

## Diagnostic evaluation of serial sections of labial salivary gland biopsies in Sjögren's syndrome

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### ABSTRACT

**Objectives:** Sjögren's syndrome is a chronic inflammatory disease. The detection of chronic inflammatory infiltrates containing >50 lymphocytes (lymphocytic focus) per 4 mm<sup>2</sup> tissue in minor salivary gland biopsies is a diagnostic parameter of the disease. The aim of the study was to examine if an increase in the tissue area of a single minor labial salivary gland biopsy through serial histological sections in patients affected by primary Sjögren's syndrome could facilitate the detection of the diagnostic focus score (grades >1 or >2).

**Methods:** We observed 24 labial salivary gland biopsies from patients affected by primary Sjögren's syndrome, diagnosed according to the clinical-laboratory criteria proposed by the American-European Consensus Group.

The analysis was carried out on sections (n= 72) obtained at three different levels at 200 micrometers from one another. The serial sections regarding the 3 levels were reviewed by the same oral pathologist, who detected both the total number of foci, and their surface, calculating a cumulative focus score.

**Results:** No significant correlation was found between the number of lobules per histological section and the focus score (Pearson correlation 0.363, p= 0.01). No significant variation in focus score distribution was identified in the three serial histological levels at 200 micrometers from one another. From the comparison between the number of lobules observed and the focus score grade, no direct proportionality between the amount of parenchyma analyzed and the focus score was found.

**Conclusions:** The focus score remained unchanged in the serial sections at different depths.

**Key words:** Sjögren's Syndrome salivary gland diagnosis.

### INTRODUCTION

Sjögren's syndrome (SS) is a chronic inflammatory disease with unknown onset and progress mechanisms, which diffusely involves exocrine glands, and which clinically presents distinctive dysfunctional signs defined as "sicca syndrome" (1-10). The disease mainly affects middle-aged women in a 10:1 ratio (3). No individual SS clinical or laboratory tests exist so far; therefore, the diagnosis is based on the combined evaluation of multiple clinical, serological, functional and morphological parameters, as originally proposed by a group of researchers supported by the European Com-

munity, and recently revised by the American-European Consensus Group (11-15).

The detection of chronic inflammatory infiltrates containing >50 lymphocytes (lymphocytic focus) per 4 mm<sup>2</sup> tissue in minor salivary gland biopsies (MSGB) is the diagnostic morphological parameter of SS (11-14, 16).

Based on the presence and number of lymphocytic foci, Daniels and Greenspan formulated a focus score (FS) ranging from 0 to >2, whose diagnostic specificity and sensitivity to SS is detectable in grades >1 and >2 (16).

Although the inflammatory involvement includes all the

exocrine glands, in SS the distribution of lymphocytic infiltrates in single glands is uneven (unifocal, plurifocal), easily leading to an over- or underestimation of the diagnostic FS in a single 4 mm<sup>2</sup> histological section (17,18). Since the method of biopsy sampling for minor salivary glands is well-established as for size (4 mm<sup>2</sup>) and number of fragments (one), in recent years the possibility was suggested to increase the tissue area, maximizing the number of lymphocytic foci, and therefore the diagnostic value of the biopsy through serial sections at different standard levels of the same biopsy sample (1,18).

The aim of this study was to examine if an increase in the tissue area of a single minor labial salivary gland biopsy through serial histological sections in patients affected by primary Sjögren's syndrome could facilitate the detection of the diagnostic focus score (grades >1 or >2) of SS.

## MATERIAL AND METHODS

From January 2000 to July 2005, we observed 24 labial salivary gland biopsies from patients affected by primary Sjögren's syndrome, diagnosed according to the clinical-laboratory criteria proposed by the American-European Consensus Group (11,14,15).

None of the biopsies were from patients with: history of radiotherapy in the head and neck region, HIV infection, sarcoidosis, amyloidosis, graft-versus-host disease. The age of the patients ranged from 30 to 77 years (average age: 53.5); there were 21 women and 3 men.

### - Basic histopathological evaluation

For the histopathological evaluation, each biopsy was fixed in 10% buffered formalin and embedded in paraffin; the approximately 4 µm thick histological section obtained was stained with hematoxylin and eosin. The number of gland lobules, the area of parenchymal section, and the presence and number of lymphocytic foci (focus score) were evaluated for each section. A lymphocytic focus was defined as a group of >50 lymphocytes (16). The focus score (FS) was classified in: FS = 0: no lymphocytic infiltration; FS = 1: less than 1 lymphocytic focus per 4 mm<sup>2</sup> (0 < FS < 1) (17,18); FS = 2: less than 2 lymphocytic foci per 4 mm<sup>2</sup> (1 < FS < 2); FS = 3: two or more lymphocytic foci per 4 mm<sup>2</sup> (FS > 2) (1). All the biopsies obtained (n= 24) were considered valid for histopathological diagnosis, since they contained at least 1 normotrophic gland lobule. The area of the biopsied section was evaluated using a morphometric software (Infometrix Inc.), capable of measuring the area of a surface circumscribed by a continuous line. The biopsies were considered compatible with the Sjögren syndrome diagnosis when the focus score was 2 or 3.

### Serial histopathological re-evaluation

The biopsies in paraffin were re-sectioned at depths of 200 and 400µm from the original section. The 4 µm thick serial sections were collected on 2 different slides and dyed with haematoxylin and eosin. Considering that the thickness of a lymphocytic focus including >50 lymphocytes has an

average diameter of 50µm (1), we considered that the interposition of 200µm between the 3 serial sections guaranteed that any foci observed at different sections were independent of one another. The serial sections regarding the 3 levels were reviewed by the same oral pathologist, who detected both the total number of foci, and their surface, calculating a cumulative focus score.

### - Statistical analysis

The potential correlation between the number of lobules per histological section and the focus score was evaluated through the Pearson correlation test. The focus score distribution at the three serial histological levels at 200 micrometers from one another was examined through the Kolmogorov-Smirnov goodness-of-fit test.

## RESULTS

No significant correlation was found between the number of lobules per histological section and the focus score (Pearson correlation 0.363, p= 0.01).

No significant variation in focus score distribution in the three serial histological levels at 200 micrometers from one another was identified (Figs 1-2).

From the comparison between the number of lobules observed and the focus score grade, no direct proportionality between the amount of parenchyma analyzed and the focus score was found (Figs 3-4).

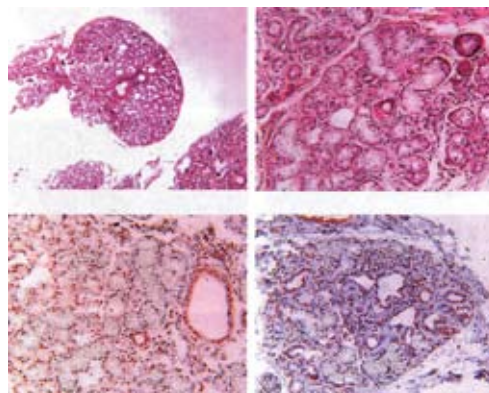


Fig. 1. Focus score grade 1.

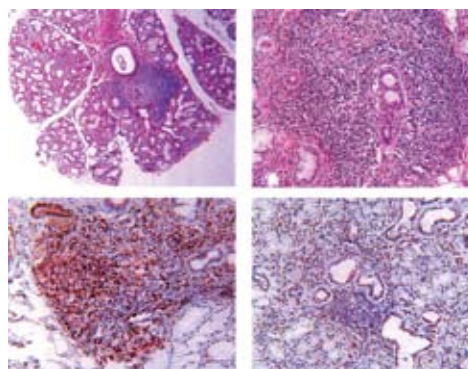


Fig. 2. Focus score grade 2.

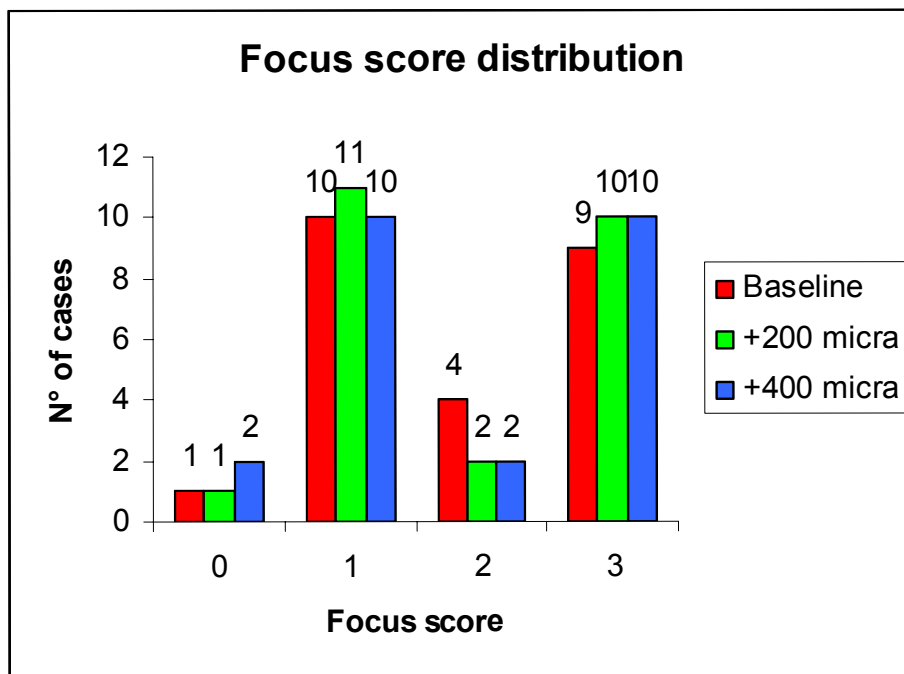


Fig. 3. The comparison between the number of cases observed and the focus score grade.

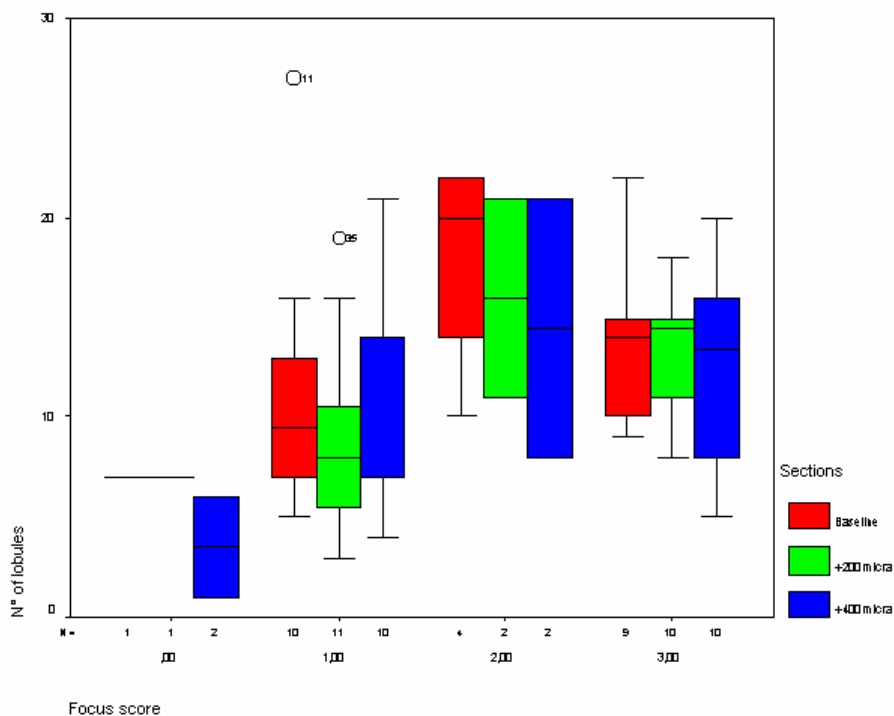


Fig. 4. The comparison between the number of lobules observed and the focus score grade.

## DISCUSSION

This study was inspired by the results obtained in recent studies present in the literature, which denied the possibility of reproducing the same focus score in serial sections obtained at different depths in biopsies of minor salivary glands of patients affected by Sjögren's syndrome (1,19). With this method, in fact, significant changes in focus score at various levels were observed, often turning a diagnostic focus score into a non-diagnostic one, and vice versa (1,18).

On this basis, we wanted to investigate literature data reproducibility, as well as the possibility that this focus score variability in the different serial histological sections compared to the basic section, may not depend as much on the increase in the total gland area analyzed, as on the number of lobules in the section. Within a single 4 mm<sup>2</sup> biopsy, we suggested a minimum of 3 section levels according to the standard procedures concerning the histopathological study of endomyocardial biopsies (20-22), assuming that the distance of 200µm prevented the diagnosis of the same lymphocytic focus in several serial sections.

The results of our study were different from those reported in the literature, since the diagnostic focus score (grades 2 and 3) and the non-diagnostic focus score (grades 0 and 1) remained unchanged in the serial sections at different depths (1,10,13,17,18).

In our view, this discrepancy did not derive from a merely statistical problem connected to the moderate number of biopsies (n= 24), and therefore of the serial histological sections analyzed (n= 72), nor from a smaller gland area examined through only 3 serial histological sections for the same 4 mm<sup>2</sup> biopsy, and therefore with a lower possibility of detecting diagnostic lymphocytic foci.

The comparison between the number of lobules observed and the focus score grade, in fact, showed no direct proportionality between the amount of parenchyma analyzed and the focus score.

We think that the persistence of the same focus score in the various serial histological sections depends on the fact that the presence and the number of diagnostic lymphocytic foci is connected to the stage of the disease.

Therefore, although considering the foci scores 2 and 3 as diagnostic morphological parameters of SS, the presence of both focus score 0 and focus score 1 would not morphologically exclude a diagnosis of SS in the cases in which the disease has been suspected through clinical-laboratory parameters.

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