Total area of inflammatory infiltrate and percentage of inflammatory infiltrate identify different clinical-serological subsets of primary Sjögren's syndrome better than traditional histopathological parameters

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ABSTRACT

Objective. *Recently, the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate have been proposed as novel histopathological parameters to improve the stratification of patients with Sjögren's syndrome (SS) in clinical trials. Both these parameters provide a more accurate assessment of the extent of the infiltrate in minor salivary gland biopsies (MSGBs) and may overcome the bias related to the Focus score (FS). To date, however, only few studies have investigated their clinical value and feasibility.*

In this study we revised consecutive MSGBs obtained routinely in a real-life clinical setting and correlated the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate both with the other MSGB histopathological parameters and with patients' clinical features in order to explore their usefulness in SS diagnostic work-up.

Methods. *We assessed the area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate in consecutive MSGBs and correlated these parameters with the number of foci, the FS and the presence of ectopic lymphoid structures (ELS). We also correlated these additional parameters with patients' clinical and biological data.*

Results. *We revised 69 MSGB samples: 46 from patients with a diagnosis of SS and 23 from subjects with no SS. The total area of inflammatory infiltrate and the percentage of inflammatory infiltrate appeared significantly higher in patients fulfilling the ACR/EULAR classification criteria for SS and correlated significantly with both the number of foci ($p<0.001$) and the FS ($p<0.001$). Particularly, they correlated better with the ELS in MSGBs than the number of foci and the FS. When we limited the analysis to the 32/69 patients with a FS<1, both the total area of the inflammatory infiltrate ($p=0.02$) and the percentage of the inflammatory infiltrate ($p=0.03$), but not the number of foci ($p=0.12$) remained significantly higher in the 10/32 anti-Ro/SSA positive patients fulfilling the ACR/EULAR classification criteria. Finally, the total area of inflammatory infiltrate and the percentage of inflammatory infiltrate correlated significantly with several biological and haematological SS-related abnormalities including hypergammaglobulinaemia, C4 levels, total number of white blood cells and the number of circulating lymphocytes.*

Conclusion. *The total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate in SS referral centres, and particularly for selected cases, may maximise the information on disease activity at tissue level, ultimately improving SS patients' assessment.*

Introduction

Sjögren's syndrome (SS) is a complex autoimmune disorder characterised by a wide spectrum of glandular and extra-glandular manifestations (1-4).

The diagnosis of disease relies particularly on minor salivary gland biopsy (MSGB) with focal lymphocytic sialadenitis (FLS), *i.e.* the presence of inflammatory foci, mainly composed of lymphocytes, which is the histological hallmark of the disease (5). A focus can be defined as a dense inflammatory aggregate composed of ≥50 lymphocytes, with plasma cells in a minor proportion, surrounding or closely associated to ducts or blood vessels, and adjacent to normal appearing acini (6, 7). At present, the most important and widely accepted parameter in defining

FLS is the focus score (FS), which expresses the number of foci per 4 mm^2 of salivary gland tissue and is calculated by dividing the number of foci by the glandular area of a sample and multiplying the result by 4 (6). As implied by the definition, the FS depends on the assessment of the number of foci and on the glandular surface area of the specimen. Therefore, in daily routine, the FS may be biased by difficulties in determining and counting the exact number of foci and by the variability in the area of the resected specimens of MSGBs, with clear clinical implications (5). Moreover, the assessment of the number of foci does not provide any information regarding the focus size since it does not differentiate smaller foci from larger ones, whereas it is widely recognised that the larger the focus size, the higher the complexity of the infiltrate and the possibility of a T/B cell segregation may be (8). In turn, the latter has been correlated with the extension and severity of SS systemic disease manifestations, implying an involvement of glandular immune responses in systemic disease features (9-12). Therefore, in the era of precision medicine it appears critical to overcome the potential pitfalls of the usual histological biomarkers to improve SS diagnosis and disease phenotyping.

Recently, it has been suggested that additional histopathological parameters could improve patients' assessment and stratification at least in clinical trials. Fisher *et al*. (6) have recently recommended reporting the presence of ectopic lymphoid structures (ELS) in MSGBs as the proportion of foci with both T/B-cell segregation and follicular dendritic cell networks by including immunohistochemical stainings for CD3, CD20 and CD21. However, a clear definition of ELS is still lacking and the frequency of these structures in real life cohorts has not yet been determined (8, 13). Moreover, it is unclear whether they indicate less common distinct SS phenotypes at higher risk for lymphoproliferative complications (12, 14-17).

Besides ELS, other complementary histological parameters are gaining increasing attention and have been proposed as histological biomarkers in clinical trials. Particularly, the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate (6, 9, 18).

The total area of the inflammatory infiltrate can be defined as the sum of the areas of each single inflammatory focus, whereas the percentage of the inflammatory infiltrate is the ratio between the total area of the inflammatory infiltrate and the total area of the MSGB surface (9).

Because both these parameters provide a more accurate assessment of the extent of the infiltrate in MSGBs and overcome the bias related to the FS, it is thought they better reflect the disease activity at tissue level. To date, however, only few studies have investigated their clinical value and feasibility in daily practice (9, 18).

In this study we aimed to investigate the correlations between the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate and the other MSGB histopathological parameters in consecutive MSGBs obtained routinely in a real-life clinical setting. Moreover, we analysed the correlations between these complementary histopathological parameters and patients' clinical features in order to explore their usefulness in SS diagnostic work-up and patients' stratification.

Methods

Patients

For the purpose of this study, we revised the latest 69 consecutive samples performed and analysed from January 2019 to April 2020 in patients with suspected SS and characterised by a FLS. Diagnosis of primary SS was made according to the ACR/EULAR 2016 classification criteria (7). In all the cases a complete rheumatological assessment was performed and the following information was collected: patients' demographic, glandular and extra-glandular clinical manifestations, biological data (particularly, complete blood count, IgG level, C3, C4 levels) and auto-antibody profiling (*i.e*. antinuclear antibody, anti-Ro/SSA, anti-La/SSB, Rheumatoid factor) and unstimulated salivary flow (USFR). The study was approved by the local ethics committee and all patients provided their informed consent.

MSGB histopathological assessment

For each of the 69 specimens the presence and number of foci, the area of the glandular tissue, the focus score (FS), the area of the inflammatory infiltrate, the percentage of the inflammatory infiltrate and the presence of ELS were assessed.

3 μm sections were cut from each formalin-fixed paraffin-embedded (FFPE) block of MSGBs and stained with haematoxylin and eosin (H&E). Immunohistochemical stainings were performed on additional 3 μm sections in order to detect T lymphocytes (CD3), B lymphocytes (CD20) and, when at least one focus was observed, the eventual presence of a follicular dendritic cells network (CD21). The slides were analysed by an expert pathologist (V.D.) in MSGB histopathology, who was blind to the clinical data.

The presence and number of foci, defined as dense aggregates of mononuclear cells, containing at least 50 lymphocytes, in a usually periductal or in a perivascular location and adjacent to normal appearing acini, was determined in the total area of the MSGB surface.

The area of the whole glandular surface and the area of the inflammatory infiltrate were assessed using a microscope (Eclipse E600, Nikon Instruments S.p.A, Italy) equipped with a digital camera (DS-Ri2, Nikon Instruments SpA, Italy) and provided with a measurement-validated software platform (NIS-Elements, Nikon Instruments SpA, Italy). In the calculation of the glandular area, spots with features of non-specific chronic sialoadenitis (fibrosis, acinar atrophy, duct ectasia) were included to avoid bias, according to Fisher *et al*. (6). The area of the inflammatory infiltrate was determined by drawing the perimeter of each focus and summing the areas of the foci, when more than one focus was present. Both the areas were calculated in mm^2 and approximated to the third decimal point.

The FS, which expresses the number of foci per 4 mm^2 of glandular tissue, was calculated by dividing the number of foci by the total glandular area and multiplying by 4 (n° of foci x 4 / glandular area), and approximated to the third decimal point. A FS≥1 was considered

a positive result for MSGB according to the ACR/EULAR 2016 classification criteria (7)

The percentage of the inflammatory infiltrate was determined as the ratio between the total area of the inflammatory infiltrate and the area of the whole glandular surface.

The assessment of ELS was determined by analysing the presence of both T/B-cell segregation and follicular dendritic cell networks in foci. The presence of T cells, B cells and follicular dendritic cells networks was evaluated by immunohistochemical staining using, respectively, an anti-CD3 rabbit monoclonal primary antibody (Clone 2GV6, ready-to use antibody, concentration 0.4 μg/mL, Ventana-Roche), an anti-CD20 mouse monoclonal primary antibody (Clone L26, ready-to use antibody, concentration 0.3 μg/mL, Ventana-Roche) and an anti-CD21 mouse monoclonal primary antibody (Clone 2G9, ready-to use antibody, concentration 1.93 μg/mL, Ventana-Roche). Immunostainings were performed by an automated slide stainer (BenchMark ULTRA IHC/ISH System, Ventana-Roche) according to the manufacturer's instructions.

Statistical analysis

We used the χ^2 test, the Mann-Whitney test, and Spearman's correlation coefficient to determine the correlations between the histopathological and clinical and serological parameters. Qualitative variables were compared using contingency table analysis and Fisher's exact test.

Results

We revised 69 MSGB samples: 46 from patients with a diagnosis of SS made according to the ACR/EULAR classification criteria and 23 from subjects with no-SS. Patients characteristics are summarised in Table I. The mean (SD) area of the samples was 8.122 (3.923) mm^2 with no statistically significant differences between samples obtained from patients fulfilling the SS criteria and no-SS subjects (Table II). The number of foci in the 69 MSGBs ranged from 1 to 9 with a mean SD of 2.45 (1.87), whereas the FS ranged from 0.257 to 5.530 with a mean (SD) of 1.388 (1.058). A FS <1 was documented in 32/69 (46%) samples. The total area of the inflammatory infiltrate ranged from 0.008 to 1.312 mm^2 with a mean (SD) of 0.193 (0.248) mm^2. The percentage of inflammatory infiltrate ranged from 0.1% to 15% with a mean (SD) of 2.7% (3.1%). ELS were detected in 43/69 (62.3%) samples and namely, isolated ELS (ELS=1) were found in 24/69 (34.8%) and multiple ELS (ELS>1) in 19/69 (27.5%) of the samples.

Table I. Characteristics of the study cohort.

	SS (46)	no-SS (23)	*p*-value
Age, mean (SD)	54 (15)	60 (12)	ns
Sex (female)	42/46 (91.3%)	20/23 (87%)	ns
Dry mouth	44/46 (95.6%)	21/23 (91.3%)	ns
Dry eye	44/46 (95.6%)	22/23 (95.6%)	ns
Salivary gland enlargement	2/46 (4.3%)	none	ns
USFR, mean (SD)	3.1 (3)	2.8 (2.4)	ns
Schirmer's test (mm/15'), mean (SD)	7.9 (4.5)	6.9 (5.1)	ns
OSS≥5	3/46 (6.5%)	2/23 (8.7%)	ns
Anti-Ro/SSA	32/46 (69.6%)	2/23 (8.7%)	0.001
Anti-La/SSB	12/46 (26%)	none	0.006
Rheumatoid factor	8/46 (17.4%)	none	0.05
IgG (n.v. 700-1600 mg/dl), mean (SD)	1388 (508)	1260 (202)	ns
C3 (n.v. 90-180 mg/dl), mean (SD)	108 (20)	103 (16)	ns
C4 (n.v. 10-40 mg/dl), mean (SD)	20 (7)	20 (5)	ns
White blood cells (n/mm3), mean (SD)	6091 (2463)	6169 (1806)	ns
Neutrophils (n/mm3), mean (SD)	3633 (1721)	3675 (1434)	ns
Lymphocytes (n/mm3), mean (SD)	1780 (800)	1865 (847)	ns

SD: standard deviation; USFR: unstimulated salivary flow; OSS: ocular staining score; n.v.: normal value.

Table II. MSGBs histological parameters in SS patients and no-SS subjects.

	SS (46)	no-SS (23)	*p*-value
Area of the sample (mm^2) mean (SD)	7.667 (3.781)	9.032 (4.125)	ns
Total area infiltrate (mm^2) mean (SD)	0.265 (0.277)	0.051 (0.034)	0.001
Percentage inflammatory infiltrate (%) mean (SD)	3.7 (3.4)	0.7 (0.6)	0.001
FS mean (SD)	1.752 (1.105)	0.661 (0.369)	0.001
N° of Foci mean (SD)	3 (2)	1 (0.5)	0.001
N° of ELS mean (SD)	1.5 (1.3)	0.4 (0.5)	0.001

SD: standard deviation; MSGB: minor salivary gland biopsy; FS: focus score; ELS: ectopic lymphoid structures.

The total area of inflammatory infiltrate and the percentage of inflammatory infiltrate reflect more accurately the extent of tissue inflammation and correlate better with the ELS in MSGBs than the number of foci and the FS

The total area of the inflammatory infiltrate correlated significantly as expected with the percentage of inflammatory infiltration (r=0.890**, $p<0.001$). Moreover, it also significantly correlated with the number of inflammatory foci (r=0.782**, $p<0.001$) and with the FS (r=0.660**, $p<0.001$); however, as shown in Figure 1, samples characterised by the same number of foci showed different areas of inflammatory infiltrate. For example, in specimens with a single focus the total area of the infiltrate ranged from 0.008 to 0.124 mm^2, whereas in samples with a number of foci=3, the total area ranged from 0.090 to 1.312. Similarly, Figure 2 shows foci of different sizes in the same MSGB. As expected, the correlation between the FS and the percentage of inflammatory infiltrate was also high (r=0.822**, $p<0.001$), but similarly samples with the same FS presented different percentage of inflammatory infiltrate (Fig. 3).

Finally, the total area of the inflammatory infiltrate showed the highest corre-

lation with the number of ELS detected in each sample (r=0.787**, p<0.001) with the total area of the infiltrate being significantly higher in samples presenting ELS >1 (0.467±0.304) with respect to samples with one single ELS (ELS =1) (0.129±0.137) or no ELS (ELS =0) (0.052±0.041) (Fig. 4); this correlation was higher than that observed between ELS and the number of inflammatory foci (r=0.588**, p<0.001). Similarly, the correlation between the number of ELS and the percentage of inflammatory infiltration was also higher (r=0.733**, p<0.001) than the correlation between ELS and the FS (r=0.548**, p<0.001).

The total area of inflammatory infiltrate and the percentage of inflammatory infiltrate appear significantly higher in patients fulfilling the ACR/EULAR classification criteria for SS, even in those with a FS <1

As shown in Table II, all the histopathological parameters were significantly higher in the 46 patients fulfilling the ACR/EULAR classification criteria for SS with respect to the 23 no-SS subjects. Moreover, the total area of inflammatory infiltrate (0.299±0.293 *vs*. 0.091±0.136, p=0.001) and the percentage of inflammatory infiltrate (4.1%±3.6% *vs*. 1.4%±1.9%, p=0.001)) appeared significantly higher in patients with a positivity for anti-Ro/SSA with respect to anti-Ro/SSA negative patients. Similar findings were obtained when we analysed the degree of MSGB infiltration stratified according to the patients' positivity for anti-La/SSB antibodies and Rheumatoid factor. Table III summarises the data regarding the total area of inflammatory infiltrate and the percentage of inflammatory infiltrate in patients subdivided according to their serological features.

When we limited the analysis to the 32/69 patients with a FS <1, both the total area of the inflammatory infiltrate (0.076±0.027 mm^2 *vs*. 0.051±0.356 mm^2, p=0.02) and the percentage of the inflammatory infiltrate (1.0%±0.4% *vs*. 0.6%±0.5%, p=0.03), but not the number of foci (1.70±0.68 *vs*. 1.27±0.46, p=0.12) remained significantly higher

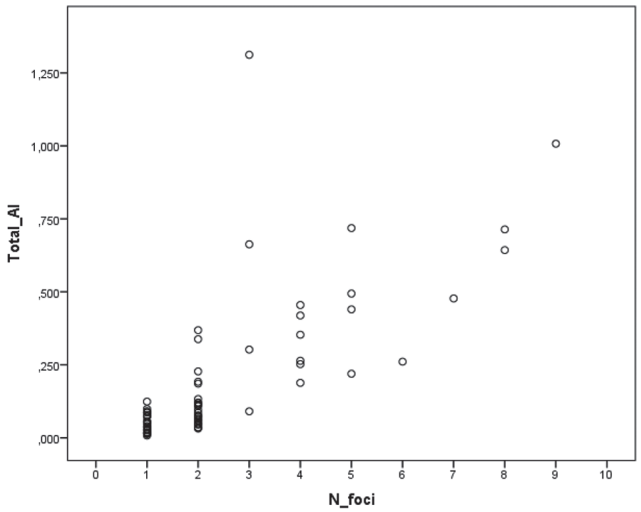

Fig. 1. Correlation between the total area of the inflammatory infiltrate (Total_AI) and the number of foci.

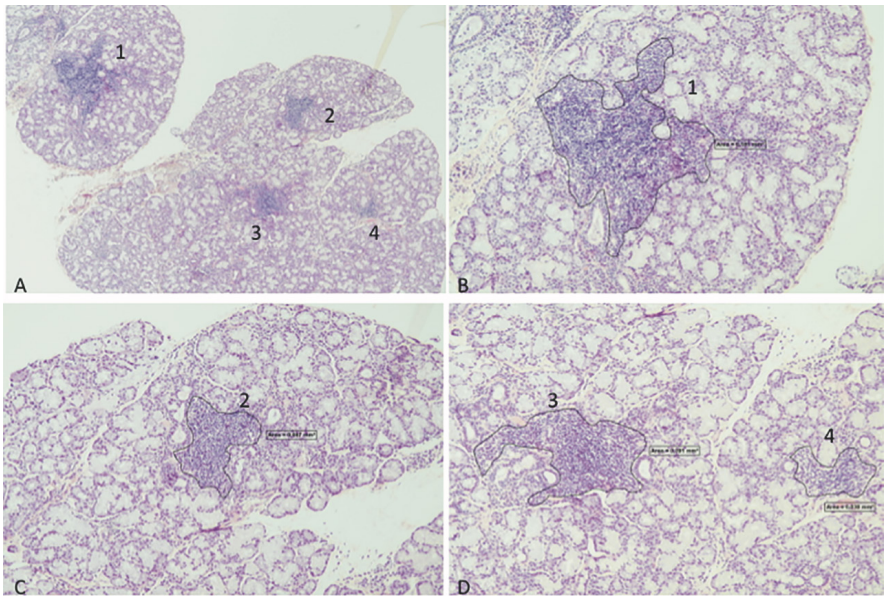

Fig. 2. Different size of foci in the same MSGB.
A: a section of MSGB with 4 foci of different size, numbered from 1 to 4. Details of the areas, circumscribed by black lines and indicated in the boxes, of focus #1 (B), focus #2 (C) and foci #3 and #4 (D). (A: H&E, magnification x40; B-D: H&E, magnification x100).

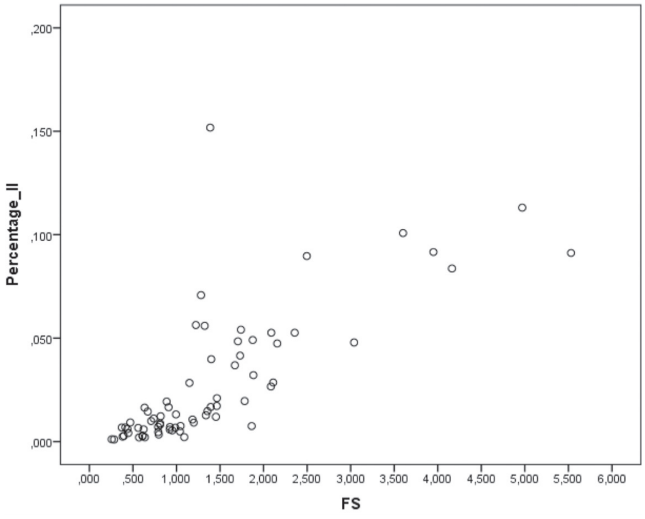

Fig. 3. Correlation between the percentage of the inflammatory infiltrate (Percentage_II) and the focus score (FS).

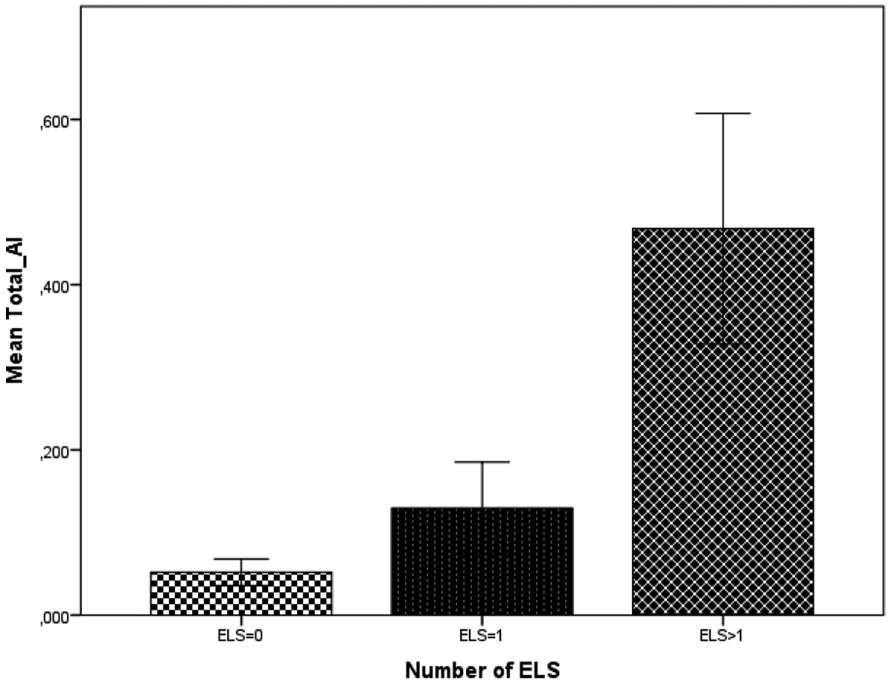

Fig. 4. The total area of the infiltrate was significantly higher in samples presenting ELS>1 (0.467±0.304) with respect to samples with one single ELS (ELS=1) (0.129±0.137) or no ELS (ELS=0) (0.052±0.041) (p=0.001).

in the 10/32 anti-Ro/SSA-positive patients fulfilling the ACR/EULAR classification criteria with respect to the 22/32 no-SS subjects. Figure 5 shows an example of two cases of MSGBs with only 1 focus and FS <1, but different areas and complexity of the inflammatory infiltrate.

By contrast, neither the total area of inflammatory infiltrate nor the percentage of inflammatory infiltrate correlated with the unstimulated salivary flow rate (USFR) and with the ocular tests results.

The total area of inflammatory infiltrate and the percentage of inflammatory infiltrate significantly correlate with SS-related biological and haematological abnormal findings

When we explored the association between the histopathological parameters and patients' clinical and laboratory features in SS patients, we observed that the total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate correlated with several SS-related biological abnormalities. Specifically, they both correlated positively with the presence of polyclonal hypergammaglobulinaemia (total area of the infiltrate: r=0.471, p=0.01; percentage of the inflammatory infiltrate r=0.382, p=0.01); moreover, the total area of inflammatory infiltrate correlated negatively with the levels of C4 (r=-0.358, p=0.03) and with the total number of white blood cells (r=-0.336, p=0.02), whereas the percentage of inflammatory infiltrate correlated negatively with the number of circulating lymphocytes (r=-0.376, p=0.01). The number of foci (r=0.472, p=0.01) and the FS (r=0.340, p=0.02) showed a significant correlation only with the polyclonal hypergammaglobulinaemia.

Discussion

In this study we explored the feasibility and the clinical value of the total area of inflammatory infiltrate and of the percentage of inflammatory infiltrate in SS diagnosis and patients' general assessment.

First of all, we confirmed that these additional histopathological parameters significantly correlated with the usual histopathological biomarkers including the number of foci and the FS. Thus, as expected, we observed that both the total area of inflammatory infiltrate and the percentage of inflammatory infiltrate were significantly higher in patients fulfilling the classification criteria for SS with respect to controls and particularly in those presenting a positivity for anti-Ro/SSA. However, despite the observed correlation, our study pinpointed that these novel histopathological parameters provided additional information on the extent of the infiltrate that in turn might improve the disease diagnostic work-up in selected cases.

In particular, we observed that the total area and the percentage of inflammatory infiltrate may vary significantly in samples showing the same number of foci or the same FS. In fact, we showed that the total area of the inflammatory infiltrate reflects and translates into a measure the differences in size among foci, distinguishing the smaller from the bigger ones, parameter not assessed by the absolute number of foci. These variations may result particularly useful in the diagnosis of patients with a borderline focus score. Noteworthy, we observed that these additional parameters were still able to differentiate SS from no-SS subjects even in those patients who fulfilled the classification criteria despite there being a FS <1 in their biopsies. Interestingly, both the total area and the percentage of the inflammatory infiltrate significantly correlated with the positivity for anti-

Table III. Total area of inflammatory infiltrate (Total_AI) and Percentage of inflammatory infiltrate (Percentage_II) in patients subdivided according to their serological features.

	SSA+	SSA-	p-value	SSB+	SSB-	p-value	RF+	RF-	p-value
Total_AI mean (SD)	0.299 (0.293)	0.091 (0.136)	**0.001**	0.409 (0.407)	0.138 (0.159)	**0.003**	0.494 (0.294)	0.139 (0.156)	**0.001**
Percentage_II % mean (SD)	4.1 (3.6)	1.4 (1.9)	**0.001**	5.3 (4.8)	2.1 (2.5)	**0.002**	5.1 (2.9)	2.4 (2.8)	**0.005**

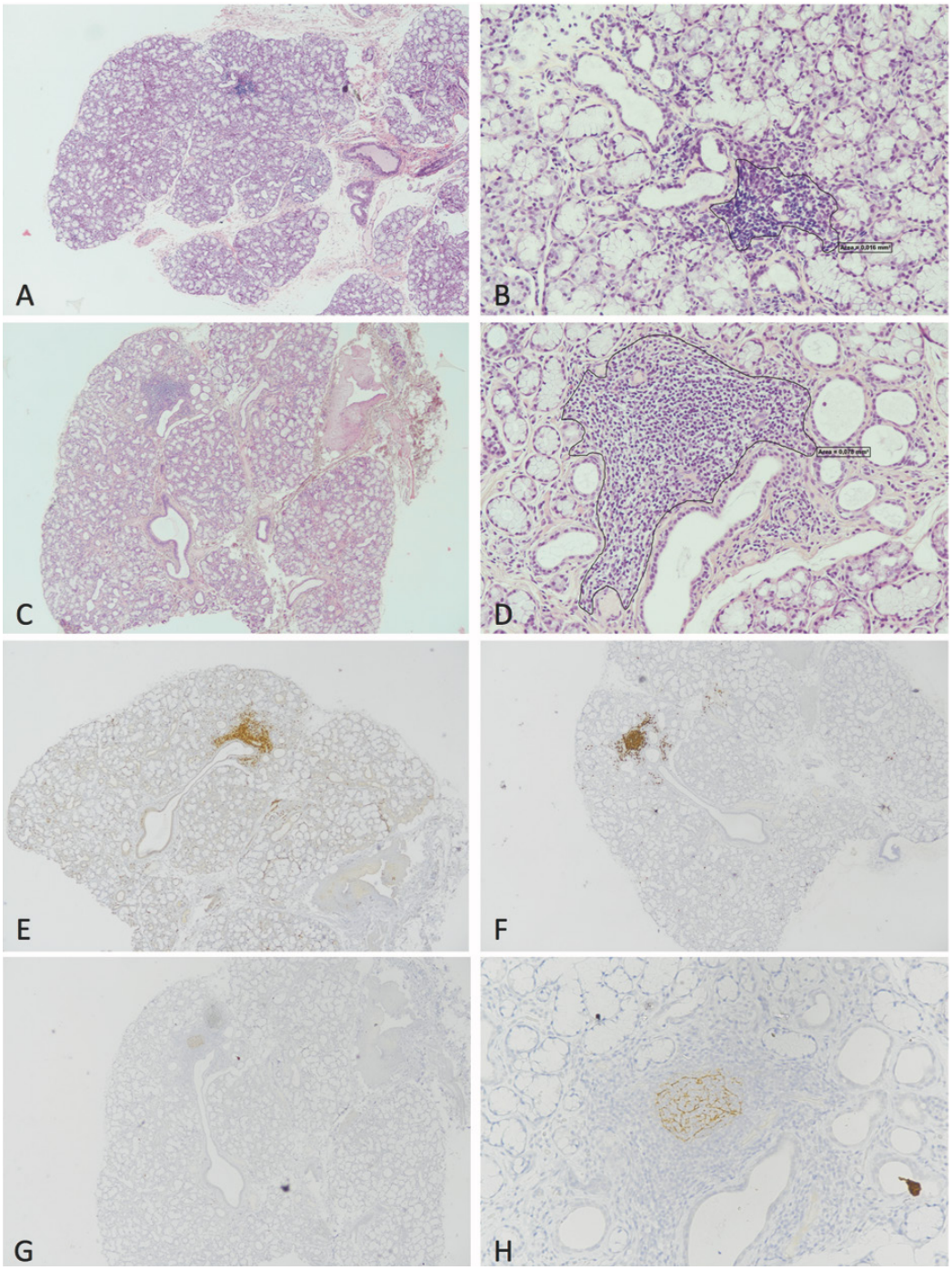

Fig. 5. Examples of two cases of MSGBs with only 1 focus and FS<1, but different areas and complexity of the inflammatory infiltrate.
A-B: a case of MSGB presenting only one focus, whose area is circumscribed by the black line and indicated in the rectangle (B).
C-H: another example of MSGB with only one focus, but bigger (C-D) than that of the previous one (A-B), and with a more complex organisation, as highlighted by the distribution of T lymphocytes (E) and B lymphocytes (F) and by the presence of a network of follicular dendritic cells (G-H)
A and **C**: H&E, magnification x40;
B and **D**: H&E, magnification x200, with the area of the inflammatory infiltrate circumscribed by the black line and indicated in the rectangle;
E: immunohistochemical staining for CD3, magnification x40;
F: immunohistochemical staining for CD20, magnification x40;
G-H: immunohistochemical staining for CD21, G magnification x40, H magnification x200).

Ro/SSA, which is a crucial item in the ACR/EULAR classification criteria, characterised by the highest average weight together with MSGB FS.

An additional aspect derived from our results is that, when compared with the number of foci and the FS, both the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate displayed a higher correlation with the presence of ELS, thus suggesting that these parameters may reflect more accurately the complexity of the inflammation in the MSGB for equal FS or equal number of foci. Whether the complexity of the infiltrate in the tissue may correlate with the systemic activity of the disease remains debatable. However, we observed that the total area of the inflammatory infiltrate and the percentage of inflammatory infiltrate significantly correlated with several items of the biological domain of the ESSDAI, including hypergammaglobulinaemia and low C4 levels. Moreover, these additional parameters also correlated inversely with the circulating white blood cells, thus indicating that patients presenting a higher total area and higher percentage of the inflammatory infiltrate in their MSGB may develop a more active disease.

These results are in line with previous studies that highlighted the relationship between MSGB histology and the disease phenotyping and pointed out the

value of the histology in predicting the disease expression and prognosis. From this perspective, ELS have been associated, although controversially with lymphoma risk (14, 20-25). Similarly, the distribution of the infiltrating-cell-types in MSGBs and the infiltrate complexity have been also correlated with distinct systemic disease manifestations (10).

Regarding the total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate, Gerli *et al*. (9) observed a correlation between the degree of the infiltration and both the age of the patients and the presence of extra-glandular manifestations including Raynaud's phenomenon, vasculitis, lymph node or spleen enlargement, leucopenia, and positivity for anti-SSA/Ro and anti-SSB/La autoantibodies. In our study we did not observe a correlation between the total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate and the age of the patients but we confirmed the correlation between the degree of inflammation and the presence of leucopenia or positivity for anti-SSA/Ro and anti-SSB/La autoantibodies.

Overall, both the total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate may provide useful information to improve patients' assessment and stratification, particularly for clinical research.

In addition, as it has been suggested in the literature, these two parameters may be useful in overcoming the issue correlated with the FS calculation biased by variability in the interpretation of the number of foci or by an insufficient surface area (5). Indeed, determining the exact number of foci in MSGBs may be a critical issue, and subjective to the interpretation of pathologists (19). Therefore, the calculation of the total area of inflammatory infiltrate instead of the total number of foci may be more accurate and less subjective to interpretation (26). Secondly, the glandular surface area represents undoubtedly a critical aspect for the FS calculation. In clinical trials it has been proposed to examine a minimum glandular surface area of 8 mm^2 to facilitate agreement, but in real life a larger variability in the area of the MSGB can be found and these additional histopathological parameters may help to quantify the inflammation in those inadequate cases.

Despite the complementary information that the total area of the inflammatory infiltrate and the percentage of the inflammatory infiltrate may offer, we recognise that the feasibility of the assessment of these two parameters can be limited. In fact, the accurate calculation of the total area of the inflammatory infiltrate, which is the sum of the areas of usually very small foci, requires specific instruments such as a microscope equipped with a digital camera and a measurement-validated microscope-associated software, which may not be present in all pathology laboratories, and can be time consuming. Considering these limitations, it is unlikely that these parameters can be assessed routinely in daily practice. However, on the basis of our results we may speculate that in SS referral centres and particularly for selected cases such as those with a borderline FS or for patients candidate to receive biological therapies, these additional biomarkers may maximise the valuable opportunity of capturing the disease in its target organs, in a complementary and even more accurate way than usual histopathological parameters.

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