Review

Imaging in primary Sjögren’s syndrome: the “obsolete and the new”

C. Baldini¹, A. Zabotti², N. Filipovic³, A. Vukicevic³, N. Luciano¹, F. Ferro¹, M. Lorenzon⁴, S. De Vita²

ABSTRACT
Primary Sjögren’s syndrome (pSS) is a complex systemic autoimmune disease primarily characterised by a focal chronic inflammation of glandular parenchyma, with chronic and persistent involvement of major salivary gland remaining a key element of the disease. Indeed, classification criteria proposed for pSS have always included items for histological and/or imaging salivary gland assessment. Over time, the approach to the definition of glandular involvement in pSS is constantly evolving. In this review we will therefore illustrate the state of the art of imaging techniques in pSS, focusing on conventional and novel modalities and discussing their advantages, drawbacks and possible future developments.

Introduction
Primary Sjögren’s syndrome (pSS) is an heterogeneous systemic disorder potentially involving any organ and system (1-3). The “autoimmune exocrinopathy” remains, however, the key manifestation of the disease (4). Inflammation and dysfunction of salivary and lacrimal glands have been described in the vast majority of the patients (5, 6) and dryness represents the most frequent presenting symptom of pSS. Indeed, classification criteria proposed for pSS have always included items allowing histological and/or imaging salivary gland assessment (7-11). Over time, the approach to the definition of glandular involvement in pSS has significantly changed and different tools have been proposed with the ultimate aim of enabling an early recognition of the disease and a better stratification of its phenotypes (12). Recently, new insights have been gained in salivary glands histopathology in order to standardise the reading of minor salivary glands (13), and “-omics” techniques have been extensively applied to the study of glands dysfunction (14); in parallel, a critical reappraisal of the imaging modalities for detecting salivary glands involvement in pSS has also been made (12). In this review we will specifically focus on the state of the art of imaging techniques in pSS. In the first part, we will discuss the advantages and disadvantages of salivary glands sialometry and scintigraphy. The core part will be devoted to salivary gland ultrasonography focusing on the efforts made on the standardisation of the procedures, the elaboration of a common score and on the novel perspective in its applications. Finally, we will refer to the use of MRI and to its complementary role in salivary gland assessment.

Salivary gland scintigraphy and sialography
Salivary glands scintigraphy and sialography have been used for many years for the assessment of salivary gland involvement in pSS and had been included in several criteria classification sets for the disease in the past (15). Major salivary glands scintigraphy is a nuclear imaging technique that, through radioactive tracer infusion (Technethium-99 pertechnetate), permits to study glandular function by evaluating the distribution and speed of elimination of the radio-tracer after a secretive stimulation (i.e. lemon juice). In 1971, Shall et al. first proposed a quantitative score system (from 0 to 4) for the assessment of xerostomia in pSS patients (7); this grade classification system was considered valid enough to be included in 1993 in the Preliminary European Classification Criteria for

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Sjögren’s Syndrome (9). Subsequently, this quantitative score system was surpassed, being included as a purely qualitative interpretation of the scintigraphic images. According to the 2002 American European Criteria Group (AECG) (10), a positive scintigraphy was defined as a test characterised by delayed uptake, reduced concentration and/or delayed secretion of tracer. So far, several studies (8, 16-19) have tried to evaluate the diagnostic performance of this technique, showing a sensitivity up to 89%. The specificity of salivary gland scintigraphy is lower, being described as around 50%, making this tool apparently not able to distinguish the peculiar functional alterations of pSS from other major salivary gland diseases characterised by a secretive dysfunction.

More recently, Salaffi et al. have evaluated the diagnostic performance of salivary gland scintigraphy in a monocentric population of patients clinically suggestive of pSS and candidates for minor salivary gland biopsy, showing a specificity of 70% and an unexpected high specificity of 82% (20). These results are probably due to a patient selection bias. Interesting data also come from a large study by Ramos-Casals et al. conducted on 405 pSS patients; their results showed a significant correlation between a severe scintigraphy pattern at diagnosis and several widely recognised predictive factors for lymphoma, including parotid enlargement, extra-glandular involvement, anti-Ro/SSA, anti-La/SSB, rheumatoid factor positivity and hypocomplementaemia (21).

Currently, salivary gland scintigraphy is no longer part of the recent classification criteria for pSS (22). However, it is possible that this technique, monitoring salivary gland functioning over time, might still have some potential indications during patients’ follow-up to objectively evaluate changes of their secretory function after treatment.

Sialography is a traditional radiographic test based on the cannulation of the main salivary ducts and the subsequent injection of iodinated contrast medium, allowing the visualisation of the architecture of the entire ductal system and its typical pSS-related anatomical changes. The results are interpreted through the score system of Rubin and Holt firstly proposed in 1957 (12); this score consists of five classes of severity based on the morphological abnormalities and the amount of contrast material ductal retention. The sparsity of the branching pattern of the ducts and distal signs including acinar and ductal dilatations have been considered as the leading signs (23).

In 2015, Song and Lee summarised in an interesting meta-analysis the diagnostic power of sialography in pSS patients. The meta-analysis included 488 patients and 447 controls from two European and four Asian studies and analysed the diagnostic accuracy of sialography in comparison to AECG criteria for the European population and to Japanese criteria for the Asian population. The authors showed that sialography had an overall sensitivity of 80% and a specificity of 89% (24). Although sialography is considered a reliable and accepted method for pSS diagnosis, it has limitations in terms of invasiveness and radiation exposure. In fact, cannulation of the salivary duct might cause complications such as sialadenitis and sialoectasia; furthermore, sialography is contraindicated in patients with infection, inflammation or allergy to iodine. For these reasons, sialography has been excluded from the novel pSS ACR / EULAR criteria as well (22).

**Salivary gland ultrasonography**

Salivary gland ultrasonography (SGUS) was proposed for the diagnosis and follow-up of pSS many years ago (25). In particular, its evaluation in pSS was developed not based on US abnormalities established a priori, as putatively more distinctive of pSS, but after adequate stepwise discriminant analyses of multiple echographic abnormalities possibly detected. By this methodological approach, glandular inhomogeneity was selected as the most useful US abnormality to distinguish pSS patients from controls, and a very simple scoring system was developed (25). Later, parenchymal inhomogeneity was indeed confirmed to be of primary importance to score SGUS in pSS.

In recent years there has been a renewed interest in SGUS, to guide clinician in the diagnostic process of pSS (26, 27). Table I summarises the pivotal initial study and most relevant SGUS studies performed in pSS in the recent past (20, 24, 25, 28-40). Despite the many differences between these studies in terms of aims and scoring systems used, they all confirmed the high specificity and the good sensitivity of SGUS supporting the hypothesis of including this tool in the diagnostic algorithm of pSS (36, 41). A recent systematic review by Delli et al. which included 29 studies, found a pooled sensitivity of 69% and a specificity of 92%, despite limitations related to both the different scoring systems adopted and the diverse definitions of the ultrasound elementary lesions considered (42).

Moreover, when compared to sialography and sialoscintigraphy, SGUS displayed a similar diagnostic accuracy with respect to the other techniques, with the advantage of lower invasive-ness and costs (20, 32, 34).

In 2012, an international study group was created with the ultimate aim of verifying whether SGUS could be included into the classification criteria for pSS. The first step was a literature systematic review aimed at defining the elementary lesions that may have the better psychometric properties for the ultrasonographic evaluation of pSS patients. Elementary lesions were identified and a preliminary reference atlas was created (41). The subsequent step was to assess the inter and intra-observer reliability of different SGUS items on both static and real time acquisition images in order to identify the most reliable parameters. The list of parameters explored included for each gland the echogenicity, homogeneity, number of hypo or anechoic areas, measure of the biggest hypo or anechoic area, location of the an (hypo) echoic areas in the gland, calcification, posterior border and measure of the gland.

When static images were analysed, the highest agreement was reached in scoring the heterogeneity of parenchyma defined as the presence of hypoechoic/anechoic areas with or without hyperechoic bands. The acqui-
The authors showed that the agreement between SGUS and parotid as well as labial gland biopsies was good but was slightly higher for the former (40).

**Salivary gland ultrasonography as a prognostic tool**

In medicine the identification of activity and damage biomarkers is an essential issue in research and clinical practice and in pSS, SGUS is a promising imaging biomarker with a recently established role for diagnostic purpose, yet to be proven as a prognostic tool (45). Focusing on SGUS as a prognostic tool in pSS patients, three important issues need to be addressed: a) the identification and differentiation of active inflammatory lesions (theoretically reversible with therapy) and damage related lesions; b) the correlations of US lesions with histological findings; and c) the sensitivity to change of US glandular lesions in prospective cohorts of pSS patients during treatment. Actually, the majority of SGUS studies have a diagnostic purpose, mainly focussing on hypoechoic areas that are the main pathological features of pSS and considered a marker of activity (25, 43, 45). These lesions are easy to detect, have an optimal specificity for diagnosis and for this reason became pivotal elementary lesion for diagnostic scores. Since salivary glandular damage is the cause of dryness in pSS patients, the ultrasonographic imaging of the amount of the glandular damage could be essential for evaluating the response to therapy. In pSS the damage could be found particularly in glandular tissues, mainly in salivary glands and fibrosis is the most common consequence of tissue damage. The major cause is related to tissue inflammation and depends on the degree and duration of disease activity, nevertheless, theoretically other causes of damage could be suspected, e.g. individual predisposition to fibrosis, or as results of the neoplastic process. Currently, the damage could be visualised mainly as an increased echogenicity due to hyperechoic bands, probably related to glandular deposition of high-density fibrous tissue, and only partly as a reduction of glandular size (43). However, the evaluation of hyperechoic bands is still challenging as their detection shows low intra and inter-reliability and, furthermore, their development could also be ascribed to physiological glandular damage accrual (43). Moreover, the

### Table I.

<table>
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<tr>
<th>Authors and year</th>
<th>n. pts</th>
<th>Aim of the study</th>
<th>Sp (%)</th>
<th>Se (%)</th>
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<tbody>
<tr>
<td>Song et al. (2014) (24)</td>
<td>488</td>
<td>Meta analysis on the diagnostic accuracy of US compared to sialography</td>
<td>81.5</td>
<td>77.4</td>
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<tr>
<td>Hammenfors et al. (2014) (44)</td>
<td>97</td>
<td>Correlation between US score and clinical, laboratoristic and histological parameters</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Theander et al. (2014) (37)</td>
<td>162</td>
<td>Diagnostic accuracy and prognostic value of SGUS</td>
<td>98</td>
<td>52</td>
</tr>
<tr>
<td>Luciano et al. (2015) (38)</td>
<td>109</td>
<td>Diagnostic accuracy in SS and UCTD patients</td>
<td>96</td>
<td>65</td>
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<tr>
<td>Baldini et al. (2015) (39)</td>
<td>107</td>
<td>SGUS in pSS early diagnosis</td>
<td>98</td>
<td>66</td>
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<tr>
<td>Mossel et al. (2017) (40)</td>
<td>103</td>
<td>Comparison SGUS with major salivary gland biopsy</td>
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In particular, the work by Theander et al. in a cohort of 105 pSS patients found that SGUS correlated with ESSDAI, CD4-T cell lymphopenia and the presence of more germinal centre-like structures in salivary gland biopsy findings (37). Similarly, Mossel et al. compared the validity of SGUS with parotid and labial gland biopsy outcome. The authors showed that the agreement of all items in real time showed lower κ-values with a inter and intra-observer reliability from moderate to fair for both parotid and submandibular glands, but once again the two most reliable SGUS items were echogenicity and homo-geneity (43).

The diagnostic value of SGUS has been proved also in the differential diagnosis with other autoimmune disease. Wernicke et al. (31), Corne et al. (36) and Luciano et al. (38) confirmed the good performance of SGUS in differentiating pSS from patients affected by secondary SS or other inflammatory rheumatic diseases and undifferentiated connective tissue diseases. Additional studies have demonstrated a good correlation between SGUS inhomogeneity, patients’ serology, clinical features and minor salivary gland focus score (44).

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<tr>
<td>De Vita et al. (1992) (25)</td>
<td>27</td>
<td>SGUS diagnostic accuracy</td>
<td>84.6%</td>
<td>88%</td>
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<tr>
<td>Niemela et al. (2004) (29)</td>
<td>81</td>
<td>Comparison SGUS with parotid MR and MR sialography</td>
<td>94</td>
<td>78</td>
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<tr>
<td>Hocevar et al. (2005) (30)</td>
<td>218</td>
<td>SGUS diagnostic accuracy</td>
<td>98.7</td>
<td>58.8</td>
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<tr>
<td>Wernicke et al. (2008) (31)</td>
<td>316</td>
<td>SGUS diagnostic accuracy</td>
<td>93</td>
<td>48</td>
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<tr>
<td>Salaffi et al. (2008) (20)</td>
<td>156</td>
<td>Comparison SGUS with sialography and scintigraphy</td>
<td>83.5</td>
<td>75.3</td>
</tr>
<tr>
<td>Takagi et al. (2010) (32)</td>
<td>360</td>
<td>Comparison SGUS with sialography</td>
<td>73</td>
<td>82</td>
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<tr>
<td>Milic et al. (2009) (33)</td>
<td>135</td>
<td>Comparison SGUS with minor salivary gland biopsy</td>
<td>90.8</td>
<td>87.1</td>
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<td>Milic et al. (2012) (34)</td>
<td>190</td>
<td>Comparison SGUS with scintigraphy</td>
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<td>91.4</td>
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<td>158</td>
<td>SGUS diagnostic accuracy</td>
<td>95</td>
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<td>Corne et al. (2014) (36)</td>
<td>101</td>
<td>Diagnostic performance of SGUS + 2012 ACR criteria</td>
<td>87.5 (SGUS) &amp; 89.3 (SGUS+ACR criteria)</td>
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authors of the TRACTISS study have recently suggested that hypoechoic areas, possibly for their highest grades, might also reflect a combination of damage and inflammation (46). On the basis of the above data, it seems significant that the differentiation of US lesions of activity and damage lesions still needs a great deal of research: starting from a correlation with other imaging techniques (e.g., MRI), histological findings of either major and minor salivary glands and the continuing evaluation of new scores and different US techniques (e.g., automated medical image segmentation techniques, sonoelastography). Lee han et al. have recently demonstrated that fibrosis is part of the pSS process and not only a consequence of aging. Furthermore, they highlighted that minor salivary gland fibrosis was positively associated with biopsy focus score but not with patient-reported disease duration, suggesting a close relationship between lymphocytic infiltration and fibrotic tissue replacement (47).

These data strongly suggest that tissue fibrosis in pSS is a common consequence of chronic inflammation, thus supporting the desirability to identify imaging biomarkers of irreversible damage. Up to now, two major studies have evaluated the responsiveness to treatment of SGUS, both trials being on the efficacy of rituximab compared to placebo in pSS. In the TEARS, a randomised controlled trial comparing rituximab vs. placebo, twenty-eight patients with pSS were enrolled and underwent SGUS before treatment and 6 months later. SGUS showed a greater improvement in parotid gland echostructure (i.e. defined abnormal echostructure as the typical heterogeneous gland characterised by a honeycomb appearance with hypoechoic or anechoic areas) in the rituximab group than in the placebo group. In contrast, rituximab therapy did not significantly change salivary gland size or vascularisation (48). These results seem to support for the first time the reversibility of some of the salivary gland changes and suggest which US lesions could reflect disease activity rather than glandular damage. Recently, in another randomised controlled trial comparing rituximab with placebo (TRACTISS study) the authors demonstrated a statistically significant improvement in a total ultrasound score of SGUS after rituximab therapy compared with placebo, but unlike the TEARS study, the improvement was only attributed to the definition domain, associated with the visibility of the salivary gland posterior border (46). In conclusion, at present, SGUS cannot be considered as a prognostic biomarker, probably because of the need to identify the correlations between histological features and SGUS lesions, and consequently clearly stratify active and damage salivary glands lesions. Furthermore, in our opinion, the improvement in differentiating between glandular inflammatory/lymphoproliferative activity and damage, as evaluated by SGUS, is of primary importance, and should be more properly evaluated in separate dedicated scores in additional investigations.

**Magnetic resonance imaging of the salivary glands**

In the '90s, the role of MR imaging in the diagnosis of pSS was assessed in great detail. In the beginning of the '90s it became clear that ultrasound, thanks to its high spatial and contrast resolution in superficial organs such as parotid and submandibular glands, was the imaging modality of choice in this clinical scenario. Nonetheless, changes in major salivary glands of patients with pSS have an MR imaging correlate, which is interesting to understand. In the early stages of the disease, the oedema caused by active inflammation results in glandular enlargement, which can be observed with MR imaging. Damage progression results in lobular destruction, associated with deposition of fibrous tissue and fat. This pathological condition has an MR imaging correlate: lobular destruction results in diffuse micro- and/or macro-cystic changes, which on T2 weighted images with inversion recovery or fat-sat technique are recognised as hyper-intense areas. On T1 and T2 weighted images without fat-suppression techniques, the typical feature of salivary glands is the so-called salt-and-pepper appearance, consisting in small areas of hypo- and hyper-intensity. Heavily T2 weighted images can provide sialography-like, non-invasive information. With regards to diffusion-weighted imaging, some authors have correlated changes in Apparent Diffusion Coefficient (ADC) with the severity of glandular damage (49-52). In addition to diffuse changes in salivary glands, it is well known that pSS significantly increases the risk of parotid lymphoma (53). This information is of primary importance in the case of pSS patients with a dominant, painless mass in a parotid gland. However, other benign and malignant lesions of major salivary glands do exist. In all these cases, MR imaging features are non-specific, with significant overlap between benign and malignant lesions. On non-enhanced T1 weighted images, almost all masses are hypo-intense compared to salivary glands, and on contrast-enhanced images almost all salivary gland neoplasms enhance. On T2-weighted images, the general rule is that hyper-intense masses should be benign, while intermediate and hypo-intense lesions should be malignant; however, this information is not reliable enough to adequately diagnose a single mass in a single patient (54, 55). Even morphologic features, such as shape and margins, exhibit considerable overlap between benign and malignant lesions (56, 57). Therefore, in the case of a dominant, painless mass in a parotid gland of a patient affected by pSS, cytological or histological assessment is mandatory. The specimen should be obtained under ultrasound guidance: current evidences suggest that histological assessment has superior diagnostic potential and it is safe, since the direct observation of the principal vessels enables the location of the facial nerve branches. Seeding can occur both after cytological or histological percutaneous assessment, but the risk is small and apparently does not justify the surgical excision of the biopsy tract, routinely (58). However, even if it is not useful in the diagnosis in cases of painless masses in the parotid glands, MR imaging can be
useful in local staging, i.e. in the assessment of eventual perineural spread and infiltration of adjacent structures, especially deeper ones.

Image segmentation of salivary gland ultrasound

Image segmentation analysis of SGUS pictures should provide the possibility to evaluate and score SGUS lesions automatically, based on computer algorithms analysis and computer training with SGUS standardised images provided by expert sonographers. In this way SGUS evaluation may be used by all clinicians, although the expert sonographer will remain in any case the key referent for the final SGUS evaluation (e.g. controlling and validating the final image segmentation result).

This novel field of investigation in pSS was originally proposed within the HarmonicSS project of joint European research, Horizon 2020 (http://harmonicss.eu/). As recently highlighted, reliability still remains a challenge for SGUS in pSS; thus, image segmentation analysis could represent an important step beyond (45, 59). Image segmentation analysis studies objective parameters such as the pixel values of the images; then the extracted features are used to develop artificial intelligence (AI) algorithms for texture analysis to assist human experts in scoring SGUS lesions and to overcome human-dependent assessment of echostructural parameters of SGUS (45, 60). Segmentation, reconstruction and scoring of salivary gland (SG) involvement in pSS from SGUS images remained as underestimated topics among computer-aided diagnosis (CAD) community. Previous studies were not focused on pSS assessment, but they proved the efficiency of SGUS texture characterisation to evaluate various SG diseases. The aim of texture-characterisation analysis is to use texture features to develop validated systems to discriminate between healthy and pathological salivary glands (61, 62). For example Chikui et al. 2005 suggested using the fractal analyses to characterise SG tumours (62) and Siebers et al. performed multi-feature tissue characterisation to differentiate malignant and benign parotid gland lesions using maximum likelihood supervised classifier (63). Murakami et al., applied 2D wavelet analysis to SGUS images for the diagnosis of pSS, reaching the diagnosis sensitivity and specificity of 78% and 95%, respectively (64). In the recent two-centric study, the authors evaluated a series of radiomics-based AI algorithms for the assessment of pSS from SGUS using the De Vita scoring system (25) (personal observation by Vukicevic AM, Milic V, Andjelkovic-Cirkovic B et al.) Among the considered algorithms, the best training and test performances were obtained by using the following classifiers: K-nearest Neighbour (k=0.74/0.56), Multilayer perceptron (MLP) (k=0.80/0.70) and Random forest (k=0.79/0.56). Among these, the MLP classifier showed the best accuracy for SGUS lesions grading. Moreover, a couple of studies investigated the possibility of using the elastography techniques to diagnose pSS in SGUS (65, 66) by mapping the elastic properties and stiffness of soft tissue from images (11). Overall, currently there is a lack of automated methods for the segmentation and reconstruction of SGUS images in pSS. Further development of a dedicated computerised software tools may significantly advance the diagnosis of pSS from SGUS by reducing the screening time and dependency on experts. Their current lack may be justified with a few facts: first, most of the previous studies have been performed independently using relatively small cohorts (up to 100–200 patients), which are separately insufficient for the development of a robust automated procedure. Moreover, it is difficult for a single institution to collect a sufficient number of pSS patients appropriate for the training of robust AI based algorithms with balanced distribution of pSS-grade classes, sufficient amount of data, with no outliers, etc. Furthermore, it is advisable to do an analysis of the data from different pSS cohorts. In order to overcome these obstacles, leading SS experts have recently started the HarmonicSS initiative with dedicated workpackage and task, with the aim of enveloping independent cohorts and metacentric data for the proper development of image segmentation for SGUS in pSS (67). With the course of HarmonicSS, given the further increase of data from different European cohorts of patients, and improvements of AI methods used, image segmentation in SGUS will be likely shown to greatly help SGUS itself as an effective imaging tool for pSS clinical and research issues.

Conclusions

The existing literature and ongoing research have had a common leitmotiv in dealing with the challenge of capturing disease activity and damage in major salivary glands, the key pSS target organs. The acquisition gained in the past has paved intriguing avenues for novel imaging modalities. It is likely that in the near future an improved evaluation of the morphological changes of salivary glands may lead to having valid instruments to ameliorate pSS diagnostic work-up, assessment of patients and therapeutic follow-up.

References

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