Review

Nuts and bolts of salivary gland pathology in primary Sjögren's syndrome

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a chronic, systemic, inflammatory autoimmune disease characterised by lymphocyte proliferation and progressive damage to exocrine glands. Salivary gland histopathology based on salivary gland biopsy is relevant for the diagnosis of pSS and therefore broadly applied in clinical practice. Tissue can be obtained from labial salivary glands (LSG) biopsy or from major salivary glands (MSG) biopsy, namely the parotid; in this latter scenario, the procedure can be either an open surgical biopsy or a US guided core needle biopsy.

In this review we will: i) present the histopathological findings that may be encountered by pathologists on biopsies from pSS patients; ii) discuss the advantages and disadvantages of the surgical and/or imaging guided procedures to obtain tissues from LSG or MSG; iii) describe the histopathological features of lymphoma of MSG in pSS patients.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic connective tissue disease characterised by a wide spectrum of clinical features, from exocrine to extra-glandular involvement (1). The exact pathogenesis of pSS is not completely understood but appears to be multifactorial and to involve both T and B lymphocytes (2-4). Infiltrates of inflammatory cells within the salivary glands (SGs) may create clusters, structured as ectopic germinal centres (GCs), which might lead to chronic stimulation and triggering of B-cells (5-7). Inflammation of the salivary and lacrimal glands is the characteristic feature of the disease, which has also been referred to as 'autoimmune epitheliitis' (8). The T-cell-mediated hyperactivation of B-cells contributes to evolve from asymptomatic conditions to systemic complications and even lymphoma development (9). The B-cell hyperactivity and the abundance of inflammatory cell infiltration into the glandular tissue place individuals with pSS at a higher risk to develop lymphoproliferative disease (10). This risk is notably the highest among a range of autoimmune conditions (11).

The glandular involvement in pSS is usually assessed mainly with biopsy and imaging, however the diagnosis relies on a set of clinical, laboratory, imaging, and pathological characteristics, since no single test can be diagnostic alone.

Labial salivary gland (LSG) biopsy is considered the gold standard tool and plays a key role in the classification criteria for pSS (12).

At present, major salivary gland (MSG) biopsy is mainly reserved to patients exhibiting clinical signs and symptoms of lymphoma development (13). Limited data are available to assess the feasibility of MSG biopsy for diagnostic purposes in pSS, primarily due to its historical underuse owing to the risk of facial nerve injury in the case of parotid biopsy and the higher surgical expertise required compared to the LSG biopsy (14, 15).

Imaging modalities to assess the MSG are currently available in pSS management. In the past three decades ago, the significance of salivary gland ultrasound (SGUS) in patients with pSS was emphasised (16). Since then, it has proven to be a valuable tool in the evaluation of structural abnormalities and tissue lesions within the MSG. Ultrasound is a non-invasive, non-irradiating, and inexpensive technique which has gained extreme interest focusing

on the evaluation of structural abnormalities and parenchymal lesions in the MSG, such as parotid and submandibular glands (17-21). SGUS can assess the typical structural abnormalities and detect parenchymal lesions of the MSG (22-24), allowing a targeted histological sampling by ultrasound-guided core needle biopsy (US-guided CNB), as recently proposed (15, 25, 26).

Furthermore, pSS is known to harbour a high risk of lymphoma development, and various biomarkers have been associated with an increased risk of lymphoproliferative disease (27). The parotid glands are the main primary sites where lymphoma usually arises, and therefore an inexpensive imaging modality to assess the glandular parenchyma such as SGUS is desirable as a screening tool. The aim of this review is to explore the histopathological examination of the major and labial salivary glands in the clinical scenario of pSS patients.

Minor salivary glands: histopathological features and pearls for an optimised sampling

Anatomic perspective

The primary function of the minor salivary glands is to moisten the mucous membranes of the upper aerodigestive tract. In humans, this function is fulfilled by the continuous secretion of numerous (600 to 1000) minor salivary glands. These glands, ranging in size from 1 to 5 mm, are located in the submucosa throughout the oral cavity, pharynx, and upper airways with the greatest density in the lips, tongue, buccal mucosa, and palate.

Salivary glands are defined as exocrine glands that secrete saliva through ducts from a secretory structure called the salivary acinus; the acinus itself can be divided into three main types: serous, mucous, and mixed. Serous acini in salivary glands are roughly spherical and are composed of pyramidal cells, with basally located nuclei surrounded by dense basophilic granular cytoplasm and secretory granules. On the other hand, mucous acinar cells are commonly simple columnar cells with flattened, basally situated nuclei and water-soluble granules that make the intracellular cytoplasm appear clear. Mixed, or seromucous, acini contain components of both types, but one type of secretory unit usually dominates. The majority of LSGs are either mucous or seromucous. Between the epithelial cells and basal lamina of the acinus lies the flat myoepithelial cells network that, with contraction, can force secretion of the acinus. The other important component of the salivary gland parenchyma is the salivary gland duct system. The acini first secrete through small canaliculi into the intercalated ducts, which in turn empty into striated ducts within the glandular lobule and then into the interlobular excretory ducts. The lobular architecture of all the salivary glands is well defined by the anastomosed connective tissue trabeculae carrying the vascular and neural branches, as well as the excretory ducts (28-30).

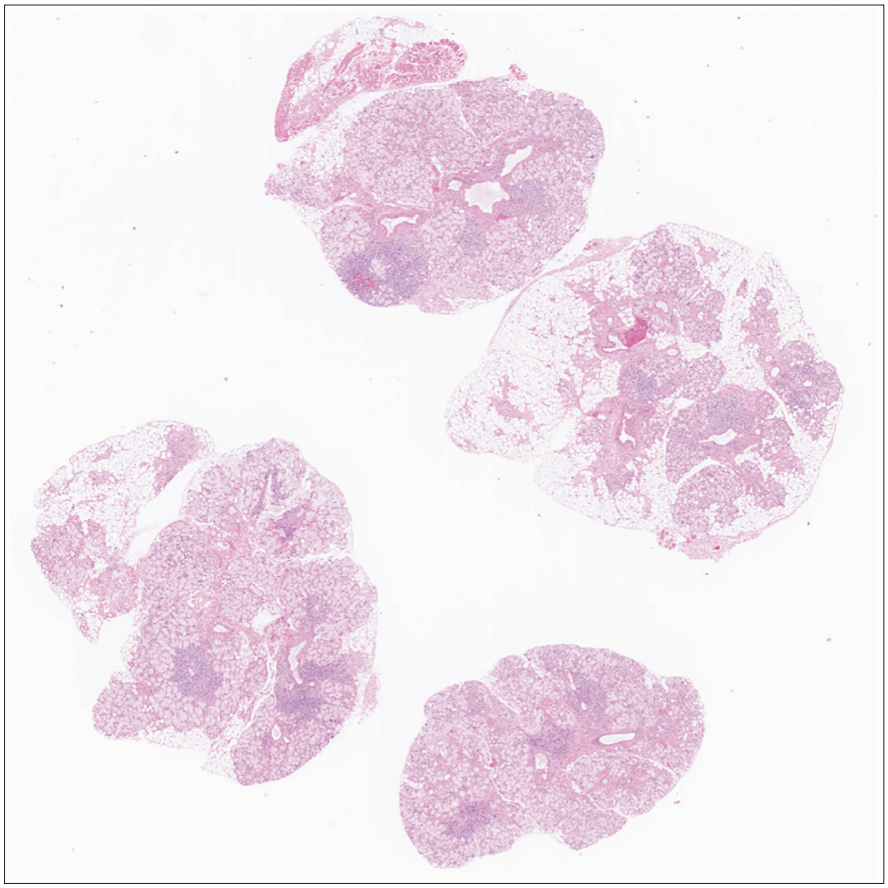

Fig. 1. Microphotograph at low magnification illustrating minor salivary gland biopsy obtained from a patient with primary Sjögren's syndrome with FLS, stained with H&E.

Tissue sampling and handling

LSG biopsy can be obtained in a day-care facility with minimal invasiveness and discomfort; the glands are easily accessible either via the buccal mucosa or through the skin. This procedure usually gives enough tissue for the diagnosis, is less invasive compared to the open surgical biopsy of the parotid gland and the possible complications are more commonly minor and transient (31).

Adequate material is essential to allow a robust and reliable analysis. According to the guidelines, the proposed minimum number of LSG is from four to six and the minimum surface area of gland sections should be 8 mm^2 (32) (Fig. 1).

Recently, ultra-high frequency ultrasonography (UHFUS) has been introduced in various medical fields due to the possibility to obtain up to 30-μm resolution imaging of structures located within the first centimetre from the surface. UHFUS has been tested as an intraoral tool in pSS and preliminary data show how ultrasonography guide may help in obtaining a higher glandular

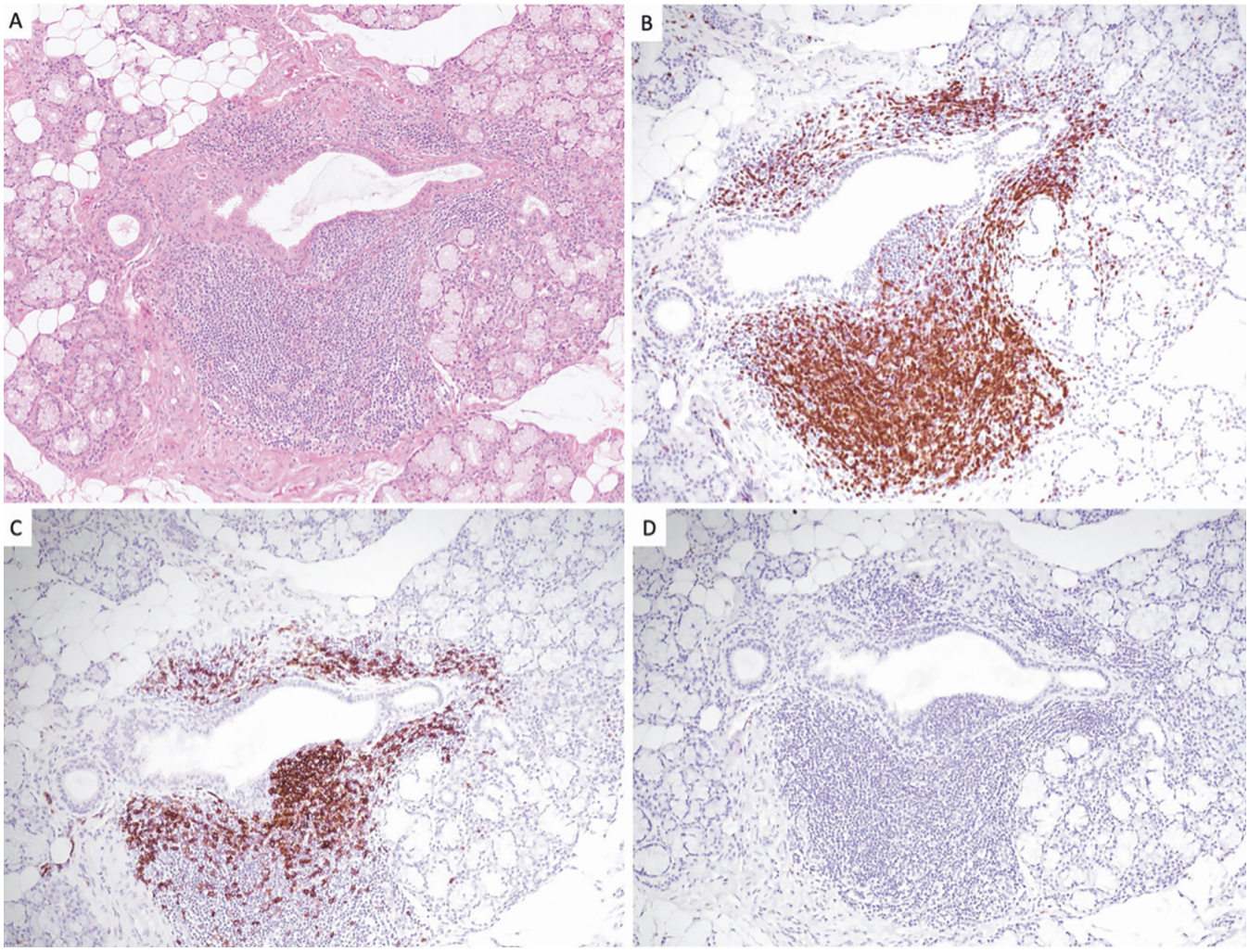

Fig. 2. A: Higher magnification of the minor salivary glands showing a lymphoid focus in a periductal location (H&E, 100x magnification). **B**, **C**, **D**: immunohistochemical staining showing the distribution of T lymphocytes (Cd3a) and B lymphocytes (Cd20a) in the lymphoid focus. The staining for CD21 is negative in this early lesion.

surface area to be examined with a good correlation between UHFUS score and LSG histopathology (33). The histological analysis remains crucial for the pSS management, however UHFUS may optimise the information obtained by LSG biopsy and may become a valid support for LSG procedure.

Biopsy can be used to confirm a pSS clinical diagnosis, at any time during the disease course to evaluate disease progression (34), or to monitor disease changes upon therapeutic intervention or during clinical trials (35).

The appropriate handling of the tissue is very important. In particular, the so-called "pre-analytical phase", which includes specimen handling issues occurring prior to the arrival time in the laboratory, is a crucial step in the pathology workflow, and must be completely and correctly managed by the clinician. To preserve tissue morphology and tissue antigenicity for immunohistochemical and molecular exams, the biopsy material must be readily placed in an adequate amount of fixative (usually 10% neutral buffered formalin); moreover, the fixation time must be controlled and standardised to avoid under- or over-fixation issues; both these conditions can affect the final diagnosis if ancillary tests are applied. In particular settings (if other exams are planned to be performed, especially for research purposes, or in centres where a tissue biobank is established), fresh biopsy material can be sent to the pathology department where part of the fragments will be snap-frozen and part processed for routine histology. Fresh tissue can also be sent for flow cytometry if a haematological disease is suspected. After fixation, the tissue fragments are routinely processed, embedded in paraffin (care should be given to preparation of paraffin blocks, with smaller glands set higher) (32), cut in 3.5–4 μm thick sections, placed on a glass slide and stained with Haematoxylin & Eosin (H&E).

Histopathological features

The salivary glands of most pSS patients are characterised by the presence of chronic lymphocytic infiltrates (the so-called lymphocytic foci), mainly composed of T and B lymphocytes with few other mononuclear cells. While T-cells predominate in mild lesions, B-cells are the most represented cell subset in the advanced lesions (36). The lymphocyte infiltration of glandular tissue in pSS is

a result of chronic antigenic stimulation from various causes involving different molecular pathways, and ultimately produces exocrine dysfunction and the sicca syndrome. Salivary gland epithelial cells in pSS provide key signals that enable the recruitment of proinflammatory chemokines and adhesion molecules thus facilitating the organisation of lymphocytic foci, and playing an active role in pSS pathogenesis (37-39). The triggers that initiate the process are not known but some of the viruses and bacteria that display specific epithelial tropism seem to be implicated in the pSS pathogenesis (40). Although a single responsible agent has not been identified, there are reports of viral involvement in support of the persistent antigenic exposure to locally recruited lymphocytes (41).

With the prolonged activation of the glands, the relatively unorganised infiltrate may transform towards more organised ectopic lymphoid structures that resemble secondary lymphoid organs with segregated T and B cell areas, high endothelial venules and follicular dendritic cell networks in which GSs may develop (5).

Since the presence of lymphomononuclear cell infiltrates in the salivary glands is a characteristic feature of pSS, the histopathological evaluation of salivary gland tissue plays a crucial role in diagnosis and classification; indeed, according to the ACR/EULAR guidelines, a positive histopathology finding is a requirement for the classification of pSS in the absence of anti-Ro/SSA antibodies (12).

The histological lesion called focal lymphocytic sialadenitis (FLS) is considered the histopathological hallmark of pSS. FLS is characterised by the presence of lymphoid foci in a periductal or perivascular glandular localisation (13, 32). A lymphoid focus is defined as a dense aggregate of at least 50 mononuclear cells, usually placed around ducts (striated or intercalated) or vessels, while the surrounding tissue is mainly composed of unaffected parenchyma (Fig. 2). Foci are composed of T and B lymphocytes, the former prevalent, arranged in a non-segregated manner, with plasma cells aligned at the periphery (32). Some plasma cells may be detected at the periphery of the largest aggregates in the glands; however, the finding of high number of plasma cells is still considered an exclusion criterion for pSS diagnosis.

The concept of Focus Score (FS), introduced by Greenspan in 1974 (42) in order to establish a histological index of severity of the salivary gland involvement in pSS, is calculated by dividing the number of foci by the total glandular surface area in mm^2 multiplied by 4 to yield the number of foci per 4 mm^2.

A biopsy is considered positive if a FS≥ 1 is detected, and this parameter is used for the classification of pSS, according to the 2002 AECG and 2016 ACR/ EULAR criteria (12, 43). The FS ranges from 0 to 12: above a FS of 10, foci are typically confluent and a maximum score of 12 is applied (35). It is currently recommended that the presence of FLS, based on strict histological features, should be determined prior to FS calculation. The whole of the glandular surface area in the denominator should be included. If the glandular tissue is below the limit (less than 8 mm^2), two additional cutting levels at 200 μm intervals should be obtained. The fragments obtained from this procedure are usually wide and well-preserved so the features of FLS can be easily identified in the parenchyma and the FS can be easily calculated. No data are available regarding how measurements are carried out except by means of an eye piece grid or, more precisely, by a measurement-validated microscope-associated software (32). In a fair number of patients, the calculated FS is <1 and these patients may be wrongly misclassified as non pSS according to the ACR-EULAR (44,45). The reason for that is that lymphoid foci may be scattered throughout the parenchyma, and there is always a risk of sampling error, especially in patients with few foci. Furthermore, lymphoid foci may also be found in healthy people: from 5% to 9% of non pSS patients have a FS>1 in their salivary gland tissue (44,46). The clinical diagnosis based on expert opinion is therefore very important in both these settings that might be confounding. According to the systematic literature review of Guellec et al. based on 9 selected studies, LSG biopsy sensitivity ranges from 63.5% to 93.7%, while its specificity comprises between 61.2% and 100%, when compared to expert opinion or classification criteria fulfillment (47). The positive predictive value and negative predictive value range from 74.2% to 100%, and 39.1% to 96.1%, respectively (47). A series of manuscripts have correlated the presence of high focus scores with indices of local or systemic disease activity. For instance, recently, Chatzis *et al*. (48) established that a high focus score (≥4) is an independent lymphoma associated risk factor and may serve as a predictive biomarker for the early diagnosis of SS-associated lymphomas.

Whilst the focus score has been proven as a functional diagnostic and prognostic tool, it presents obvious limitations. Problems in assessing FS arise when other histopathological patterns such as nonspecific chronic sialadenitis is present (49). An important drawback of the focus score is that it is only based on the number of foci and does not include the size of these foci. For a better estimation of the level of inflammation in the salivary gland biopsy the area of infiltrate should be evaluated (32). This gives a more precise indication on the extension of tissue involved in the inflammatory process. For this purpose, digital image analysis is a promising and valuable tool to improve inter-rater agreement and multicentre data harmonisation and it should be routinely implemented in the histological evaluation of LSG (50). Likely, such an approach is also more sensitive to change, in case sequential biopsies are taken for evaluation of treatment effects or follow up of disease progression (13). For these latter purposes, LSG biopsy is very suitable. The introduction of such additional measurements as well as the degree of lymphocytes organisation in order to increase the information provided by the simple focus score, is currently debated in the pSS community (51).

Historically, in addition to the FS, other histological scores have been reported in the literature to describe glandular involvement during pSS. The Chisholm

and Mason grading system considers MSGs with normal architecture (grade 0), slight and moderate infiltrate (grades 1 and 2, respectively), presence of 1 focus (grade 3), and more than 1 focus in 4 mm^2 (grade 4) (52). The Tarpley score highlights only the absence (score 0) or the presence (scores 1-4) of foci in 4 mm^2 of glandular tissue, with the higher scores identifying glands with many foci and consequent progressive destruction of glandular tissue (53). Both Chisholm and Mason's and Tarpley's grading system show limitations, but since they are still in use in many centres, the pathology report must state which system has been used.

With the progression of the disease, in approximately 20–25% of SS patients, the salivary gland aggregates undergo an organisation into segregated T- and B-cell areas, and are characterised by the formation of follicular dendritic cell (FDC) networks within areas of active B-cell proliferation defined as GC-like structures (54). GCs can be found in LSG and in the parotid parenchyma of pSS patients.

In a meta-analysis of 16 articles performed by Risselada *et al*., the authors concluded that patients with GCs are characterised by more severe disease: the presence of GC is more frequent in patients with positivity for RF and anti SSA/SSB antibodies; moreover, these patients show a higher risk of lymphoma development (55). However, the presence of GCs and their role as predictors of disease progression, needs to be clarified with further research since the studies published so far showed discrepant results (34, 35, 56, 57). The reason for that may be related to the fact that GCs are focal and scattered structures within the salivary parenchyma and their detection is affected by biopsy sampling. Even if in the literature no mention is made on the development of secondary lymphoid follicles within or adjacent to foci in the calculation of the FS, a statement regarding the presence/absence and the number of GCs should always be present in the pathology report.

Hematoxilin and eosin-stained sections are considered sufficient to detect a fully formed GC by a pathologist, although additional immunostainings can be used to better identify the lymphoid structure. CD3 and CD20 highlight the T-cell and B-cell segregation respectively within the secondary follicle and CD21 stains the follicular dendritic network in the GC (Fig. 3) (32).

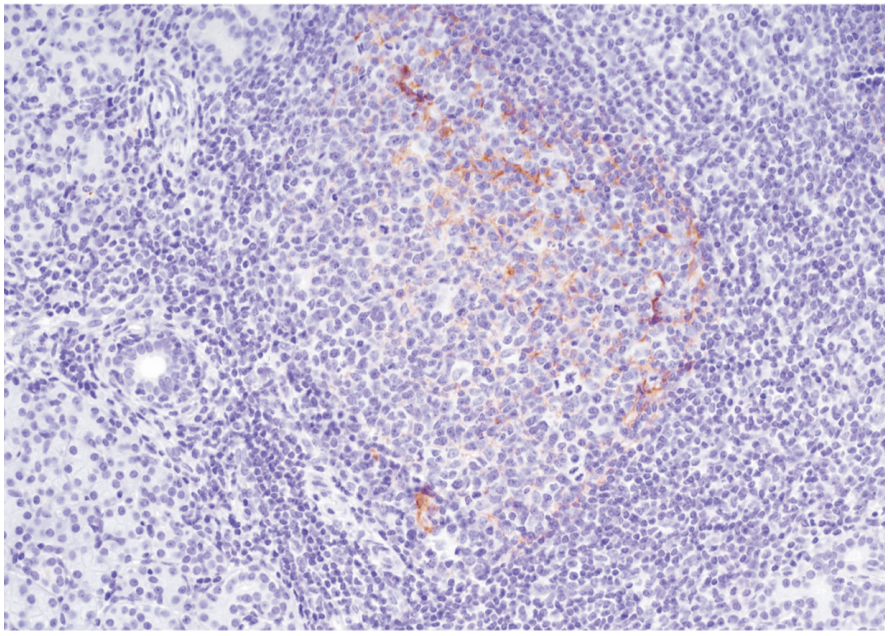

Fig. 3. CD21 immunohistochemical staining for CD21 highlights the follicular dendritic cell network inside the germinal centre.

Another histological feature that can develop in pSS patient and must be reported is the presence of the so-called lympho-epithelial lesions (LELs) or epimyoepithelial islands, characterised by a proliferation of the ductal epithelium ultimately obliterating duct lumina, associated constantly with intraepithelial lymphoid exocytosis and adjacent foci or rim of lymphocytes. Since these lesions are rarely found in the LSG compared to the parotid, it's authors opinion that LSG biopsy is not recommended when the target is the detection of LELs in pSS patients. Additional data are available further in the text.

It is important to point out that, when evaluating salivary biopsies from suspicious pSS patients, the pathologist should recognise the features of non-specific chronic sialadenitis (NSCS) (Fig. 4). NSCS is a lesion characterised by acinar atrophy, interstitial fibrosis, and ductal dilatation, along with scattered or focal infiltration of lymphocytes, macrophages, and plasma cells, which are usually not adjacent to normal acini and located in the lobules of acini (26). Those features are so common in the population and so may coexist with pSS. NSCS may also be associated with infiltration of lymphocytes and even aggregation, thus raising issues for interpretation and focus score calculation (32).

The features of acinar atrophy, interstitial fibrosis, duct dilation and adipose metaplasia should be assessed in the pathology report as absent/ present and furthermore they may be graded as mild, moderate or severe.

Major salivary glands: histopathological features and pearls for an optimised sampling

Anatomic perspective

The major salivary glands (MSG) are a crucial part of the digestive and oral health system and are known as major because of their larger size compared to the numerous minor salivary glands scattered throughout the oral cavity. The three pairs of MSG (the parotid, the submandibular, and the sublingual gland) share the same basic salivary gland unit described for the LSG, but their acini are of serous or seromucous type. MSG produce most of the saliva during meals and are primarily involved in food digestion. The parotid glands are the largest and produce a watery, se-

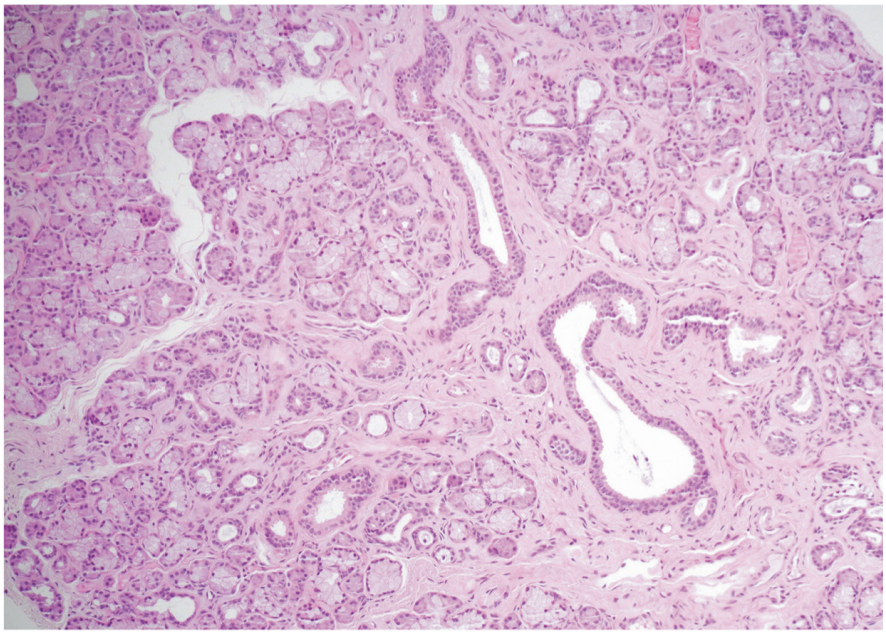

Fig. 4. Histological pictures showing features of non-specific chronic sialadenitis (NSCS) characterised by mild duct dilation, acinar atrophy and periductal and interstitial fibrosis in a minor salivary gland (H&E, 100 x magnification).

rous secretion rich in enzymes (particularly amylase). Submandibular glands produce a mixed secretion that contains both serous and mucous components, serving in the digestion and providing lubrication for the oral cavity. The sublingual glands are the smallest, and produce a mucous secretion, serving as a moisten for the oral cavity.

The three pairs of MSG share the same basic salivary gland unit described for the LSG, but their acini are of serous or seromucous type.

Sampling and handling

Nowadays, the application of MSG biopsy, especially of the parotid gland, is limited to some specialised centers, and it is mostly reserved for cases with persistent salivary gland swelling to rule out a diagnosis of lymphoma. Furthermore, salivary gland histological examination is usually undertaken to improve the diagnostic process of pSS through differential diagnosis of possible mimickers, such as sarcoidosis or IgG4-related disease, and identification of lymphoma associated to pSS.

Tissue from the parotid gland can be obtained by two different methods: the open surgical biopsy and the recently introduced technique of the ultrasound guided needle core biopsy (15, 26).

The open surgical biopsy involves making an incision and directly accessing the parotid and/or submandibular gland tissue. This procedure is typically performed by an oral and maxillofacial surgeon, head and neck surgeon, or a skilled otolaryngologist, and is performed under local anaesthesia. The open surgical parotid biopsy approach is not commonly used mainly because it's an invasive procedure and because of the risk of surgical complications, such as facial nerve damage, sialoceles, salivary fistulae, as well as the frequently described temporary change in sensation of the skin area around the incision (44, 58). The most feared complication is the injury of the facial nerve, which presents a strict anatomical relation with the parotid gland. However, when performed by experts, the risk of perioperative complications is usually minimal (14).

This procedure usually gives a large amount of well-preserved tissue for histology and even if the main indication is the diagnosis of a suspicious lymphoma, also the FS can be calculated with results comparable to LSG tissue, considered the gold standard (44).

The ultrasound guided core needle biopsy (US-guided CNB) is a less invasive and well-tolerated procedure that can be performed by a radiologist with expertise in ultrasonographic technique in an outpatient setting under local anaesthesia. The procedure is conducted under real-time ultrasound guidance with an aseptic, free-hand technique in a non-operating room. US-guided CNB has proven to provide adequate sampling for histological examination, immunohistochemical staining and flow cytometry (15, 31).

To avoid injuries of the facial nerve, knowledge of nerve anatomy is crucial and allows to guide the biopsy in the 'safety zone' (15). This area is located 1 cm anterior and 1 cm below the ear lobe and represent the safe area to perform the US-guided CNB since the facial nerve is still deep in the parotid parenchyma.

Maintaining a superficial depth of the biopsy needle within 1 cm from the gland's surface Zabotti *et al*. demonstrated remarkable feasibility and tolerability of the procedure (15, 31). As a result, we can use a mnemonic approach to define the 'safety zone' as 'the role of one,' indicating 1 cm anterior and 1 cm below the earlobe, with a depth of 1 cm from the parotid surface (31) (Fig. 5).

US-guided CNB represents the optimal approach for obtaining tissue from a suspicious focal lesion, improving the differential diagnosis process (59-62). Since tissue can be sampled multiple times from the same gland, this approach is also suitable for disease progression monitoring, and treatment efficacy (59-62). Compared to the surgical approach, the US-guided CNB seem to be associated with a low risk of complications in expert hands (31). Since the tissue sampled by CNB is not as wide as that sampled by the open surgical biopsy, a possible flaw is that the detections of unequivocable features of FLS and the calculation of the FS might be not straightforward (15).

As previously reported, the main indication for MSG biopsy in pSS is persistent swelling of the MSG to rule-out a lymphoid proliferation of various nature. The parotid gland indeed, and not the LSG, is the anatomical site in which those lesions mostly arise (63). Lymphoid proliferation in the setting

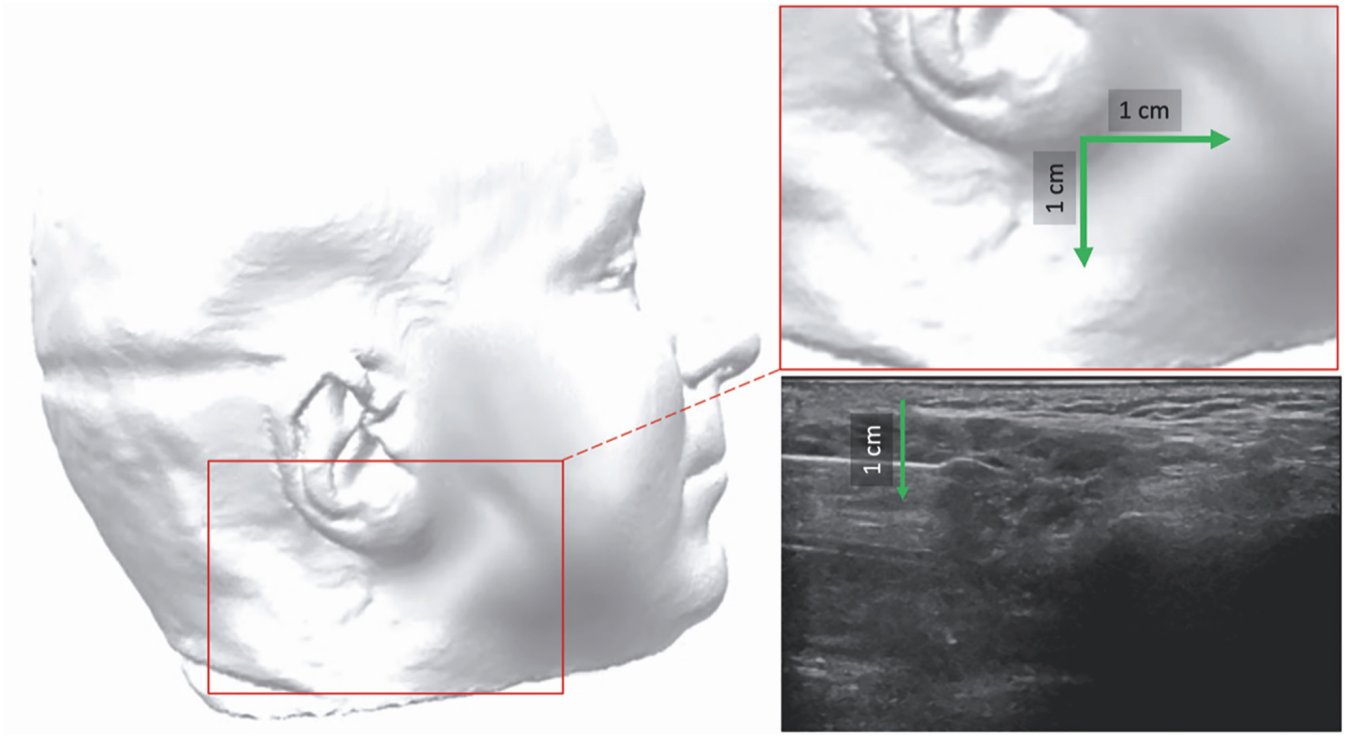

Fig. 5. 'Safety zone': Area located 1 cm anterior and 1 cm below the ear lobe, representing a safe area to perform US-guided CNB when the biopsy is performed within 1-1.5 cm from the gland surface. In this area the facial nerve is still deep in the parotid parenchyma.

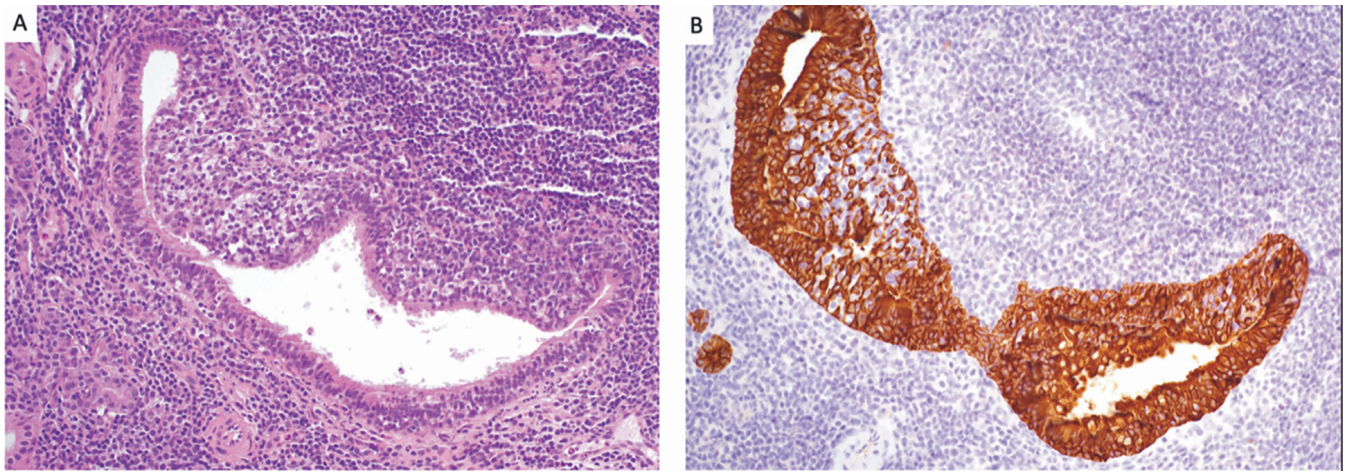

Fig. 6. A: Picture showing a lymphoepithelial lesion (LEL) with hyperplasia of the ductal epithelium associated with intraepithelial lymphoid exocytosis (H&E, 200x magnification).
B: Immunohistochemical staining for cytokeratin AE1/AE3 highlights the epithelial proliferation.

of pSS can pose formidable issues in differential diagnosis since it ranges from a seemingly reactive process to a neoplastic lesion with overlapping morphological features.

Histopathological features

The MSGs of most pSS patients are characterised by a various set of histopathological abnormalities such as NSCS and lymphocytic Infiltration with all the features of the focal lymphocytic sialadenitis as previously described in the LSG chapter.

Differently from LSG tissue, lesions that are mostly found in parotid biopsies, are the 'lymphoepithelial lesions' (LELs) (Fig. 6), defined as striated ducts infiltrated by lymphocytes with concurrent marked hyperplasia of the epithelial cells, ultimately obliterating the duct lumen (44). In order to grade this histopathological lesion, the following classification of LELs has been proposed: i) stage 1: partial LEL, involving <50% of the epithelium; ii) stage 2: developed LEL, involving 50–100% of the epithelium; iii) stage 3: occluded LEL, fully circumferentially affecting epithelium without lumen. With increasing severity of LEL, the relative number of B cells is also expanding, suggesting a crucial role of B cells for epithelial proliferation (13). Although considered by many authors one of the classic histologic compo-

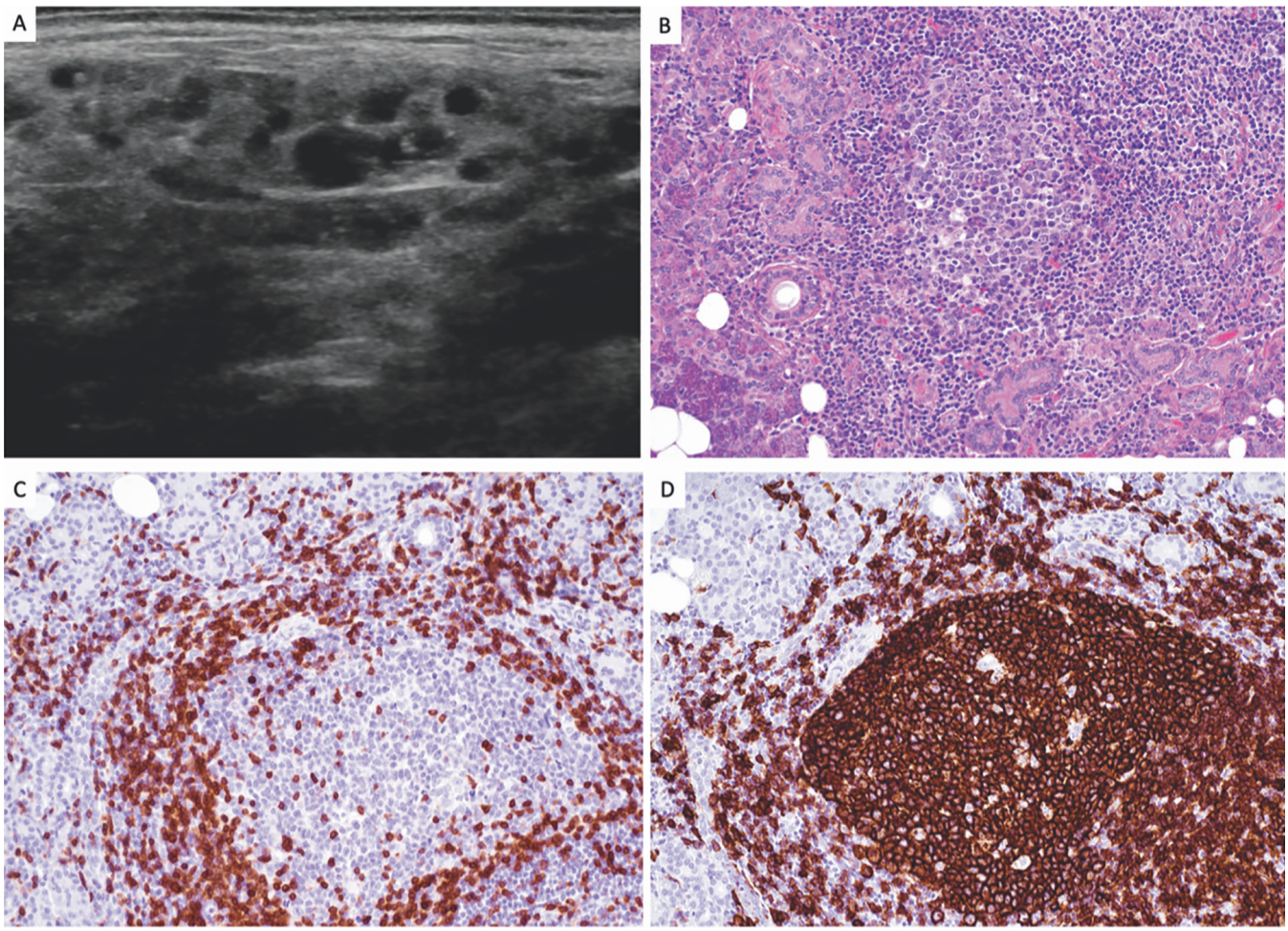

Fig. 7. C: Ultrasonographic image of a MESA/LESA in a parotid gland of a patient with pSS.
D: A case of lymphoepithelial sialadenitis (LESA) in the parotid gland with germinal centre formation (H&E, 200x magnification).
E, F: immunohistochemical stainings for CD3 and CD20 showing segregation of the T and B lymphocytes respectively in this secondary lymphoid follicle.

nents of pSS, LELs must be always associated with lymphoid infiltrates; LELs alone are indeed not diagnostic of pSS since not all patients with this histologic finding possess the clinical signs, symptoms, and laboratory findings of the disease. LELs contribute to the definition of a specific histopathological lesion called lymphoepithelial sialadenitis (LESA), one of the lymphocytic proliferations that can occur in the parotid gland (Fig. 7).

According to De Vita *et al*. (64) and Carbone *et al*. (65), three histological patterns can be identified within the spectrum of salivary gland lymphoproliferation in pSS. The first one is the stage in which LESA/MESA presents a 'fully benign lymphoid infiltrate', characterised by a preserved lobular architecture of the gland, prominent reactive lymphoid follicles without expansion of the mantle or marginal zone, and evident LELs acquiring a monocytoid and/or centrocyte-like appearance (restricted to the LELs). The B cell population is polyclonal. The second stage considers LESA/MESA as a 'lymphoproliferative lesion', characterised by a diffusively or multifocally lymphoid involvement of the gland (with preservation only of the normal acini island), a more aggressive LEL appearance, aggregates of centrocyte-like cells within the diffuse lymphoid infiltrate, and nonconfluent centrocyte-like cell 'halos' surrounding the LELs. Areas of immunoglobulin light chain restriction may be present, and molecular analyses may show either oligo-polyclonal or monoclonal B-cell expansion (64, 66). The third stage represents a clear-cut lymphoproliferation, with B-cell clonal expansion, resulting in uncontrolled progression towards B-cell lymphoma (see next chapter).

LESA is a benign lymphocytic infiltration of salivary gland tissue characterised by ductal hyperplasia and the formation of lymphoepithelial lesions. The term myoepithelial sialadenitis (MESA) has also been used for this same disease process but this has been shown to be a misnomer since there is little if any proliferation of myoepithelial cells within this lesion (65). LESA is characterised by chronic inflammation, parenchymal atrophy, and LELs. The lobular architecture of the parotid gland is typically preserved, and the number of lymphocytic follicles and the size of germinal centre are considerably variable. Advanced disease shows diffuse parenchymal lymphocytic infiltration and atrophy of normal acinar tissue.

LELs consists of proliferating basaloid ductal epithelial cells with a monotonous appearance and pale, regular, rounded nuclei with minimal cyto-

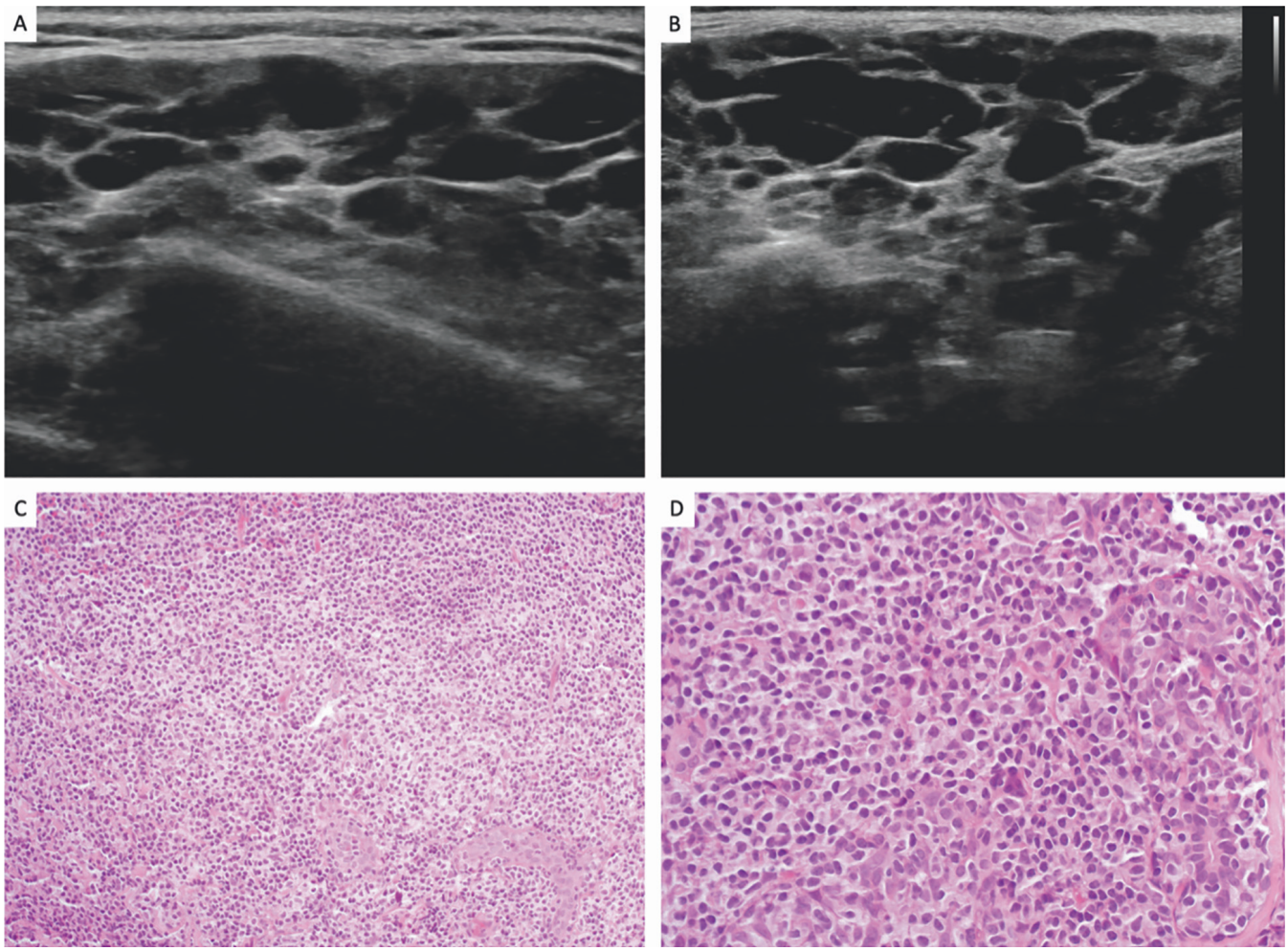

Fig. 8. A, **B**: Ultrasonographic image of a typical MALT lymphoma in a parotid gland, showing a complete disarrangement of the salivary gland parenchyma **C**, **D**: histological pictures at various magnification (200x and 400 x respectively) of a MALT lymphoma diagnosed by US-guided CNB of the parotid gland. The images show a disruptive proliferation of centrocyte-like and monocytoid B-cells with features of coalescence and LELs.

plasm, surrounded and penetrated by a mixture of centrocytic and monocytoid lymphocytes. This island-like structures vary substantially in both size and shape. Some LELs are nodular while others display irregular misshapen contours that are angulated. The lymphoid infiltrate in LESA has a predominance of T cells, but within the lymphoepithelial lesions, lymphocytes have features of marginal zone B cells. The distinction of LESA from incipient MALT lymphoma can be difficult and sometimes subjective. A combination of a monocytoid B-cell proliferation with a broad "halo" around the epithelial cell nests, wide strands between lymphoepithelial lesions, and extensive parenchymal destruction strongly favours a diagnosis of MALT lymphoma (Fig. 8). Caution should be taken when evaluating B-cell clonality since it may be present in a high percentage of LESA cases (65,67).

The presence of a focus score (FS) ≥1 in the parotid gland is considered as a positive biopsy and used for the classification of pSS (44). However, according to Pijpe *et al*. both a focus score ≥1 or the presence of small lymphocytic infiltrates in combination with benign LELs are adequate criteria for diagnosing pSS, reporting a sensitivity of 93% and a specificity of 95% (44).

Recently, a preliminary study by Nakshbandi *et al*. shows in LSG biopsies of non-pSS sicca patients more signs of unspecific inflammation compared to parotid biopsies. In parotid gland biopsies signs of B-cell hyperactivity (CD20+B-cells, GCs/mm^2 and LELs/mm^2) are more pronounced, compared to LSG biopsies (68). These histopathological differences should be considered in the choice of salivary tissue to biopsy when diagnosing and classifying Sjögren's disease (69, 70).

Histopathological features of salivary gland lymphoma and clinical aspects

Primary salivary gland malignant lymphomas represent from 6 to 26% of all extranodal lymphomas in the head and neck region (71). Although normal salivary gland parenchyma contains no lymphoid tissue, intraglandular lymph nodes are common, especially within the parotid gland. To be considered truly extranodal, these lymphoid neoplasms should contain no residual normal lymphoid tissue and lack a fibrous capsule. Malignant lymphoma secondarily involving the salivary glands also occurs but is less common than primary salivary gland lymphoma.

Patients with pSS are at a higher risk to develop lymphoproliferative disease (10). This risk is notably the highest among a range of autoimmune conditions (11) and is likely linked to prolonged inflammation, B-cell hyperactivity and lymphocyte infiltration (2-4). Non-Hodgkin lymphoma (NHL) is the most fearable and severe complication, occurring around 5-10% of pSS patients (11). Because it is the most common salivary gland affected by pSS, the parotid gland is also the major site for the development of primary salivary gland lymphoma (63). Although any subtype of lymphoma may involve salivary glands, the major subtypes of primary salivary gland lymphoma are B-cell malignancies especially the marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), occurring in 75% of lymphoma cases (66, 72-74) (Fig. 5).

Lymphoma presents as a firm, painless mass of variable duration. Microscopically it recapitulates Peyer's patch-type lymphoid tissue and is composed of morphologically heterogeneous small B cells, including marginal zone (centrocyte-like) cells, cells resembling monocytoid cells, small lymphocytes, and scattered immunoblasts and centroblast-like cells (75). In some cases, a plasmacytic differentiation is found, with light chain restriction shared by lymphocytes and plasma cells. The neoplastic cells are mostly located in the marginal zones of reactive B-cell follicles but can extend into the interfollicular region, as well as colonising the germinal centres. In epithelial tissues, like the salivary glands, neoplastic cells typically invade and partially destroy the epithelium, forming LELs. The presence of a monocytoid B-cell proliferation with a broad "halo" around the epithelial cell nests, and extensive parenchymal destruction are characteristic features of MALT lymphoma, as stated earlier.

Malignant B-cell populations seem to arise from precursor cells that have already infiltrated the salivary glands in patients with pSS during the polyclonal phase of the local humoral response. This enrichment of malignant B-cell clones continues throughout the progression from LELs to MALT lymphoma (76, 77).

In some cases, low- grade malignant B-cell clones can further undergo monoclonal transformation into diffuse large B-cell lymphoma (DLBCL); the term "high grade MALT lymphoma" should not be longer used (78). Neoplastic cells stain with B-cell markers, CD20, CD19, Cd79a, PAX5, but typically do not express CD10, bcl1, CD23. While CD5 is rarely express, cases positive for CD5 were shown to be associated with a more aggressive behaviour. MALT lymphomas are usually positive for IgM and occasionally for IgG or IgA, but not for IgD, with kappa or lambda light chain restriction. IRTA1/FCRLF4 (CD307d), a marker of marginal zone cell differentiation, is expressed in 40–90% of cases. Although various chromosomal translocations are found in MALT lymphoma, the t(14;18)(q32;q21)/IGH-MALT1 is most often associated with salivary gland MALT lymphoma (79, 80).

For the diagnosis of lymphoproliferative lesions, the open surgical biopsy is the first-choice procedure but, in our experience, also ultrasound-guided CNB has shown good diagnostic performances especially in overt MALT lymphoma (15). The tissue obtained from multiple passages was in most cases sufficient for morphological diagnosis and for performing ancillary studies such as immunohistochemical stainings and molecular biology assays. The prevention of lymphoma in pSS continues to represent a significant challenge that has not been adequately addressed. Treatable infections may trigger lymphoproliferation and have been identified in peculiar microenvironments in MALT NHLs, but the role of infection remains undefined in pSS (81, 82).

Epidemiological, clinical, laboratory, histological, and imaging features predictive of lymphoproliferative disease have been widely studied (27). The two strongest predictors for lymphoma development in pSS patients are the glandular swelling and mixed cryoglobulinaemia (83); these manifestations, closely related from a biological point of view, well reflect the degree of salivary inflammation and MALT lymphoproliferation (83, 84). A precise clinical record of salivary gland swelling and an early and repeated screening of serum cryoglobulinaemia are mandatory for a correct assessment of pSS-related lymphoproliferative risk (84, 85). However, other factors associated with higher development of lymphoma are male gender, rheumatoid factor positivity, low C4 levels, anti SSA/SSB positivity, histopathological changes (high FS and GC-like structures associated with LELs) and ultrasonography abnormalities (27, 86). In the latest years, a model has been proposed to assess the probability of NHL development in pSS patients according to 7 risk factors: salivary gland swelling, lymphadenopathy, Raynaud phenomenon, anti-SSA/SSB autoantibodies positivity, rheumatoid factor positivity, monoclonal gammopathy, and C4 hypocomplementaemia (87). Fragkioudaki *et al*. reported a risk of 3.8% when 2 risk factors are present, 39.9% when 3–6 risk factors are present and 100% when all 7 risk factors are present (87). Further researches should also be conducted to evaluate eventual different clinical and laboratory features between pSS patients developing MALT lymphoma and different subtypes like Diffuse Large B-cell Lymphoma (DLBCL). The ultimate objective is to achieve an early diagnosis, enhance patient management, and improve prognosis by employing a rational patient stratification strategy in the context of pSS (63).

Conclusions

Salivary gland histopathology based on salivary gland biopsy is crucial for the diagnosis of pSS and for the assessment of lymphoproliferative lesions associated with pSS. For diagnostic purpose, tissue can be obtained either from LSG or from MSG, preferably parotid glands.

The FLS is the hallmark of pSS and it is usually highlighted in both LSG and parotid gland biopsy. Recent studies highlight that LSG biopsy and parotid gland biopsy have similar accuracy and safety for pSS diagnosis. However, parotid gland biopsy may offer some advantages in the pSS management,

mainly the direct comparison between imaging and histology at diagnosis and during the follow-up, the early detection of lymphoma, and, for imaging guided procedures, the opportunity to sample suspected areas.

Regarding the MSG biopsy, the choice between the open and the US-guided technique relies on the expertise of each centre. In favour of the former, the larger amount of sampling; of the latter, the possibility to be performed directly by rheumatologist. Moreover, US guided approach can be considered the method of choice for submandibular enlargement.

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