Rheumatology International (2022) 42:1411–1421
https://doi.org/10.1007/s00296-021-04949-6

Rheumatology
INTERNATIONAL

OBSERVATIONAL RESEARCH

Focal lymphocytic sialadenitis and ectopic germinal centers in oral reactive lesions and primary Sjögren's syndrome: a comparative study

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Received: 13 April 2021 / Accepted: 13 July 2021 / Published online: 20 July 2021

Abstract

Focal lymphocytic sialadenitis (FLS), an important diagnostic criterion for Sjögren's syndrome (SS) diagnosis, can also be observed when assessing minor salivary gland (mSG) biopsies from healthy asymptomatic individuals (non-SS patients). Fifty cases of primary SS (pSS group) and 31 cases of oral reactive lesions (non-SS non-sicca group) containing also typical FLS features, were assessed by morphological and immunohistochemical (CD10, CD23 and Bcl-6) analysis, aiming at the detection of GCs. All pSS cases showed FLS with focus score (FS) ≥ 1. In the non-SS non-sicca group, 12, 10 and 9 cases showed FLS with FS ≥ 1, FLS with FS < 1 and FLS associated with chronic sclerosing sialadenitis with FS < 1, respectively. The morphological analysis revealed similar frequency of GCs in pSS (20%) and non-SS non-sicca group (19%). The area ($p = 0.052$) and largest diameter ($p = 0.245$) of GCs were higher in pSS than non-SS non-sicca group. The FS and number of foci were significantly higher in pSS than non-SS non-sicca group with FS < 1. Immunohistochemistry confirmed all morphological findings (GCs showing CD23 and Bcl-6 positivity, with variable CD10 expression) and additionally in 3 and 1 cases of the pSS and non-SS non-sicca group, respectively. Moreover, another 6 and 2 cases of the pSS and non-SS non-sicca group with FS ≥ 1, respectively, showed positivity only for CD23. FLS can also be observed when assessing oral reactive lesions, which showed similar frequency of GCs with those found in pSS patients. Further studies, including functional analysis of lymphocytic populations and GCs in FLS, are encouraged.

Keywords Sjögren's syndrome · Non-Sjögren's non-sicca patient · Oral reactive lesions · Focal lymphocytic sialadenitis · Ectopic germinal centers · Immunohistochemistry

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease that mainly affects the salivary and lacrimal glands, causing xerostomia and ceratoconjunctivitis sicca, respectively [1]. The SS prevalence in a general population varies from 0.5 to 3%, with an incidence of 4 cases per 100,000 per year. Moreover, the frequent reported male/female ratio of 1:9 seems to be more suitable in the range of 1:20. The SS is predominantly observed in middle-aged adults and may manifest alone [primary SS (pSS)] or associated with other autoimmune disease [secondary SS (sSS)], especially rheumatoid arthritis and systemic lupus erythematosus [2]. Extraglandular manifestations, such as myalgia, arthralgia and fatigue are not uncommon findings (5–10% of pSS patients) and immunological disturbances, such as hypergammaglobulinemia, rheumatoid factor, hypocomplementemia, cryoglobulinemia and autoantibodies directed against SSA/Ro and SSB/La autoantigens are frequently detected [3].

Notably, the histopathological analysis of the minor salivary glands (mSGs) showing focal lymphocytic sialadenitis (FLS) with focus score (FS) ≥ 1, is an important diagnostic criterion for defining SS [4]. Germinal centers (GCs), which are specialized microenvironments within lymphoid tissues,

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where B cells undergo extensive proliferation, somatic hypermutation and antigen affinity selection, can be visualized when assessing mSG biopsies from SS patients [1, 5–11]. Noteworthy, the presence of GCs has been linked to higher risk of lymphoma development [5, 10–13], as well as higher FS, reduced saliva production and higher levels of rheumatoid factor (RF), anti-SSA antibodies, anti-SSB antibodies and proinflammatory mediators [1, 5–10, 14, 15]. However, other studies indicate that GCs are not predictive for lymphoma development in SS patients [3, 16, 17].

Previous studies in pSS, through hematoxylin and eosin (H&E) staining and some of them also by immunohistochemistry, have shown that GCs can be detected in approximately 25% of mSG biopsies [5, 9, 10], ranging from 16.5 to 54.5% [11, 18]. In addition, other studies have also evaluated the presence of GCs in parotid gland biopsies [12, 19–21], which presented similar [19, 21] or high (76% and 100%) [12, 20] frequency of GCs when compared with mSGs in pSS. By immunohistochemistry, the GCs were assessed through CD21, CD23, CD35 and Bcl-6 markers [12, 14, 16, 22–24], with a recent study [21] indicating Bcl-6 as a sensitive and specific marker for unequivocal identification of GCs in mSG and major salivary gland (MSG) biopsies obtained from pSS patients.

Interestingly, similar with the previous studies [25–33] (Supplementary Table S1), we have observed typical microscopical features of FLS assessing intraoral biopsies from healthy asymptomatic, non-SS patients in our Oral Histopathology Laboratory. In fact, in the studies previously mentioned, 8 out of 54 (15%) mSGs of healthy volunteers [33] and 6 out of 40 (15%) labial salivary glands of coroner's autopsies with no clinical findings suggesting SS [29] were microscopically diagnosed as FLS with FS $\geq$ 1. However, relevantly, the prevalence of GCs in these cases [25–33], which appears to have a prognostic impact in SS patients [5], is unknown.

Thus, the aim of the current study was to comparatively analyze mSG biopsies presenting FLS obtained from patients with SS and non-SS (healthy asymptomatic individuals) and to determine the frequency of GCs, aiming at a better understanding of the prognostic impact of this morphological finding.

Materials and methods

Patients and biopsy samples

This study was approved by the Research Ethics Committee of the Ribeirão Preto Medical School, University of São Paulo (protocol number: 68748117.0.0000.5440) and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. We conducted a retrospective study including 81 cases, being 50 pSS cases (SS group) diagnosed according to previously established criteria [4] and 31 cases of healthy asymptomatic patients (non-SS non-sicca group). These 31 cases were selected after careful microscopical analysis of 792 oral biopsies [563 females (mean age, 55.4 years) and 229 males (mean age, 55.8 years)], clinically and microscopically diagnosed as mucocele, inflammatory fibrous hyperplasia or ranula, which also presented typical FLS features at the periphery of the specimen and away from the main lesion [29, 33]. For non-SS non-sicca group, inclusion criteria were patients with available clinical data, as well as formalin-fixed paraffin-embedded (FFPE) tissue suitable for histopathological and immunohistochemical (IHC) analysis. Exclusion criteria were autoimmune diseases, endocrine disorders, immunodeficiency disorders, smokers and former smokers, ethilists and former ethilists, as well as use of antibiotics, corticosteroids or non-steroidal anti-inflammatory drugs.

Histopathological analysis

Sections of 5-µm were obtained from all cases and stained with H&E for diagnostic confirmation. All 81 cases presented mSGs with a diagnosis of FLS, in which the FS and presence of GCs was assessed. FS is the total number of foci (one focus is a cluster of $\geq$ 50 lymphocytes adjacent to normal-appearing salivary gland parenchyma) per 4 mm^2 of glandular tissue examined [4]. GC was defined as a well-circumscribed, round to oval shaped structure of at least 50 mononuclear cells and presenting features indicative of lymphoid organization, such as a densely packed dark zone and a light zone within otherwise normal salivary gland epithelium [3, 10].

Immunohistochemistry

For IHC analysis, consecutive (serial) histological sections of 3-µm were placed on organosilane coated slides (Sigma-Aldrich, St. Louis, MO). The deparaffinization using xylene solution was performed and the rehydration of the sections occurred through the passage in ethanol solution with serial concentrations. The antigen retrieval involved the immersion of the sections in citrate buffer (target retrieval solution, pH6). After, the sections were submitted to the immunohistochemistry technique by streptavidin–biotin-peroxidase method (Universal LSAB™ + Kit/HRP, Dako, Carpinteria, CA, USA) to evaluate the presence of GCs using the primary antibodies: Bcl-6 (Clone LN22, dilution 1:500, Leica Biosystems, United Kingdom), CD10 (Clone 56C6, dilution 1:500, Leica Biosystems, United Kingdom) and CD23

(Clone 1B12, dilution 1:500, Leica Biosystems, United Kingdom).

The IHC staining was evaluated by three experienced pathologists (RC, JEL and ARS) blinded to the clinicopathological features, using a computerized system consisting of a light microscope (Leica DM500), adapted to a high-resolution camera (Leica ICC50) and a color video monitor. The images were obtained using the Leica IM50 Image Manager Program and the processing was done through the Leica QWin Image Processing and Analysis System. After evaluation of the slides at × 100 magnification, areas presenting positive immunostaining at × 400 magnification (0.25 mm^2) were analyzed, for confirmation of the GC structures.

The antibodies were assessed as being positive or negative, within a focus. CD23 marker was considered positive when highlighted the follicular dendritic cell network, preferably in concentric arrangement [14]. CD10 marker was considered positive when GC B cells showed a cytoplasmic and/or cell membrane staining pattern [34]. Bcl-6 marker was considered positive when a cluster of ≥ 5 adjacent GC B cells showed a nuclear staining pattern [16, 21]. Next, on each IHC slide, the frequency of immunopositive areas was recorded.

Statistical analysis

Statistical analysis was performed using the IBM SPSS 20.0 software. The Shapiro–Wilk's test for assessing the normal distribution was used. The Student's *t* test and Pearson's correlations were applied in samples with a normal distribution, whereas the Mann–Whitney *U* test and Spearman's correlation were applied in samples with a non-normal distribution. For categorical data, the chi-square test was applied. A probability (p) value < 0.05 was considered statistically significant.

Results

SS group

This group included 50 patients; 46 were women and 4 were men, with an age range from 12 to 81 years (mean age, 55 years). Only one pediatric case affecting a 12-year-old female patient was included in the current study. When assessing the lower lip mSG biopsies, all pSS cases showed FLS with FS ≥ 1. The FS ranged from 1.2 to 10 (mean, 2.7) (Table 1 and Fig. 1). The serological analysis revealed that 78%, 50%, 40% and 30% of the patients presented positivity for anti-SSA, ANA, anti-SSB and RF, respectively. A significant positive correlation ($p < 0.05$) was observed only when comparing anti-SSA with the number of foci and FS.

Non-SS non-sicca group

This group included 31 cases; 18 were women and 13 were men, with an age range from 9 to 69 years (mean age, 42.3 years). These cases were clinically and microscopically diagnosed as inflammatory fibrous hyperplasia ($n = 17$), mucocele ($n = 13$) and ranula ($n = 1$). Of them, 12 cases (10 women, 2 men; mean age, 36.7 years) also showed FLS with FS ≥ 1 (ranging from 1.1 to 2.7; mean, 1.4), 10 cases (6 women, 4 men; mean age, 30.1 years) also showed FLS with FS < 1 (ranging from 0.1 to 0.6; mean 0.2) and 9 cases (6 women, 3 men; mean age, 56 years) also presented FLS associated with focal areas of chronic sclerosing sialadenitis (CSS) (FLS/CSS) with FS < 1 (ranging from 0.1 to 0.7; mean, 0.25) (Table 1; Fig. 1).

The FS and number of foci were significantly higher in SS rather than non-SS non-sicca group with FS < 1 ($p < 0.05$).

Ectopic GC frequency

In the SS group, the morphological analysis revealed that 20% (10/50) of the cases presented GCs (ranging from 1 to 10; mean, 3.8). Moreover, in 3 cases, the presence of GCs (ranging from 1 to 3) was identified only by immunohistochemistry. In addition, there was a statistically significant correlation when comparing GCs with FS and number of foci ($p < 0.05$).

In the non-SS non-sicca group, the morphological analysis revealed the presence of GCs (ranging from 1 to 6; mean, 1.8) in 5 out of 22 (23%) cases which presented FLS (4 with FS ≥ 1 and 1 with FS < 1) and in 1 out of 9 (11%) cases (3 GCs), which presented FLS/CSS with FS < 1. Moreover, in 1 case, which presented FLS with FS ≥ 1, the presence of 2 GCs was identified only by immunohistochemistry. There was no statistically significant correlation when comparing GCs with FS and number of foci.

All cases presenting GCs on morphological analysis, also exhibited GCs immunopositive for CD23 and Bcl-6. Of them, only 3 and 2 cases of the pSS and non-SS non-sicca group, respectively, also showed positivity for CD10 (Fig. 2). All 3 and 1 cases of the pSS and non-SS non-sicca group with FS ≥ 1, respectively (identified only by immunohistochemistry), were positive for CD23 and Bcl-6. In addition to these findings, another 6 and 2 cases of the pSS and non-SS non-sicca group with FS ≥ 1, respectively, showed positivity only for CD23.

Moreover, through morphometric analysis, the area and diameter of the GCs were greater in pSS than non-SS non-sicca patients, but without statistically significant differences (Table 1).

Table 1 Clinicopathological features of the cases included in the current study

Clinicopathological features	pSS group (no. patients = 50) (%)	Non-SS non-sicca group (no. patients = 31) (%)
Mean age/range (years)	55/12–81	42.3/9–69
Gender		
Female	46	18
Male	4	13
Diagnosis		
pSS	50	–
Inflammatory fibrous hyperplasia	–	17
Mucocele	–	13
Ranula	–	1
Serological parameters[§]		
Anti-SSA positive, no. (%)	39/50 (78)	–
Anti-SSB positive, no. (%)	20/50 (40)	–
RF positive, no. (%)	9/30 (30)	–
ANA positive, no. (%)	15/30 (50)	–
Histopathological findings		
FLS with FS ≥ 1	50	12
FLS with FS < 1	–	10
FLS/CSS with FS < 1	–	9
GCs based on H&E, no. (%)	10/50 (20)	6/31 (19)
Range of GCs (mean)	1–10 (3.8)	1–6 (1.8)
Morphometry of GCs[#]		
Area ± SD (mm^2)	0.11 ± 0.08	0.07 ± 0.04
Largest diameter ± SD (μm)	408.5 ± 136.6	356 ± 95.8
GCs based on IHC analysis, no. (%)*	13/50 (26)	7/31 (22)

ANA antinuclear antibodies, *CSS* chronic sclerosing sialadenitis, *FLS* focal lymphocytic sialadenitis, *FS* focus score, *GC* germinal center, *H&E* hematoxylin and eosin stain, *IHC* immunohistochemical, *pSS* primary Sjögren's syndrome, *RF* rheumatoid factor, *SD* standard deviation, *SSA* Sjögren's syndrome antigen A, *SSB* Sjögren's syndrome antigen B

*IHC analysis included CD10, CD23 and Bcl-6 markers. All GCs identified by H&E stain were also CD23 and Bcl-6 positive, with variable CD10 expression

[§]Anti-SSA titer > 25 EU/ml, Anti-SSB titer ≥ 50 EU/ml, ANA titer ≥ 1:320, and gM RF > 12.5 IU/ml titer, were considered positive. A significant positive correlation ($p < 0.05$) was observed only when comparing anti-SSA with the number of foci and FS

[#]No statistically significant differences were observed when comparing the area ($p = 0.052$) and largest diameter ($p = 0.245$) of GCs between pSS and non-SS non-sicca patients

Discussion

To the best of our knowledge, this is the first study that evaluates a large series of pSS and oral reactive lesions, both presenting FLS. This sialadenitis was assessed through morphological and IHC analysis, aiming at the detection of GCs, which seems to have a prognostic impact in pSS patients [5]. Noteworthy, we have shown a similar frequency of GCs in these two populations; however, without significant differences, a morphometric analysis revealed that the area and diameter of the GCs were greater in pSS than non-SS non-sicca patients, suggesting that some differences (perhaps qualitative or functional), other than quantitative, should be considered. Considering serological parameters, a significant positive correlation only when comparing anti-SSA levels with the number of foci and FS, was observed. In addition, the information available on the follow-up of patients indicates that none of them developed lymphoma and that in all non-SS non-sicca patients there was complete resolution of the lesion after biopsy.

We have previously shown that inflammatory fibrous hyperplasia, a very common oral reactive lesion, can present oncocytic metaplasia areas and inflammatory lymphoid infiltrate associated with GCs [35]. Moreover, in our Oral Pathology Laboratory, we have had the opportunity to evaluate oral reactive lesions presenting mSGs, at the periphery of the specimen and away from the main lesion, presenting typical microscopical features

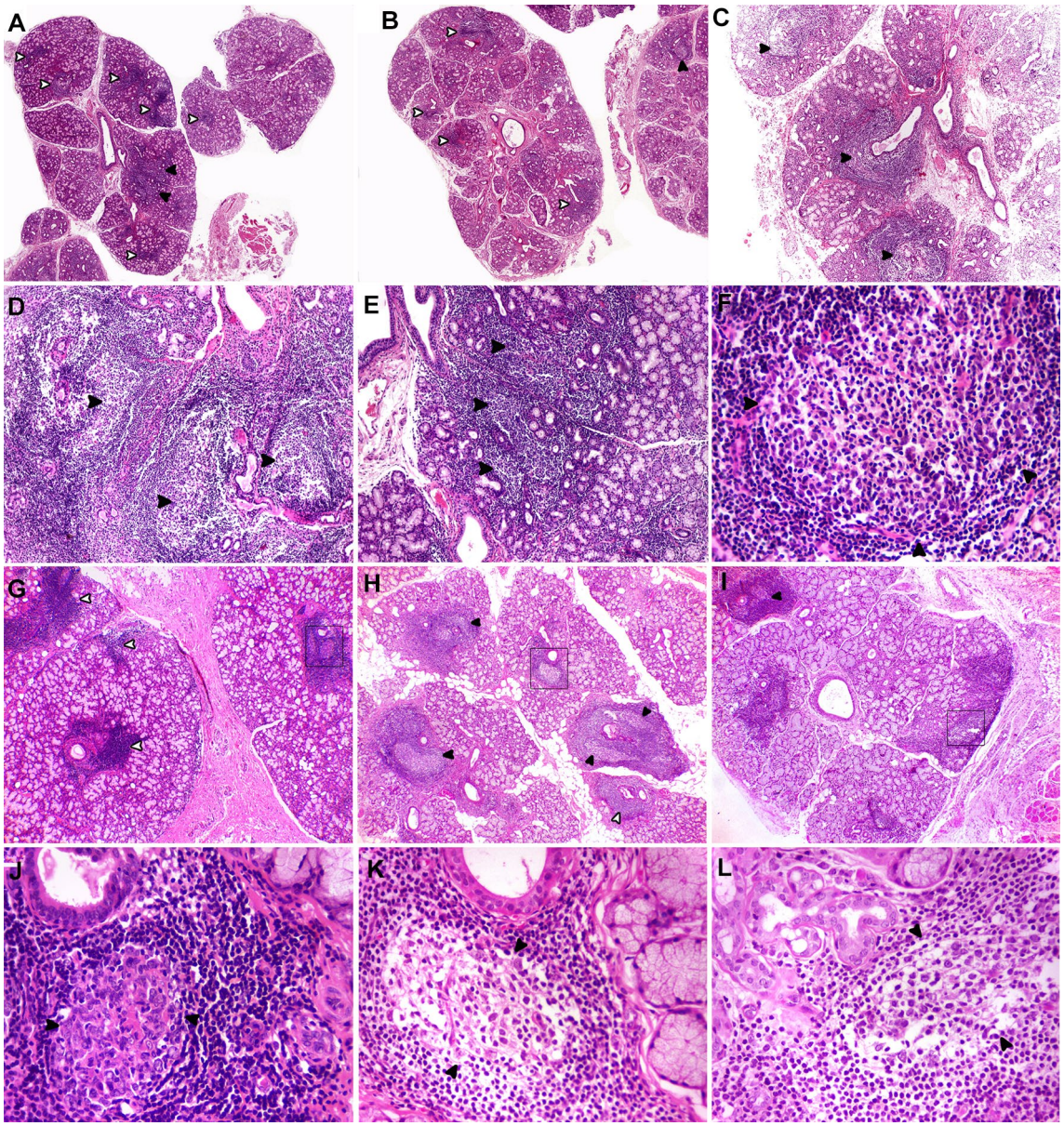

Fig. 1 Histopathological analysis on hematoxylin and eosin (H&E) staining showing focal lymphocytic sialadenitis in minor salivary gland biopsy specimens obtained from primary Sjögren's syndrome patients. In these cases, several foci were observed (white arrowheads), containing germinal center-like structures (black arrowheads) (**A** and **B**, ×2.5; **C**, ×5; **D** and **E**, ×10; and **F**, ×40). Similarly, notice focal lymphocytic sialadenitis in oral reactive lesions obtained from non-Sjögren's syndrome non-sicca patients. In these cases, several foci (white arrowheads) were observed, containing germinal center-like structures (black arrowheads) (**G**, **H** and **I**, ×4; **J**, **K** and **L**, ×40). The squares in G, H and I are shown in increasing magnification in **J**, **K** and **L**, respectively

of FLS. Noteworthy, FLS is an important criterion for SS diagnosis. After an extensive review of the literature, we have found previous studies emphasizing the FLS when evaluating mSGs, MSGs and lacrimal glands from healthy patients [26, 27, 29, 33] and postmortem assessment [25, 28, 30–32]. These findings show that FLS can also be detected in non-SS non-sicca patients, such as shown in the present study. Moreover, these previous studies showed that the percentage of cases with FS ≥ 1 varied between 8.3% and 32%, whereas in the current study 12 out of 31 (38%) patients presented FS ≥ 1.

Another interesting finding in our study is the presence of CSS areas in the non-SS non-sicca group, which occurred in 9 out of 31 (29%) cases. These CSS areas represented less than 10% of the whole of the glandular surface area. Similarly, in the current pSS group, 5 out of 50 (10%) cases presented focal CSS areas, representing less than 7% of the whole of the glandular surface area. In fact, parenchymal

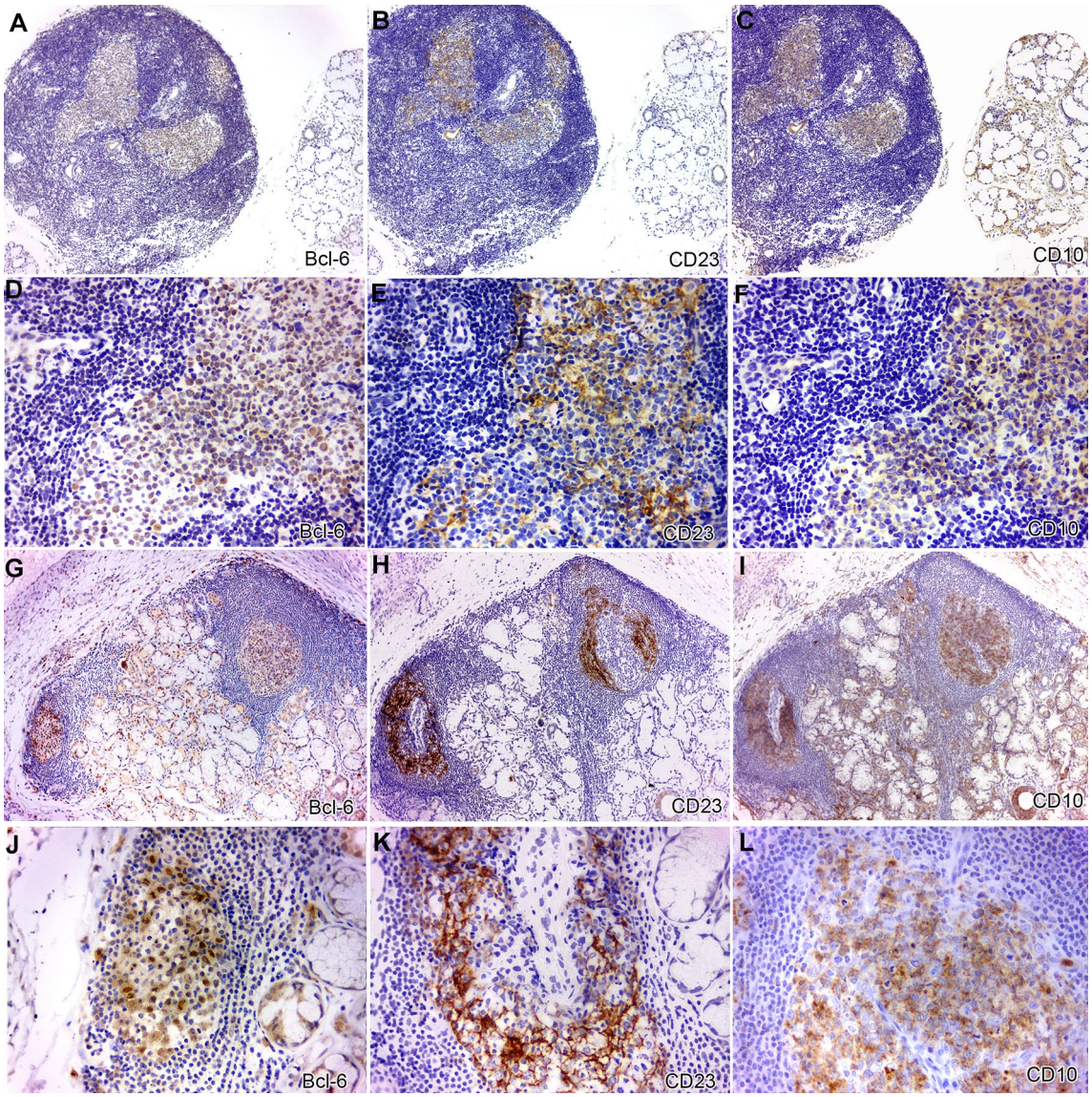

Fig. 2 Immunohistochemical analysis in consecutive (serial) sections of focal lymphocytic sialadenitis in minor salivary gland biopsy specimen obtained from primary Sjögren's syndrome patient **A**–**F**. In this case, notice germinal center-like structures showing strong positivity for Bcl-6 (**A** and **D**), CD23 (**B** and **E**) and CD10 (**C** and **F**) (**A**, **B** and **C**, × 10; **D**, **E** and F, × 40). The **D**, **E** and **F** photomicrographs represent higher magnification of **A**, **B** and **C** photomicrographs, respectively. Similarly, notice the immunohistochemical analysis in consecutive (serial) sections of focal lymphocytic sialadenitis in oral reactive lesion obtained from non-Sjögren's syndrome non-sicca patient, which showed germinal center-like structures with positivity for Bcl-6 (**G** and **J**), CD23 (**H** and **K**) and CD10 (**I** and **L**) (**G**, **H** and **I**, × 10; **J**, **K** and **L**, × 40). The **J**, **K** and **L** photomicrographs represent higher magnification of **G**, **H** and **I** photomicrographs, respectively

atrophy and large area of fibrosis, alongside areas of FLS, can be detected in mSG biopsies obtained from pSS patients [36]. Moreover, from our results, it is possible that CSS areas in the non-SS non-sicca group will have the potential to reduce the FS, such as previously commented [36].

Such as above commented, GCs can be visualized when assessing mSG biopsies in SS patients [1, 5–11] which have been linked to higher risk of lymphoma development [5, 10–13], as well as higher FS, reduced saliva production and higher levels of antibodies and proinflammatory mediators [1, 5–10, 14, 15]. However, other studies have not supported this proposal indicating that GCs may not be predictive for lymphoma development [3, 16, 17]. In this context, it is relevant that most studies indicate that GCs can be detected between 16.5% and 54.5% (mean, 25%) of mSG biopsies in pSS [5, 9–11, 18]. Therefore, these findings show the need for standardisation of the detection method of GCs in SG biopsies from pSS patients [16, 36].

In fact, such as shown in Table 2, there are several studies assessing GCs in mainly mSG biopsies, through morphological (H&E stain) and IHC analysis. The percentage of cases presenting GCs on H&E-stained slides varied between 16.5 and 54.5% in mSGs and between 50 and 75% in MSGs. The IHC analysis, in general, showed an

Table 2 List of studies showing clinicopathological features and frequency of germinal centers in primary Sjögren syndrome patients

No	Author [ref.]	pSS cases/ female (*n*)	Age at diagnosis (mean ± SD, median, range) years	FS ≥ 1 (mean ± SD, range, %)	SG biopsy[a]	GCs (H&E) (%)	GCs (IHC)[b] (%)	Immunomarkers used
01	Szodoray et al. [56]	19/19	51 ± 15	3.75 ± 2.52 GC+ 4.81 ± 3.06 GC−	Minor	8/19 (42.1)	ND	–
02	Jonsson et al. [1]	130/NS	51 ± 13	4.1 GC+ 3.6 GC−	Minor	33/130 (25.4)	10/18 (55.5)	CD35, CD20, Ki-67
03	Jonsson et al. [18]	22/22	50 ± 3	4.0 ± 1.0	Minor	12/22 (54.5)	ND	–
04	Daridon et al. [22]	18/15	range 32–77	NS	Minor	NS	7/18 (39)	CD21, CD35, Ki-67
05a	Bombardieri et al. [12]	19/NS	53; range 21–72	NS	Minor	NS	8/19 (42.1)	CD3/CD20, CD21
05b	Bombardieri et al. [12]	5/4	52 ± 4	NS	Major	NS	5/5 (100)	CD3/CD20, CD21
06	Jonsson et al. [6]	169/160	54; range 45–62	2.0 ± 1.0	Minor	47/169 (28)	9/11 (82)	CD21
07	Manoussakis et al. [58]	21/19	49; range 28–80	range 1.0–12.0	Minor	NS	4/21 (19)	CD3, CD20, Ki-67
08	Pijpe et al. [19]	15/NS	NS	87%	Major	6/12 (50)	ND	–
09	Jonsson and Skarstein, 2008 [14]	60/58	55 ± 2	2.6 ± 0.24	Minor	11/60 (18)	12/60 (20)	CD21, CD23, CD35
10	Gatumu et al. [57]	17/16	51 ± 15	range 1.0–7.0	Minor	NS	10/17 (59)	CD21
11	Le Pottier et al. [23]	40/40	range 31–72	NS	Minor	NS	11/40 (27.5)	CD21, CD35
12	Reksten et al. [7]	115/NS	51 ± 1	2.42 ± 0.16	Minor	27/115 (23.5)	ND	–
13	Christodoulou et al. [13]	39/NS	55; range 24–70	3.38; range 1.0–8.9	Minor	8/39 (20.5)	ND	–
14	Szyszko et al. [15]	21/19	55 ± 9	range 1.0–12.0	Minor	7/21 (33.3)	ND	–
15	Theander et al. [10]	175/161	51 ± 13	78%	Minor	43/175 (25)	ND	–
16	Fei et al. [24]	103/100	44 ± 11	89.5%	Minor	NS	22/103 (21.1)	CD21
17	Johnsen et al. [3]	49/ 42	51; range 29–85	2.0; range 0–10	Minor	17/40 (43)	27/40 (68)	CD21
18	Delli et al. [20]	25/NS	NS	1.6; range 0.8–3.3	Major	19/25 (76)	ND	–
19	Lee et al. [8]	93/91	44 ± 11	2.71 ± 0.66 GC+ 1.65 ± 0.97 GC−	Minor	28/93 (30.1)	28/93 (30.1)	CD21
20a	Haacke et al. [16]	11/11[c]	47 ± 14	1.8; range 0.8–4.0	Minor	2/11 (18)	3/11 (27)	Bcl-6
20b	Haacke et al. [16]	22/22[d]	48 ± 17	2.7; range 1.4–3.5	Minor	4/22 (18)	5/22 (23)	Bcl-6
21	He et al. [9]	126/124	49 ± 11	2.02 ± 0.71 GC+ 1.78 ± 0.78 GC−	Minor	36/126 (28.6)	36/126 (28.6)	CD21
22	Sène et al. [11]	115/100	53; range 50–56	76.5%	Minor	19/115 (16.5)	ND	–
23a	Nakshbandi et al. [21]	36/NS	NS	100%	Minor	3/210 foci	50/210 CD21+ 9/210 Bcl-6+	CD21, Bcl-6
23b	Nakshbandi et al. [21]	31/NS	NS	100%	Major	9/141 foci 69/141 CD21+ 22/141 Bcl-6+		CD21, Bcl-6

FS focus score, *GCs* germinal centers, *H&E* hematoxylin and eosin stain, *IHC* immunohistochemical analysis, *NS* not specified, *ND* not done, *pSS* primary Sjögren's syndrome, *SD* standard deviation, *SG* salivary gland

[a]All biopsies were obtained from lower lip (minor SG) and parotid gland (major SG)

[b]Only in #4 and #11 studies, the GCs were assessed by immunofluorescence

[c]pSS patients diagnosed with parotid MALT lymphoma

[d]pSS patients without lymphoma

increase in these values (between 19 and 82% in mSGs and 100% in a single study on MSGs). The immunomarkers also showed variability, since lymphocyte surface (CD3, CD20), follicular dendritic cell (FDC) (CD21, CD23, CD35), GC B cell (Bcl-6) and cell proliferation (Ki-67) markers were used to detect GCs (Table 2). Taken together, these data evidently show a notable variation in the frequency of GCs, possibly reflecting a lack of standardization in both morphological and IHC criteria, in pSS patients.

Notably, the IHC analysis is a powerful tool that complements the morphological analysis; however, it is important to carefully interpret their results. A recent study has emphasized that positivity for CD21 (FDC marker) does not necessarily indicate the presence of GCs, proposing to use Bcl-6 as a sensitive and specific marker for unequivocal identification of GCs, when assessing mSG and MSG biopsies from pSS patients [21]. Accordingly, in the current study, all GCs showed positivity for both CD23 and Bcl-6, whereas 8 cases exhibited positivity only for CD23, which after evaluating consecutive serial sections (H&E, CD10-negative and Bcl-6-negative slides), providing rich details of cell morphology, absence of B cell blasts was noticed.

The FDCs are a unique type of cell located within lymphoid follicles, containing and retaining immune complexes and expressing molecules involved in the proliferation and differentiation of B cells. FDC markers include antibodies to complement receptors, such as CD21 and CD35, IgE FC receptor (CD23) and IgG FC receptor (CD32). In both primary (without GC) and secondary (with GC) follicles from reactive lymph nodes, FDCs are CD21 and CD35 positive. FDCs in GC light zone additionally upregulate CD23 and CD32. Notably, upregulation and downregulation of CD23 by FDCs appears to be related to B cell centrocytic and centroblastic differentiation, respectively [37, 38]. Such as shown in Table 2, eleven, four and two studies have assessed CD21, CD35 and Bcl-6, respectively, aiming to detect GCs in pSS. It is possible that primary follicles or FDC networks have been interpreted as GCs [14, 16, 21]. Such as shown in previous study, we have used CD23, which seems to have apparent restriction to GCs, for comparative analysis with studies assessing CD21 and CD35 and we have found similar results [19, 38].

The CD10 antigen is a cell surface zinc-dependent metalloprotease, which is expressed in several cell types, including hematolymphoid, epithelial and mesenchymal origin. In normal lymph node, CD10 expression is preferentially confined to GCs (i.e., secondary follicles). Stromal cells, scattered lymphocytes and granulocytes in the interfollicular areas are also CD10 positive [39]. Thus, it is due to the preferential expression in the secondary follicle that in the current study we decided to evaluate CD10 expression. However, our results showed variable CD10 expression in GCs, being positive in only 3 out of 13 and 2 out of 7 cases of the pSS and non-SS non-sicca group, respectively.

The BCL-6 gene encodes a 95-kD nuclear phosphoprotein, belonging to the BTB/POZ/ZincFinger (ZF) family of transcription factors, which is essential for GC B cell and follicular helper T (Tfh) cell development [40]. In GC, unlike from centroblasts and centrocytes, Tfh cells are not organised in clusters and usually show a rounded shape [21, 41]. Thus, it has been proposed that a cluster of ≥ 5 adjacent Bcl-6 positive cells within a focus be classified as a GC [16]. Similarly, in the current study, clusters of centroblasts were efficiently detected through Bcl-6 expression. Moreover, in our experience, the CD57 expression can be used to assist in the identification of Tfh cells within GCs (data not shown).

Such as previously commented, while some studies concluded that the presence of GCs in mSG biopsies from pSS patients was predictive of lymphoma development [10–12], other studies showed opposite results [3, 16, 17]. In this context, other findings such as FS ≥ 3 [42, 43], elevated FcRL4 + expression [44], higher pSTAT-3 expression [45], weak or absent A20 immunostaining [46], lymphoepithelial lesion [47], recurrent or permanent swelling of MSGs, lymphadenopathy, cryoglobulinemia, splenomegaly, low complement levels of C4 and C3, skin vasculitis [48–54], lymphopenia [52, 53], M-component in serum or urine, peripheral neuropathy, glomerulonephritis, elevated beta2-microglobulin and CD4 lymphocytopenia [48, 49, 52, 53], have been proposed to identify SS patients with an increased risk for lymphoma development, which seems to correspond to 14% of the cases [51, 53, 55].

In summary, we have shown for the first time that FLS can also be detected when assessing intraoral reactive lesions, which exhibited similar frequency of GCs (by both morphological and IHC analysis) when comparing to mSG biopsies obtained from pSS patients. All GCs detected on morphological analysis, were also CD23 and Bcl-6 positive, with variable expression of CD10, whereas 8 cases showed positivity only for CD23. Our results suggest standardised protocols for morphological and IHC analysis when assessing GCs in mSG biopsies from pSS patients. Because the similar frequency of GCs in oral reactive lesions and pSS, further studies including functional analysis of lymphocytic populations and GCs in FLS are encouraged.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00296-021-04949-6.

Author contributions RC, EMR, FCP, ARS, and AD, provided the clinical cases for this study. EVS, KCB, IBQ and FCJ, were responsible for collecting data from patients. JEL, RC, and LYA were responsible for the methodology. The immunohistochemical analysis was made in the Oral Immunopathology Laboratory (FORP/USP), coordinated by JEL. The authors BABA, LYA, EVS, and HAS, were responsible for histochemistry and immunohistochemical reactions, including analysis of results. JEL, EMR, FCP, RC, ARS, LYA, and EVS, were in charge

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Funding This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 (Evânio Vilela Silva). Luciana Yamamoto Almeida (2016/02713-2), Heitor Albergoni Silveira (2018/12734-2), Karen Cristine Bortoletto (2016/14636-2), and Jorge Esquiche León (2016/11419-0) have received research Grants from State of São Paulo Research Foundation (FAPESP).

Data availability The data underlying this study are available in the article and in its online supplementary material.

Code availability Not applicable.

Declarations

Ethical approval This study was reviewed and approved by the Research Ethics Committee of the Ribeirão Preto Medical School, University of São Paulo (Protocol number: 60786216.8.0000.5440) and all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors have no conflict of interest in the present manuscript.

Consent to participate Appropriate.

Consent for publication Appropriate.

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