Usefulness of ultrasound guided core needle biopsy of the parotid gland for the diagnosis of primary Sjögren's syndrome

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Abstract

Objective

The diagnosis and classification of primary Sjögren's syndrome (pSS) relies on labial biopsy, whereas the role of open parotid biopsy is mainly reserved to evaluate the lymphoproliferative complications. Recently ultrasound-guided core needle biopsy (US-guided CNB) appeared as a novel and safe technique useful in lymphoma assessment, however, its potential role in the diagnosis of pSS has never been assessed. The main aim of this study was to evaluate the diagnostic value of US-guided CNB of the parotid glands in patients affected by pSS.

Methods

Patients affected by pSS who underwent US-guided CNB for a suspected glandular lymphoma were included. Adequacy of the samples and histopathological features related to pSS were analysed.

Results

US-guided CNB was performed on 29 parotid glands. The biopsied samples were adequate for diagnosis in 28/29 (96.5%) cases. Fifteen patients showed pathologic features of parotid lymphoma. Among the remaining patients, 9/13 presented focus score ≥1; LELs were present in 8/13 patients, and GCs in 11/13. In 8 cases the histological features were coherent with MESA/LESA. Acinar atrophy, fibrosis and duct dilatation were also evaluated.

Conclusion

This preliminary study suggests the possible usefulness of US-guided CNB for the diagnosis of pSS by enabling the collection of adequate salivary gland tissue to assess the FS, GCs, LELs, and other histopathologic features also useful in the management of pSS patients.

Key words

Sjögren's syndrome, core needle biopsy, diagnosis, parotid gland, histopathology

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic, systemic autoimmune disease, characterised by lacrimal and salivary gland (SG) involvement, with consequent keratoconjunctivitis sicca and xerostomia and heterogeneous glandular and extra-glandular involvement (1-3).

To date, the diagnosis and classification of pSS relies on a combination of clinical, serological, histological and instrumental parameters (4). The pSS histopathological diagnostic work-up relies on labial salivary gland (LSG) biopsy. The minor salivary glands are distributed in the oral cavity (labial, buccal and palatal mucosa) and are easily accessible, hence LSG biopsy has been included in the diagnostic work-up of pSS since 1970 (5).

LSG biopsy is a rather invasive procedure, which has a morbidity rate due to the significant risk of permanent damage to the sensory nerve supply to the lower lip (6). Previous studies, however, support the hypothesis that the minor salivary gland structure reflects the microenvironment of the major salivary gland (5, 7), including the parotid glands, where malignant lymphoma is usually localised in pSS (8).

At present, only LSG biopsy is included in the classification criteria for pSS (9), whereas open surgical parotid biopsy is mainly reserved for evaluating lymphoproliferative complications (10).

Nonetheless, parotid gland (PG) sampling is attracting increased interest as a diagnostic tool for pSS, and specific classification criteria have also been developed (10, 11). Compared to LSG biopsy, major salivary gland biopsy (and therefore PG biopsy) may offer some benefits for the management of pSS, including improved differential diagnosis, disease progression monitoring and treatment efficacy assessment (12, 13). Furthermore, since pSS patients are predisposed to lymphoproliferative disease (14, 15), the diagnosis of B-cell lymphoma must be pathologically proven.

Open surgical biopsy of the parotid gland is a safe technique when performed by expert surgeons, but presents some disadvantages, such as the need for expert surgeons themselves, an operating surgery room and possible adverse effects, which may justify its limited application up to now.

In recent years, sonographic evaluation of the parotid and submandibular glands has attracted increased interest, allowing the assessment of the glandular parenchyma and the detection of salivary gland abnormalities (16, 17). For years, ultrasound-guided core needle biopsy (US-guided CNB) has been an established method for diagnosis of breast lumps or masses in other body parts (18). Recently, US-guided CNB appeared as a novel and safe technique useful in PG lymphoma assessment in pSS patients with salivary gland enlargement (19). However, its potential role in the diagnosis of pSS has never been assessed.

This preliminary study aims to evaluate the diagnostic value of US-guided CNB of the parotid glands in pSS. To this end, the histopathological features of the biopsied samples were investigated in detail in patients affected by pSS with suspected lymphoma during their follow-up, *i.e.* the patients were suffering or had suffered from persistent parotid swelling (PSW) (>2 months), or had experienced a recent onset of a suspicious major salivary gland lesion detected by SGUS (20).

Methods

Study design

This is a retrospective cohort study including 37 consecutive pSS patients who fulfilled ACR/EULAR classification criteria for pSS (9) and underwent US-guided CNB of the major salivary glands (PG or submandibular gland) for a suspected glandular lymphoma during follow-up. Eight patients who had undergone submandibular gland biopsy were excluded for the purposes of this study. Thus, 29 pSS patients were ultimately included in the study.

Written informed consent was obtained from each patient in accordance with the Declaration of Helsinki and with local guidelines for good clinical practice. The study was conducted according to a protocol approved by the Regional Ethical Committee (CEUR-2017-Os-027- ASUIUD).

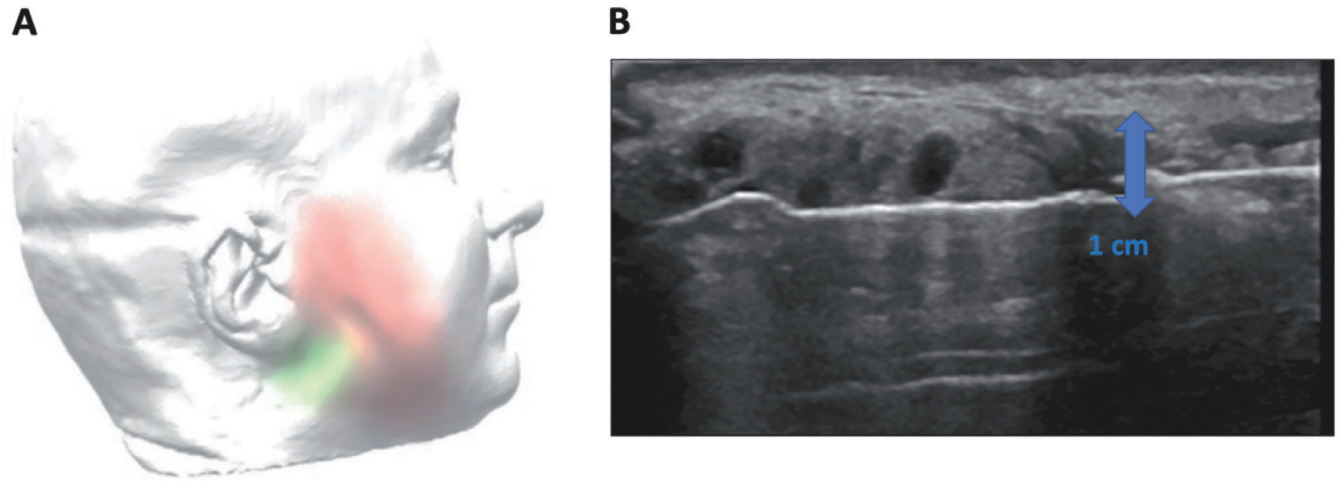

Fig. 1. The safety zone; 'the rule of one' **A.** Area located between 1 cm anterior and 1 cm below the earlobe (green area). **B**. The depth of the biopsy needle within 1-1.5 cm from the surface of the gland.

Demographic, clinical and laboratory data

Patients' clinical and laboratory data (including age, gender, disease duration, previous unstimulated sialometry and Schirmer's tests, and evidence of serum antibodies, as well as antinuclear, anti-Ro/SSA and anti-La/SSB antibodies) were collected from medical charts. The presence of lymphoma development risk factors for patients with pSS was noted at the time of participants' biopsy procedures (glandular swelling, lymphadenopathy, cryoglobulinaemia, a serum monoclonal component, rheumatoid factor, low serum C4 and leucopoenia) (15, 17).

US-guided CNB technique

US-guided CNB was performed by a radiologist with over 10 years' experience in ultrasound-guided biopsies. The procedures were conducted in a non-operating room under real-time US guidance with linear high-frequency transducers (RS85, probe LM4-15B [Samsung, Seoul, South Korea] or Affiniti 70G, probe L18-5 [Philips, Amsterdam, the Netherlands]). Under US guidance, local anaesthetic (5 mL of mepivacaine chlorhydrate) is injected in the subcutaneous tissue and in the posterior, superficial part of the parotid gland, and a small skin incision is made with a scalpel (21). Biopsies were performed with a 14-gauge, semiautomatic CNB system (Precisa 14G, HS Hospital Service, Aprilia, Italy) on the participants' clinically most swollen parotid gland, if present, or in the parotid gland more representative from a clinical (*i.e.* parotid enlargement) or sonographic point of view. To avoid facial nerve injury, the access area of the needle and the depth of the needle inside the gland complied with the safety zone and 'the rule of one' (Fig. 1). Two to four needle passes were carried out through the same skin access. Detailed information on US-guided CNB is included in previous studies (14, 19, 20).

Histopathological evaluation

All tissue samples were analysed by two expert pathologists (EP, CDL) with over 10 years of experience in head and neck pathology. All the samples were evaluated for sufficient adequate tissue (≥4 mm^2) (11) for histopathological analysis and were screened for malignancy and other diagnoses.

According to Pijpe *et al*. (11), a biopsy was considered as positive for pSS when criterion (i) or (ii) was fulfilled, as follows: (i) a focus score ≥ 1, defined as the number of lymphocytic foci (which are adjacent to normal-appearing acini and contain >50 lymphocytes) per 4 mm^2 of glandular parotid tissue (including fat tissue), irrespective of the presence of benign LELs; (ii) small lymphocytic infiltrates, not fulfilling the criterion of a focus score of >1, in combination with the presence of benign LELs.

In particular, the following histological features related to pSS were studied: presence of focal lymphocytic sialadenitis (FLS); focus score (FS); germinal centres (GCs); lymphoepithelial lesions (LELs); presence of lymphoepithelial sialadenitis (LESA). In addition, the extent of atrophy, fibrosis, duct dilatation and non-specific chronic sialadenitis were also evaluated (absent, mild, moderate, severe). Finally, since parotid lymphoma diagnosed by US-guided CNB is consistent with pSS classification (which was already satisfied before CNB parotid biopsy in this study) and parotid gland lymphoma is the main complication of pSS, such patients were considered in the initial analysis.

Safety

The participants were evaluated clinically 1 and 12 weeks after their US-guided CNB and beyond the 12-week point in case of persistent complications. All patients completed a questionnaire, reporting any intra- and post-procedural complications as well as pain, using the Visual Analogue Scale (VAS 0–10). Complications were categorized as transient (lasting <12 weeks) or persistent (lasting ≥12 weeks). Any complication in the follow-up period was assessed and recorded (including swelling, bleeding, haematoma, local infection, paraesthesia, sialocele and fistulae). Potential damage of the facial nerve was clinically assessed monitoring the onset of sensorimotor signs or symptoms.

Results

Patients

Twenty-nine consecutive, unselected patients with pSS who underwent US-

Table I. Clinical features of pSS patients.

Patient	Gender	ACR/EULAR Criteria	Positive objective tests	LSGB +	ANA	SSA	SSB	Cryo	Low C4	Low C3	RF	MC
#1	F	Yes	Yes	/	Yes	Yes	No	Yes	Yes	No	Yes	Yes
#2	F	Yes	Yes	/	Yes	Yes	Yes	No	No	Yes	No	No
#3	F	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	No
#4	F	Yes	Yes	Yes	Yes	No	No	No	Yes	No	No	No
#5	F	Yes	Yes	/	Yes	Yes	Yes	No	No	No	Yes	No
#6	F	Yes	Yes	/	Yes	Yes	Yes	No	No	Yes	Yes	No
#7	M	Yes	Yes	/	Yes	Yes	Yes	No	No	No	Yes	No
#8	F	Yes	Yes	/	Yes	Yes	Yes	No	No	No	No	No
#9	F	Yes	Yes	/	Yes	Yes	Yes	No	No	No	Yes	No
#10	M	Yes	Yes	/	Yes	Yes	Yes	No	No	No	Yes	Yes
#11	F	Yes	Yes	/	Yes	Yes	No	No	Yes	No	Yes	No
#12	F	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No
#13	F	Yes	Yes	/	Yes	Yes	Yes	No	No	Yes	Yes	No

/: not performed; ANA: antinuclear antibodies; Cryo: cryoglobulinaemia; LSGB: labial salivary gland biopsy; MC: monoclonal component; RF: rheumatoid factor.

guided CNB of the parotid glands between September 2019 and June 2022 due to suspected major salivary gland lymphoma were included in this study. Fifteen patients (15/29; 51.72%) showed pathologic features of parotid lymphoma, in only one patient (1/29; 3.4%) the CNB material was inadequate for diagnosis (see Histopathology section). By excluding these 16 patients, sub-analysis was limited to the remaining 13/29 (44.82%) pSS patients (clinical features summarised in Table I), with the aim of evaluating in detail CNB accuracy for pSS diagnosis.

At the time of the procedure, 8 out of 13 patients (61.5%) did not have parotid gland enlargement, although all these 8 patients had a history of a previous persistent PSW.

Histopathological features

Only one patient (1/29) had insufficient material for diagnosis from parotid CNB biopsy. Therefore, 28/29 patients, including 15 patients who showed histopathological features of low-grade marginal zone parotid B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), had adequate material for diagnostic purpose.

Fifteen out of 28 patients showed pathologic features of low-grade marginal zone parotid B-cell lymphoma of MALT. By including these samples, the mean area of the samples was 30.2 mm^2 (Standard deviations SD_18.6) from a mean number of passes of 2.8 (SD 1.1). When considering only the 13 pSS patients with pSS, excluding the patients diagnosed with lymphoma by parotid gland CNB, the mean surface area of the samples was 24.9 mm^2 (SD 13.4), obtained from a mean number of passes of 2.4 (SD 0.7). One out of 13 (7.69%) was <8 mm^2. Furthermore, 9/13 (69.2%) had a positive biopsy according to Pijpe *et al*. (11). All the 9 positive cases had the criterion of a FS≥1, and none of those with a FS <1 had LELs. LELs were present in 8/13 patients, and GCs in 11/13. In eight out of nine cases (88.9%) with a positive biopsy, the histological features were coherent with MESA/LESA, and multiple GCs and LELs were identifiable.

Table II.

Patient #	Parotid enlargement at biopsy time	Surface area (mm^2)	FLS (yes/no)	FS ≥1 (yes/no)	LEL (yes/no)	GC (yes/no)	MESA/LESA (yes/no)
#1	Yes	22,7	Yes	Yes	Yes	Yes	Yes
#2	No	16,5	Yes	No	No	No	No
#3	No	11,8	Yes	Yes	No	Yes	No
#4	Yes	15,2	Yes	Yes	Yes	Yes	Yes
#5	No	39,6	Yes	Yes	Yes	Yes	Yes
#6	No	8,6	Yes	No	No	Yes	No
#7	No	54	Yes	Yes	Yes	Yes	Yes
#8	No	27,8	Yes	Yes	Yes	Yes	Yes
#9	Yes	28,7	Yes	Yes	Yes	Yes	Yes
#10	Yes	25	Yes	Yes	Yes	Yes	Yes
#11	No	33,5	Yes	No	No	No	No
#12	Yes	33,7	Yes	Yes	Yes	Yes	Yes
#13	No	7,2	Yes	No	No	Yes	No

FLS: focal lymphocytic sialadenitis; FS: focus score; GCs: germinal centres; LELs: lymphoepithelial lesions; MESA: myoepithelial sialadenitis; LESA: lymphoepithelial sialadenitis.

Four cases had a negative biopsy according to Pijpe *et al*. (11). Two out of these 4 patients with a negative biopsy showed a FLS with FS <1 but with the presence of GCs.

Histopathological details, including non-specific chronic sialadenitis features (acinar atrophy, fibrosis, and duct dilatation) are reported in Tables II and III.

Safety

In all 29 patients, US-guided CNB of the parotid gland was well tolerated, and patients reported no long-term complications during the mean fol-

Table III.

Patient	Non-specific chronic sialadenitis features		
	Acinar atrophy (absent/mild/ moderate/severe)	Fibrosis (absent/mild/ moderate/severe)	Duct dilatation (absent/mild/ moderate/severe)
#1	severe	moderate	absent
#2	mild	absent	absent
#3	mild	absent	absent
#4	mild	absent	absent
#5	mild	mild	mild
#6	mild	absent	absent
#7	severe	absent	mild
#8	mild	moderate	mild
#9	severe	mild	absent
#10	severe	mild	mild
#11	mild	absent	mild
#12	severe	severe	mild
#13	mild	absent	absent

low-up period of 15.9 months (SD 9.9 months). Transient complications (lasting <12 weeks) were reported by 6 patients. In particular, there were 3 cases of local bleeding and haematoma, and 3 cases of local paraesthesia (lasting less than 3 hours) due to anaesthesia. Intraprocedural pain was evaluated by VAS and was found low (mean VAS 1.93, SD 2.5), as was postprocedural pain (mean VAS 1.58, SD 2.6). There were no significant differences in terms of safety when comparing patients with or without parotid enlargement at the time of biopsy. It is notable that patients did not report any surgical scars or wound formation after the procedure. The duration of the US-guided CNB procedure, from patients' entrance into the examination room to their exit, took a maximum of 45 minutes.

Discussion

Adequate quality of the tissue sampled for histopathological analysis is essential for diagnostic, prognostic and research purposes for any disease. In pSS patients, the quality of salivary gland tissue is even more crucial for diagnosis, for the identification of possible histopathological predictors of lymphoma development and, finally, for a possible lymphoma diagnosis. These clinical points, which could also be applied for research purposes, highlight the concept of 'the right tissue at the right time' (8, 15). Although labial gland biopsy is used in pSS (22), parotid gland biopsy is considered a possible alternative, especially in experienced hands (23). Parotid gland biopsy might not only have good diagnostic potential but might also allow for improved prognosis (24), monitoring of disease activity and tissue damage (25, 26), response to treatment (27, 28), early detection, and follow-up of suspect salivary lymphoma in pSS (24, 29).

To the best of our knowledge, this is the first report exploring the value of US-guided CNB of the parotid gland as a histopathological diagnostic tool for pSS. Through a preliminary analysis of 37 consecutive pSS patients who underwent US-guided CNB due to suspected lymphoma, the accuracy and safety of this procedure for the diagnosis of pSS is herein demonstrated.

A major issue with using a new technique such as US-guided CNB relates to the adequacy of the material obtained for histopathological evaluation and, if needed, for immunohistochemical and molecular tests, thus allowing complete characterisation from diagnosis to identification of possible lymphoproliferative disease.

In 28 out of 29 (96.5%) PG samples the material was sufficient to conclude a final histopathological diagnosis, even in the absence of parotid gland enlargement. Overall, pathologic data are consistent with pSS in 24 out of 28 cases (85.7%).

Focusing on the sub analysis restricted to the 13 pSS patients without parotid lymphoma, the rate of positive biopsies according to Pijpe *et al*. in patients with fulfilment of pSS criteria was near to 70% (9/13; 69.2%), increasing to 85% when including those with a FS <1 but with a FLS and the presence of GCs (11/13; 84.6%). With regard to this issue, GCs are usually more frequently found in PG when compared to LSG and their presence could be considered highly specific for pSS, but probably with a lower sensitivity for pSS when compared to FS (30). Currently, evidence on the role of GCs as a diagnostic marker is limited, and ectopic GCs formation in the exocrine glands, occurring in one-quarter of patients with pSS, seems to be associated with more severe disease and with an increased prevalence of lymphoma (31).

The present study confirms the excellent safety profile of the procedure. There are no persistent complications and there is optimal patient acceptance of the procedure, with low levels of pain during and after the procedure, even in those patients without clinical parotid swelling at the time of the procedure.

The evaluation of safety and patient acceptance for this procedure is the cornerstone for future clinical studies on the evaluation of US-guided CNB for the diagnosis of pSS.

The main limitation of this study is the small number of patients evaluated, due to its preliminary nature. Secondly, this study evaluated a subset of pSS patient, *i.e*. patients who already had a previous pSS diagnosis and also reasonable suspicion of lymphoproliferative disease. Therefore, pSS patients with suspected lymphoma of the parotid gland were evaluated, although excluding in sub analysis those who showed a parotid lymphoma after parotid CNB biopsy. However, it is the first study to hypothesize a possible role for US-guided CNB for the diagnosis of pSS, since it enabled the collection of adequate salivary gland tissue to assess FS, GC, LELs and other histopathologic features useful for the management of pSS patients. The next step will be the comparison of US-guided CNB with LSG biopsy in suspected cases of pSS.

References

1. MACIEL G, CROWSON CS, MATTESON EL, CORNEC D: Prevalence of primary Sjögren's syndrome in a US population-based cohort: primary SS prevalence. *Arthritis Care Res* 2017; 69: 1612-6. https://doi.org/10.1002/acr.23173
2. MAVRAGANI CP, MOUTSOPOULOS HM: Sjögren's syndrome. *Annu Rev Pathol Mech Dis* 2014; 9: 273-285. https://doi.org/10.1146/annurev-pathol-012513-104728
3. ZINTZARAS E: The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch Intern Med* 2005; 165: 2337. https://doi.org/10.1001/archinte.165.20.2337
4. BALDINI C, LUCIANO N, TARANTINI G *et al*.: Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjögren's syndrome. *Arthritis Res Ther* 2015; 17: 146. https://doi.org/10.1186/s13075-015-0657-7
5. CHISHOLM DM, MASON DK: Labial salivary gland biopsy in Sjögren's disease. *J Clin Pathol* 1968; 21: 656-60. https://doi.org/10.1136/jcp.21.5.656
6. VALDEZ RMA, MELO TS, SANTOS-SILVA AR, DUARTE A, GUEIROS LA: Adverse postoperative events of salivary gland biopsies: a systematic review and meta-analysis. *J Oral Pathol Med* 2022; 51: 152-9. https://doi.org/10.1111/jop.13229
7. CHISHOLM DM, WATERHOUSE JP, MASON DK: Lymphocytic sialadenitis in the major and minor glands: a correlation in postmortem subjects. *J Clin Pathol* 1970; 23: 690-4. https://doi.org/10.1136/jcp.23.8.690
8. DE VITA S, ISOLA M, BALDINI C *et al*.: Predicting lymphoma in Sjögren's syndrome and the pathogenetic role of parotid microenvironment through precise parotid swelling recording. *Rheumatology* (Oxford) 2022 Sep 5. https://doi.org/10.1093/rheumatology/keac470
9. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017; 69: 35-45. https://doi.org/10.1002/art.39859
10. SPIJKERVET FKL, HAACKE E, KROESE FGM, BOOTSMA H, VISSINK A: Parotid gland biopsy, the alternative way to diagnose Sjögren syndrome. *Rheum Dis Clin N Am* 2016; 42: 485-99. https://doi.org/10.1016/j.rdc.2016.03.007
11. PIJPE J, KALK WWI, VAN DER WAL JE *et al*.: Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjögren's syndrome. *Rheumatology* 2007; 46: 335-41. https://doi.org/10.1093/rheumatology/kel266
12. FISHER BA, BROWN RM, BOWMAN SJ, BARONE F: A review of salivary gland histopathology in primary Sjögren's syndrome with a focus on its potential as a clinical trials biomarker. *Ann Rheum Dis* 2015; 74: 1645-50. https://doi.org/10.1136/annrheumdis-2015-207499
13. PIJPE J, MEIJER JM, BOOTSMA H *et al*.: Clinical and histologic evidence of salivary gland restoration supports the efficacy of rituximab treatment in Sjögren's syndrome. *Arthritis Rheum* 2009; 60: 3251-6. https://doi.org/10.1002/art.24903
14. ZABOTTI A, ZANDONELLA CALLEGHER S, LORENZON M *et al*.: Ultrasound-guided core needle biopsy compared with open biopsy: a new diagnostic approach to salivary gland enlargement in Sjögren's syndrome? *Rheumatology* (Oxford) 2021; 60(3): 1282-90. https://doi.org/10.1093/rheumatology/keaa441
15. DE VITA S, GANDOLFO S: Predicting lymphoma development in patients with Sjögren's syndrome. *Expert Rev Clin Immunol* 2019; 15: 929-38. https://doi.org/10.1080/1744666X.2019.1649596
16. ZABOTTI A, ZANDONELLA CALLEGHER S, TULLIO A *et al*.: Salivary gland ultrasonography in Sjögren's syndrome: a European Multicenter Reliability Exercise for the HarmonicSS Project. *Front Med* 2020; 7: 581248. https://doi.org/10.3389/fmed.2020.581248
17. ZANDONELLA CALLEGHER S, GIOVANNINI I, ZENZ S *et al*.: Sjögren syndrome: looking forward to the future. *Ther Adv Musculoskelet Dis* 2022; 14: 1759720X221100295. https://doi.org/10.1177/1759720X221100295
18. PARKER SH, JOBE WE, DENNIS MA *et al*.: US-guided automated large-core breast biopsy. *Radiology* 1993; 187: 507-11. https://doi.org/10.1148/radiology.187.2.8475299
19. GIOVANNINI I, LORENZON M, MANFRÈ V *et al*.: Safety, patient acceptance and diagnostic accuracy of ultrasound core needle biopsy of parotid or submandibular glands in primary Sjögren's syndrome with suspected salivary gland lymphoma. *RMD Open* 2022; 8: e001901. https://doi.org/10.1136/rmdopen-2021-001901
20. LORENZON M, TULIPANO DI FRANCO F, ZABOTTI A *et al*.: Sonographic features of lymphoma of the major salivary glands diagnosed with ultrasound-guided core needle biopsy in Sjögren's syndrome. *Clin Exp Rheumatol* 2021; 39 (Suppl. 133): S175-83. https://doi.org/10.55563/clinexprheumatol/4c36nr.
21. ZABOTTI A, LORENZON M, GIOVANNINI I *et al*.: Major salivary gland biopsy in Sjögren's syndrome, with special reference to the histopathology of B-cell proliferation. *In*: BRUYN GAW (Ed.): Sjögren's syndrome and the salivary glands. Springer International Publishing, 2022, 161-70. https://doi.org/10.1007/978-3-030-90977-2_14
22. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol* 2017; 69: 35-45. https://doi.org/10.1002/art.39859
23. SPIJKERVET FKL, HAACKE E, KROESE FGM, BOOTSMA H, VISSINK A: Parotid gland biopsy, the alternative way to diagnose Sjögren syndrome. *Rheum Dis Clin N Am* 2016; 42: 485-99. https://doi.org/10.1016/j.rdc.2016.03.007
24. DE VITA S, BOIOCCHI M, SORRENTINO D *et al*.: Characterization of prelymphomatous stages of B cell lymphoproliferation in Sjögren's syndrome. *Arthritis Rheum* 1997; 40: 318-31. https://doi.org/10.1002/art.1780400217
25. BALDINI C, LUCIANO N, SEGHIERI C, FERRO F, ELEFANTE E, MOSCA M: Early damage assessment and prediction of damage accrual in primary Sjögren's syndrome using salivary gland ultrasonography during 2 years of follow-up. *Ann Rheum Dis* 2017;7 6:5 89. https://doi.org/10.1136/annrheumdis-2017-eular.4327
26. ZABOTTI A, ZANDONELLA CALLEGHER S, GANDOLFO S *et al*.: Hyperechoic bands detected by salivary gland ultrasonography are related to salivary impairment in established Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S146-52.
27. DELLI K, HAACKE EA, KROESE FGM *et al*.: Towards personalised treatment in primary Sjögren's syndrome: Baseline parotid histopathology predicts responsiveness to rituximab treatment. *Ann Rheum Dis* 2016; 75: 1933-8. https://doi.org/10.1136/annrheumdis-2015-208304
28. JOUSSE-JOULIN S, DEVAUCHELLE-PENSEC V, CORNEC D *et al*.: Ultrasonographic salivary glands response to rituximab in primary Sjögren syndrome patients in the tolerance and efficacy of rituximab in primary sjogren syndrome study is not associated with the anatomopathology changes. *Arthritis Rheum*.2013; 65: S1236. https://doi.org/10.1002/art.38216.
29. DE VITA S, DE MARCHI G, SACCO S, GREMESE E, FABRIS M, FERRACCIOLI G: Preliminary classification of nonmalignant B cell proliferation in Sjögren's syndrome: perspectives on pathobiology and treatment based on an integrated clinico-pathologic and molecular study approach. *Blood Cells Mol Dis* 2001; 27: 757-66. https://doi.org/10.1006/bcmd.2001.0446
30. KROESE FGM, HAACKE EA, BOMBARDIERI M: The role of salivary gland histopathology in primary Sjögren's syndrome: promises and pitfalls. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S222-33.
31. RISSELADA AP, LOOIJE MF, KRUIZE AA, BIJLSMA JWJ, VAN ROON JAG: The role of ectopic germinal centers in the immunopathology of primary Sjögren's syndrome: a systematic review. *Semin Arthritis Rheum* 2013; 42: 368-76. https://doi.org/10.1016/j.semarthrit.2012.07.003