The lacrimal gland in Sjögren's syndrome: can we unravel its mystery using ultrasound?

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ABSTRACT

According to a recent survey of patients with the autoimmune disease primary Sjögren's syndrome (pSS), dry eye symptoms are present in 95-98% of pSS patients. As one of the most disabling symptoms mentioned by pSS patients, dry eyes have demonstrable effects on quality of their life, leading to eye dryness, itching, and pain, with some patients describing as a recurrent sensation of sand or gravel in the eyes. The symptoms are matched only in prevalence by dry mouth and chronic fatigue. In contrast to the prevalence of dry eye symptoms in pSS and their burden on pSS patients, our comprehension of dry eye disease development is minimal; specifically how function of the tear-fluid producing gland the lacrimal gland (LG), manifests. The comparison becomes stronger again when we consider what we know about dysfunction of the salivary gland (SG) in pSS, for example the appreciation of the transcriptome of 'innately activated' B cells invading the SG, their complicity in formation of lymphoepithelial lesions, and the ability of the SG epithelium to actively contribute to the inflammatory milieu. The exploration of ultrasound imaging as an additional modality to garner information about SG dysfunction in pSS has opened many doors for non-invasive, repeatable imaging in pSS. Here we summarise SG histology and ultrasound phenotype briefly and then juxtapose this with available studies examining LG pathology and ultrasound, and our understanding of LG dysfunction in pSS.

Introduction

In order to place the current literature regarding the lacrimal gland in a relevant framework, we will first begin by recapping the current state of knowledge regarding salivary gland pathology and ultrasound in pSS.

The salivary gland in pSS

The pathology of the salivary gland in Sjögren's syndrome

The volume of both 'whole mouth' saliva, that is the combination of saliva produced by all SGs working in concert, and that of saliva specific to particular salivary glands, is decreased in pSS patients, compared to sicca controls (1-4). Lymphocytic infiltration invades the SGs, but the extent of this infiltration appears poorly correlated with the degree of salivary gland function (5, 6). Lymphocytic infiltration invades the major and minor SGs in pSS patients congregating in 'foci' around the SG striated ducts. The infiltrate consists mostly of $CD4^+$ T cells and B cells, although other immune cells, including (but not limited to) myeloid dendritic cells, plasmacytoid dendritic cells (pDCs) and follicular dendritic cells, might also be present (7). Particular attention has been paid to the role of (glandular) B cells in pSS SG pathogenesis. B cells can form germinal centers, located within the foci, and roughly one-quarter of salivary glands of pSS patients have germinal centres (8). Whether autoreactive memory B cells are generated at these sites has been suggested, but formal proof is lacking. B cells also develop within the inflamed tissue towards IgG secreting plasma cells, including plasma cells secreting autoreactive antibodies. Since in unaffected glands the vast majority of plasma cells express IgA antibodies, the presence of IgG antibodies has been considered as characteristic feature for pSS. B cells can enter also the ductal epithelium leading to proliferation of the ductal epithelial cells, finally resulting in lymphoepithelial lesions that can

occlude the ducts. Infiltrating B cells express the Fc receptor-like protein 4 (FcRL4) and exhibit an activated phenotype (high expression of *ITGAX*, *TBX21* and TACI and low expression of *CXCR5)* (9). Finally, glandular B cells in pSS are held responsible for the formation of pSS-related mucosa-associated lymphoid tissue (MALT) lymphoma and we have postulated that lymphomatous B cells originate from the intraductal epithelial B cell (1, 7). Crucially, the extent of SG infiltration is pSS is poorly correlated with the functionality of the SGs, with SG dysfunction apparent prior to substantial lymphocytic invasion (1-4, 7). In addition to infiltration with immune cells, the acinar cells also display pathological features, such as the aberrant expression of aquaporin 5 and laminin.

Ultrasound and the salivary glands in Sjögren's syndrome

Ultrasonography is a non-invasive diagnostic technology that uses sound waves to create images inside the body. Transducers producing ultrasound waves detect the echoes reflected back from the body, producing electrical signals used to create two-dimensional representations of tissues and organs depending on the degree of reflected echos. Ultrasound is simple and easy to use in daily clinical practice (10-12). Since Bradus *et al*. (13) first attempted to visualise inflammatory changes in the SGs of 6 suspected pSS patients with ultrasound in the 1980s, the use of ultrasound in assessing SG lesions has developed significantly. In the early stages of the development of salivary gland ultrasound (SGUS) in pSS (14, 15), studies suggested that the most sensitive indicator of salivary gland involvement in pSS was parenchymal inhomogeneity (16-19). Most studies of SGUS in pSS now support the observation that presence of hypoechogenic areas in the SG parenchyma is the most prominent indicator of SG involvement in pSS. Other studies included echogenicity of the parenchyma (in comparison to echogenicity of the thyroid parenchyma or healthy surrounding muscles), SG volume, maximum dimensions of hypoechoic area, visibility of the posterior boundary, the presence of areas of decreased echogenicity, hyperechoic bands with or without consequential shadowing, and the presence of normal or pathological intraglandular/extraglandular lymph (12, 20-23). Several studies have shown that including SGUS in the pSS American College of Rheumatology (ACR)-European League Against Rheumatism (EULAR) classification criteria can improve performance, diagnostic accuracy, sensitivity (64.4% *vs*. 84.4%) and feasibility, with a slight decrease in specificity (91.1% *vs*. 89.3%) (12, 24-27). Some studies also show SGUS can replace the ocular staining score (OSS), Schirmer's test, or unstimulated whole saliva flow rate in the classification of pSS (12). Mossel *et al*., on the other hand, argue that the histology, salivary flow, and ultrasonography assess separate structures, and that the 3 measures cannot be used interchangeably in the diagnosis of pSS (5). In summary, although strides have been made towards the standard employment of ultrasound in evaluating SG pathology in pSS, consensus over exactly what we are measuring is still wanting.

Ultrasound scoring systems for the salivary glands in Sjögren's syndrome

A recent meta-analysis has identified 33 different scoring systems used to evaluate the major SGs in patients with SS. Most of them evaluate parenchymal echogenicity, homogeneity, presence of hypoechogenic areas, hyperechogenic reflections and clearness of the LG border (28-32). There are several semi-quantitative scoring systems available for mapping the morphological changes of the SG in pSS. Among them, the most common one used is the scoring system developed by Hocevar *et al*. (31) in 2005, whereby the ultrasound score is calculated by summation of the grades for the five parameters listed above, for left and right parotid and submandibular SGs. The final score ranges from 0 to a maximum of 48. A score higher of 15, 16 or 17 according to different studies is compatible with pSS (33). The scoring system published by the OMERACT SGUS task force group is also widely used in clinical practice. This new four-grade semi-quantitative score showed excellent agreement intra-and inter-reader agreement results. In an attempt to increase the feasibility of SGUS in pSS clinical practice, Mossel *et al*. suggested that examination and scoring only hypoechogenic areas of parotid and submandibular glands on one side is sufficient to predict classification of patients according to the ACR-EULAR criteria (34). Considerable groundwork has thus already been laid with respect to optimisation of SGUS in pSS, and in theory, its clinical application as standard component of classification criteria can be anticipated in the near future.

(Power) colour Doppler ultrasound and the salivary gland in Sjögren's syndrome

Colour Doppler ultrasound (CDUS) detects local blood flow by converting the Doppler signal into a color and superimposing with two-dimensional black and white sonograms. A resistive index (RI) is generated, with a lower RI being indicative of inflammatory vasodilation of arteries (35-37). In healthy patients, a significant difference in RI is observed after stimulation. Interestingly, there are no significant changes in RI in pSS patients before and after stimulation, suggesting perhaps that SGs of pSS patients as baseline already display signs of vasodilation (38). One step further in CDUS, encoding of the power in the Doppler signal in colour, power doppler ultrasound (PDUS) provides better sensitivity, edge definition, and depiction of flow continuity than CDUS alone, but remains to be applied to the SG in pSS (37, 39, 40).

The lacrimal gland in pSS

The pathology of the lacrimal gland in pSS

Dry eye symptoms, as inferred from either an OSS of ≥5 measuring corneal damage or Schirmer's test result measuring tear-fluid production of ≤5mm in 5 minutes, are present in 95-98% of pSS patients. Studies suggest that dry eyes precede pSS diagnosis by up to 10 years, with the consequence that studies detailing temporal changes in tear production in pSS are not easy to find (41). Tear fluid of pSS patients contains

increased levels of the IL-1α, IL-1β, IL-1rα, IL-2, IL-4, IL-8, IL-12p70, IL-17A, IFN-γ and CXCL10/IP-10 cytokines/chemokines when compared to non-SS sicca or healthy controls patients (42, 43). Curiously, and in contrast to what we know about the SG and saliva in pSS, TNF-α was not increased in tears of pSS patients compared to healthy control (42). Levels of IL-1α, IL-2, IL-4, IL-8, IL-12, IL-17A, and IFNγ in tears correlated with clinical parameters of reduced tear production, less stable tear film, greater ocular surface damage (43). Cathepsin S (CTSS) in tears, a protein with a critical role in antigen presentation, has been suggested as a biomarker for SS, with mean CTSS activity from tears being on average 4.1-fold higher than non-SS autoimmune disease patients, and not correlated with Schirmer's test results or circulating SSA or SSB antibodies (44).

Owing to the technical difficulties and risk of complications, histopathological studies of LGs in pSS patients are very limited. Reflecting this, to date only the Japanese criteria includes a role for the LG biopsies in pSS patient classification (45). The majority of studies examining the pathology of the LG in pSS are relatively old, include few patients, and involve individuals with dry eye conditions other than pSS. Patients with LGs demonstrating poor reflex tearing were more likely to have systemic autoantibodies and lymphocytic infiltration of the LGs consistent with pSS (46). According to the available research (42), and also to our observations (Fig. 1), B and T lymphocytes and other immune cells infiltrate the LG in pSS patient, identical to the SG. Similarly, studies of LG immune infiltration in pSS have reported a composition dominated by B cells and both $CD4^+$ T helper and $CD8^+$ T cells (47). T cell receptor (TCR) β usage of infiltrating $CD4^+$ T cells in LGs of SS is diverse, with no identical clone shared by LGs, SGs, or peripheral blood mononuclear cells within the same patient, although T cell clones may be directed against the same autoantigen in general (48, 49).

Lack of function of the LG dictates that a degree of damage of the LG epithelium must occur in pSS. The tear-producing parenchyma of the LG are the acinar cells, tubular in nature. Destruction of the tubuloacinar architecture of LG tissue has been reported in pSS and has been suggested to appear secondary to infiltration of B cells and T-helper cells (47), different to the SGs where lack of gland function appears to begin pre-lymphocytic infiltration. In addition to T helper cells ,that constitute the majority of T cells, a potential role for both major pathways employed by cytotoxic T cells to induce acinar cell death has been suggested to be active in the LG in pSS. Firstly, binding of integrin $\alpha_E\beta_7$ on $CD8^+$ T cells to E-cadherin on LG acinar cells may induce acinar cell death via the Fas-Fas ligand system (50). Secondly, perforin and granzyme B pathway expression was shown to be upregulated extracellular to acinar cells of the LG in pSS, presumed to emanate from $CD8^+$ T cells, and potentially playing a role in the induction of LG acinar cell apoptosis (50, 51). In terms of trying to explore the order of tissue damage in pSS, Xuan *et al*. described findings in the histological changes in major SGs and in LGs in mouse models of SS. At ages of 3, 6, 9, 12, 15, and 18 months in both sexes (52), early inflammation concurrently occurs in submandibular SGs and LGs, around the age of 6 weeks in these mice. The parotid SGs were involved much later in the course of SS, as the inflammation could be seen from week 9 and was less severe overall. If validated in pSS patients, this interesting phased involvement of the SGs and LGs may be an extremely useful tool central to capture pSS patients early in disease progression, and increase the chances of being able to halt or reverse disease progression (45).

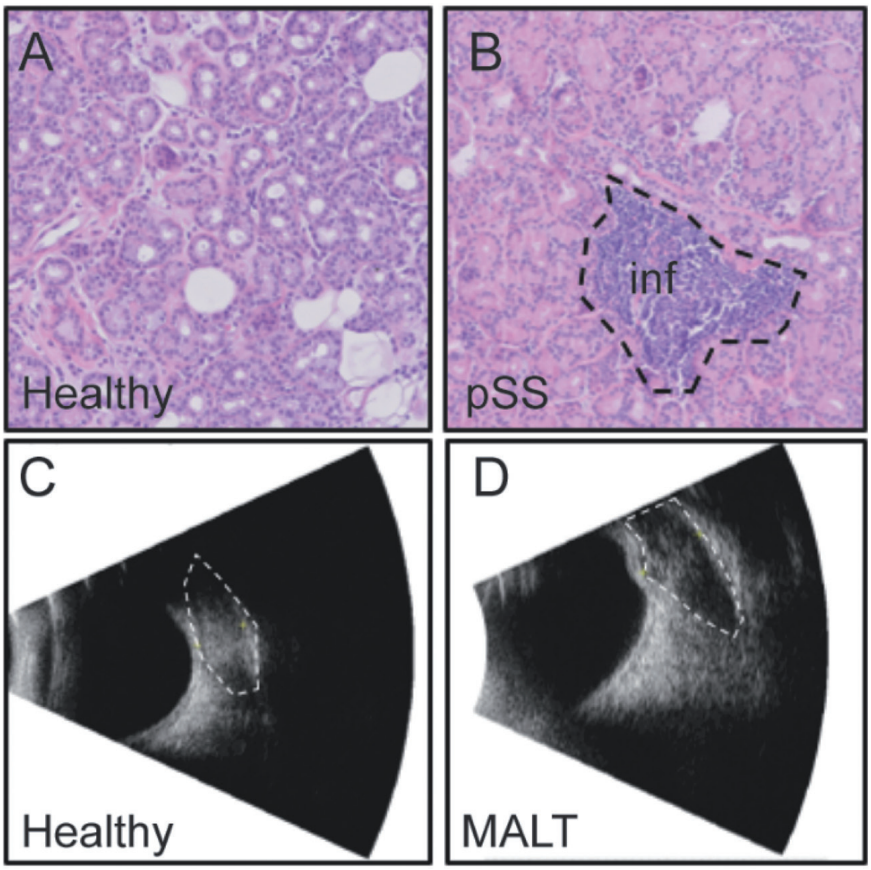

Fig. 1. Visualising abnormalities of the lacrimal gland via histology and ultrasound imaging. **A**: H&E-stained section of LG from healthy patient. **B**: H&E-stained section of LG from pSS patient. **C**: Ultrasound of healthy LG. **D**: Ultrasound of LG from patient with a MALT lymphoma in the LG. inf: infiltrate; dashes white lines: outline of LGs.

Ultrasound and the lacrimal gland in Sjögren's syndrome

A small number of studies have examined abnormalities of LGUS fibrous

Table I. Ultrasound characteristics of LGs in pSS patients.

Date	author	Type	number of pSS patients	control	Characteristics
2021	X. Liu *et al*. (62)	case-control study	51	60 pSS (without elastography)	higher Emean
2019-2020	O. De Lucia *et al*. (54)	case-control study	17	57 (health)	glandular parenchyma inhomogeneity, fibrous gland appearance
2012	A. Seceleanu *et al*. (55)	case report	1	___	enlarged masses of a cystic structure
2000	F. Giovagnorio *et al*. (53)	case-control study	15	15 (health)	oval shaped hypoechoic structures, larger than normal
1996	A.T. Ahuja *et al*. (56)	case report	1	___	reticulated appearance with intervening hypoechoic septa.

gland appearance, inhomogeneity, enlarged masses of cystic structures, and/or reticulated appearance (Table I), but so far we are still unaware of the relationship between LGUS characteristics and LG histology. The only case-control studies in the field of LGUS in pSS were performed by Giovagnorio *et al*. (53) and by De Lucia *et al*. (54). Giovagnorio *et al*. studied LGUS in 15 healthy controls and 15 pSS patients. According to their research, LGs can be visualised bilaterally in part of pSS patients (6 of 15) and appeared as oval-formed hypoechoic structures which had a larger size than healthy controls. The remaining 9 pSS patients had invisible glands (53). De Lucia *et al*. analysed LGUS in 17 patients with pSS and 57 healthy individuals and showed that for homogeneity and fibrous presentation, there were significant differences between the two groups, with poor homogeneity and fibrous glandular presentation in the pSS patient group, but no significant differences in glandular size, hyperechoic spots, and hypoechoic areas (54). Some additional case reports noted that LGUS in pSS patients, the glands were lobulated in outline with the parenchyma divided into multiple, small, relatively echogenic lobules separated by well-defined, or had enlarged cystic structures in the lacrimal fossa (55, 56).

Lack of ultrasound scoring systems for the lacrimal glands in Sjögren's syndrome

The reliability of SGUS has been extensively examined and determined to be highly reliable in assessing patients with pSS (57-59). There is, however, still no study exploring the reliability of LGUS in patients with pSS. One study (54) conducted a web-based reliability evaluation on images of healthy individuals, and suggested good inter and intra-rater reliability of LGUS. As for the intra-rater reliability, each reader involved in assessing the ultrasound images scored each patient in two rounds, and the result showed excellent reliability regarding glandular parenchyma visibility (with an agreement of 95%), glandular size (94%), homogeneity (92%), hypoechoic areas (94%), hyperechoic spots (95%), fibrous gland appearance (99%), and fatty deposition (98%) with high agreement. The inter-rater reliability was excellent for the hypoechoic areas in the first round (84%), the hyperechoic spots (86% and 85%) and fatty deposition (90% and 90%)in both rounds, and was good regarding glandular parenchyma visibility (79% and 77%), homogeneity (71% and 72%), and size (80% and 79%) in both rounds. The inter-rater also presented an excellent agreement for hypoechoic areas in the second round. Considering fibrous gland appearance, the agreement for both rounds was moderate (51% and 51%). The study of De Lucia *et al*. presented a preliminary demonstration of the inter and intra-reliability of LGUS, while further studies are needed to explore the inter and intra-reliability of LGUS in patients with Sjögren's syndrome assessed by LGUS.

(Power) colour Doppler ultrasound in the lacrimal glands in pSS

As for LGs, PDUS was used in one study and demonstrated a significant difference between pSS and healthy controls in intraglandular branches of the lacrimal artery (53). CDUS might also represent an interesting imaging technology to evaluate vascularisation and inflammation characteristics of a possible lesion in the LGs of patients with pSS. In the same research, CDUS of pSS patients showed scarce diastolic flow and therefore the RI was higher than normal (53). Considering its application in observing blood flow, P/CDUS could be future useful imaging techniques to assess the activity of pSS and response to treatment (60). However, so far there are still no studies evaluating the validity of Doppler evaluating strategy for the assessment of LGs involvement.

Conclusions and further research

Much of the volumetric and structural information required by clinicians when assessing the LGs may be obtained noninvasively via ultrasonography. Changes of echotexture, fatty infiltration or atypical lymphocytic proliferation due to lymphoma, can in general be identified using US. Consensus remains to be reached within the field of SGUS as to what exactly the hypoechoic regions represent, which appear to characterise the SG in pSS.

Broadly speaking, however, ultrasonography is a viable technology for monitoring morphologic, volumetric, and structural changes that may occur during disease progression, either as a result of therapy or as a possible degeneration of long-standing inflammation. In addition, LGUS is a feasible, easy to perform, non-invasive, with a good inter and intra-rater reliability and low cost, then it could be the imaging exam

of choice in front-line clinical practice to evaluate LGs lesions in pSS patients. Supporting this notion, enlargement of the LG, in one study, was associated with lacrimal involvement in pSS (lymphocytic infiltration with a 100% specificity) (61).

Despite these encouraging first steps, there are still significant challenges to overcome in LGUS. Specifically, the current studies include very limited ultrasound features. Many ultrasound characteristics such as margin definition, parenchymal echogenicity have not been addressed. The relationship between LGUS features and LG pathology, and indeed tear production still needs to be further explored. We have recently shown in the salivary gland of pSS patients that ultrasound, histology and function are 3 independent measures, depicting apparently distinct pathological events in pSS (5). If this also applies to the LG with relation to Schirmer's tests or the OSS test remains to be investigated, as indeed does that between SGUS and LGUS of the same patient. From the perspective of clinical application, the potential role of LGUS as a predictive tool of disease progression, a guide to therapeutic strategy, and as an assessment of treatment efficacy still needs to be confirmed by large well-designed randomised controlled trials. In addition, there is still no reliable or valid scoring system for LGUS and the differentiation from other diseases in pSS patients, a final hurdle that will present a roadblock in standardisation of clinical practice.

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