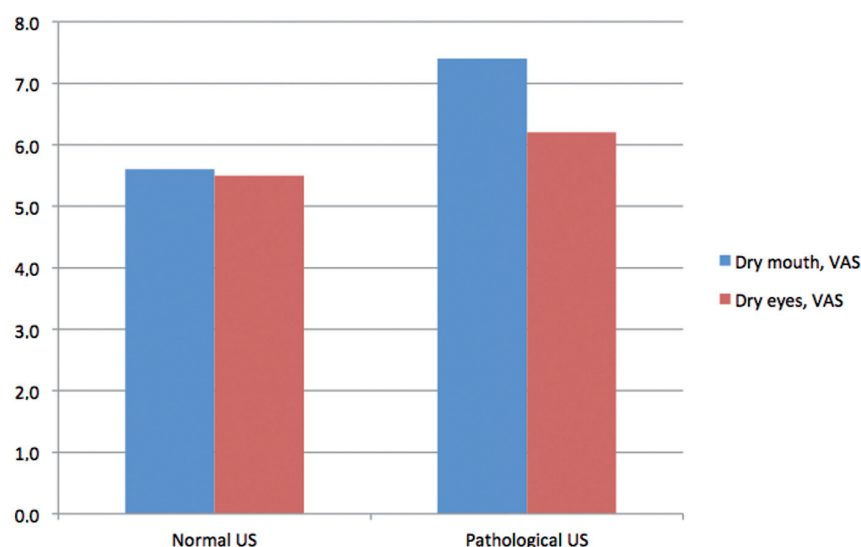


Fig. 2. The patients' own perception of oral and ocular dryness symptoms were recorded using a visual analogue scale (VAS). Patients with pathological US findings in the major salivary glands more often reported dryness symptoms in the mouth and there was an association between reported symptoms in the eyes and mouth.

Tear secretion by the Schirmer I-test correlated in the right and left eye ($p<0.001$, $r=0.720$, $n=95$). Positive Schirmer I-test in one or both eyes were observed in 42/50 patients with pathological US findings, compared to 24/45 patients with normal/non-specific US findings ($p<0.01$), and US scores correlated with tear secretion ($p<0.01$, $r=-0.343$, $n=95$) and $p<0.01$, $r=-0.309$, $n=95$), right and left eye, respectively. Unstimulated saliva secretion correlated with tear secretion ($p<0.01$, $r=0.297$, $n=92$) and ($p<0.01$, $r=0.287$,

$n=92$), right and left eye, respectively. Stimulated saliva secretion also correlated with tear secretion ($p<0.05$, $r=0.263$, $n=92$) and ($p<0.05$, $r=0.238$, $n=92$), right and left eye, respectively.

Minor salivary gland inflammation

Focus score was available in 81/97 patients, and correlated with US score ($p=0.05$, $r=0.219$, $n=81$). Mean focus score was 2.3 in patients with US pathology and 1.6 in patients with normal/non-specific US ($p=0.069$). Lymphoid organisation in the form of germinal

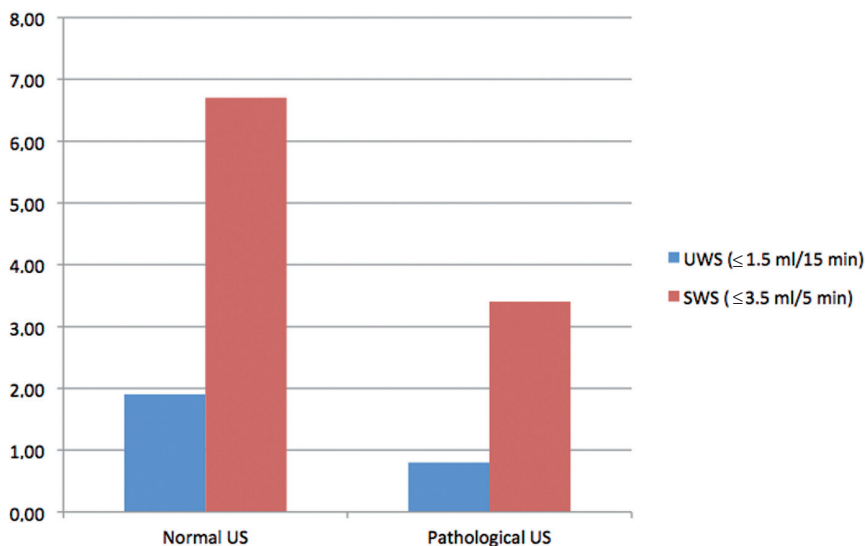


Fig. 3. Salivary capacity was evaluated using unstimulated and stimulated salivary secretion. Mean un-stimulated saliva levels were 0.8 ml/15 min in patients with pathological US compared to 1.9 ml/15 min in patients with normal US ($p=0.002$). Mean stimulated saliva levels were 3.4 ml/5 min in patients with pathological US compared to 6.7 ml/5 min in patients with normal US ($p<0.001$).

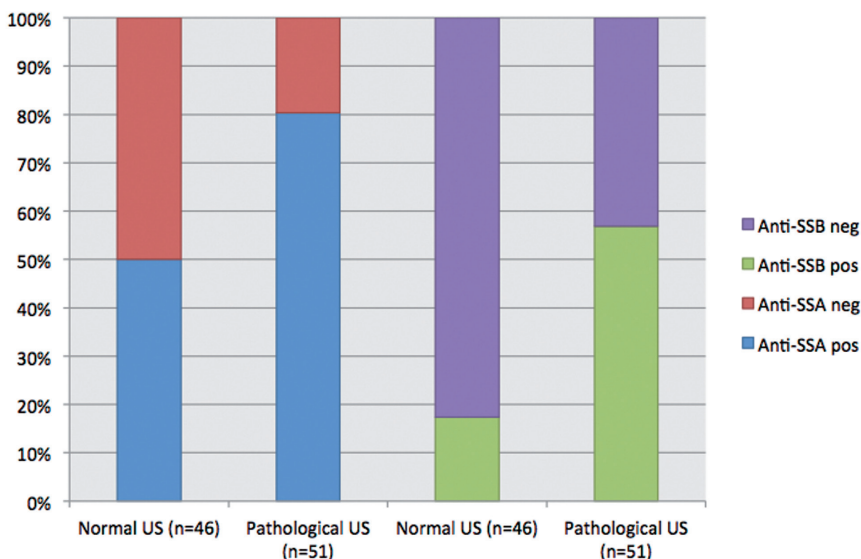


Fig. 4. Systemic autoantibodies against anti-to/SSA and/or anti-La/SSB were determined in 70% of the cohort. Elevated serum levels of autoantibodies to Ro/SSA and/or La/SSB were associated with US pathology ($p<0.001$). Serum titres of anti-Ro/SSA were elevated in 41/50 patients with US pathology compared to 23/46 patients with normal appearing major salivary glands ($p<0.01$). Anti-La/SSB titres were elevated in 29/50 patients with pathological US findings compared to 8/46 with normal appearing glands ($p<0.001$).

centre (GC)-like structures were observed in 15/72 (21%) patients; 10/15 GC-like structures were observed in patients with US pathology ($p=0.090$).

Serum autoantibodies

In the whole cohort, pathologically elevated serum levels of autoantibodies to Ro/SSA and/or La/SSB were determined in 68/96 (71%) patients. Slightly more than 50 % of the patients ($n=33$)

were positive for both anti-Ro/SSA and anti-La/SSB, the remaining were only anti-Ro/SSA positive ($n=31$) or only anti-La/SSB positive ($n=4$). Serum autoantibody status was associated with US pathology in 44/50 patients ($p<0.001$) (Fig. 4). Specifically, serum titres of anti-Ro/SSA were elevated in 41/50 patients with US pathology compared to 23/46 patients with normal appearing major salivary glands

($p<0.01$). Anti-La/SSB titres were elevated in 29/50 patients with pathological US findings compared to 8/46 with normal appearing glands ($p<0.001$).

Ultrasonography of major salivary glands in recent and well established primary Sjögren's syndrome

Patients in the investigated cohort were either previously diagnosed with pSS ($n=82$) and part of a well characterised pSS cohort, or recently diagnosed and included in an extension of the same cohort ($n=15$). 43/82 previously diagnosed patients and 8/15 of the newly diagnosed patients had pathological findings by US, indicating an even distribution of pathological changes in the major salivary glands in the patient population. Apart from the mean age being 9 years younger in the newly diagnosed cohort, no significant differences were observed. This finding most likely represents a selection bias due to the fact that many of the patients in the older cohort have been followed at the Department of Rheumatology, Haukeland University Hospital, for up to ten years.

Discussion

Pathological changes in the major salivary glands were determined in 51/97 (52.6%) of the patients in our pSS cohort, and in similar levels in the 43/82 (52.4%) previously and the 8/15 (53.3%) recently diagnosed patients. Interestingly, in this cohort, patients with severe changes determined by US tended to be younger and had a higher degree of subjective and objective findings, indicating a patient sub-group with a more severe pSS. The patient population consisted of 97 patients and, although subjective and objective items were observed and measured in the cohort as whole, these findings seem to be more pronounced in the patients with positive imaging findings. Pathological US findings changes in the major salivary glands were found to correlate with both subjective symptoms such as VAS dry mouth, and objective organ-specific features such as hyposalivation, reduced tear secretion, focal chronic inflammation of the minor salivary glands, as well as autoimmune features such as serum autoan-

tibodies directed against Ro/SSA and La/SSB.

Poorer oral symptoms and dysfunctional secretory capacity are possibly linked to salivary gland atrophy and the US hypoechogenicity and inhomogeneity observed of the glands. US of the major salivary glands in patients suffering from pSS may show inhomogeneity and hypoechogenic areas which are considered to correspond to pathological changes. We are not aware of current studies that have thoroughly examined the histology or the significance of such areas, and it is tempting to speculate whether the hypoechogenic areas may be due to atrophy of the gland resulting from the chronic autoimmune inflammation in pSS.

The mean VAS dry mouth was increased in patients with pathological US, whereas interestingly an inverse correlation was observed in VAS fatigue and age. This could be due to the multifactorial background of fatigue, in contrast to VAS dry mouth which may be more directly linked to decreased saliva production, or altered saliva quality/composition (20, 21). We did observe a correlation between VAS dry mouth and VAS fatigue which is consistent to the findings described by Haldorsen *et al.* (21). Although the cohorts are based on the same study population, the difference in number of patients may explain why the association between UWS and VAS fatigue is not confirmed. The observed associations between secretion of tears and saliva, by Schirmer I-test, unstimulated and stimulated whole sialometry, respectively, and US findings, further point to a general affection of the exocrine glands in the cohort.

In line with this, salivary gland US findings correlated with elevated serum titres of both anti-Ro/SSA and anti-La/SSB, indicating a more severe disease in this group of patients. US may thus prove useful in selecting subgroups of patients in need of closer follow-up. Patients with pathological US findings were younger, possibly explained by the fact that patients with US findings suffer from a disease with earlier onset of clinical symptoms and thus diagnosis of disease; the correlation between VAS fatigue and age where younger pa-

tients experienced more severe symptoms of fatigue, further strengthens this hypothesis. It is tempting to speculate whether the time of onset of symptoms until the diagnosis is established is a factor of importance. In future studies, serum biomarkers (3) in combination with non-invasive methods such as US of the major salivary glands may prove useful in early detection of patients with pSS. Indeed, serum autoantibodies were associated with positive imaging findings in 44/50 patients.

Minor salivary gland biopsies are important in the diagnostics of pSS (22). Patients with GC-like structures (GC+) in the minor salivary glands have previously been described to have a more severe disease, with a possible increased risk of lymphoma development. Interestingly, the GC+ patients more often have pathological changes by US in the major salivary glands. Similar findings have recently been reported in a Swedish cohort (18) and in concert with our findings indicates that US examination of major salivary glands may eventually serve as a supplement or even an alternative to minor salivary gland biopsy in cases where the biopsy is not possible to perform.

Our cohort consisted of hospital-referred patients, and this may contribute to a more severely affected patient group that is not fully representative for the general pSS population and the patients diagnosed and treated by a general practitioner. The simplified US scoring system in this study and in the recent study by Theander *et al.* (18) uses fewer parameters as compared to other studies (17, 23-25), deliberately selected with the intention of accessibility and use in clinical daily practice. It may, however, be less accurate for minute changes than the more complex scoring systems (23). US may also be considered as a possible parameter or end point in therapeutic studies (24), in such studies a more complex scoring system may be warranted. The issue of intra- and inter-observer variation also needs to be addressed. A more complex scoring system with more precise criteria might reduce the difference but the clinical application would be more challenging.

In all but one of the 51 patients pathological changes were observed in the submandibular glands, with or without concomitant changes in the parotid gland. Overall, severe changes were more frequent in the submandibular glands than in the parotid glands. Only one patient had more severe changes in the parotid glands than observed in the submandibular glands. This would indicate that in most patients, the submandibular gland is either affected earlier, or more severely than the parotid gland. Thus, it seems necessary to examine all 4 glands to get an overall impression. In this study both parotid glands and both submandibular glands were evaluated. When changes representing score 2 were observed in at least one of the glands, findings were considered to represent pathological changes and representative for pSS. Another possibility could be to add the grades of all glands and set a cut-off value to indicate the diagnosis.

Conclusion

In summary, the observed associations between US findings, severity of dryness symptoms, exocrine function, glandular inflammation and systemic autoantibodies indicate the usefulness of US as a tool for assessing salivary gland involvement in pSS. Further studies are needed to elucidate the potential role of major salivary gland US imaging as a diagnostic item for pSS.

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