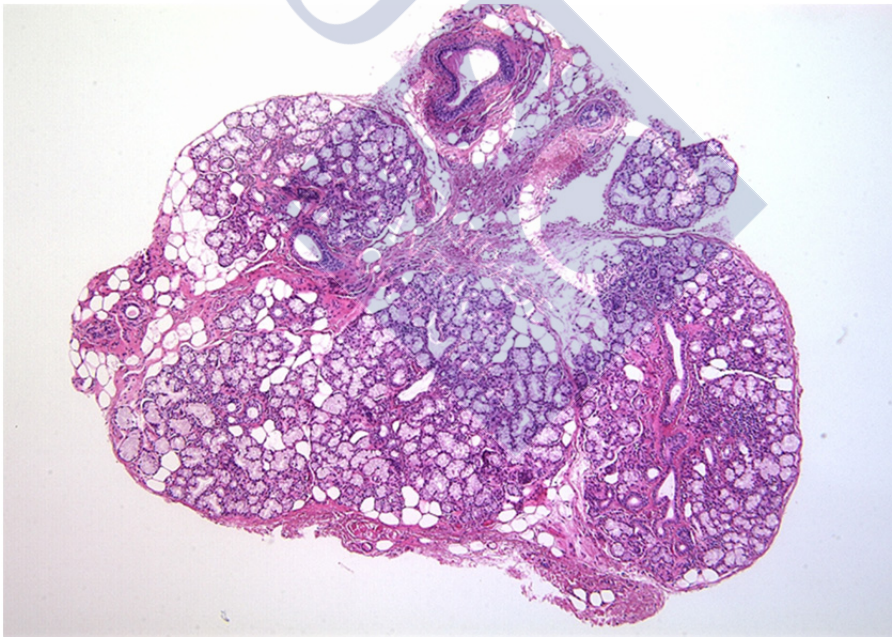
Universidad de Santiago de Compostela
Facultad de Medicina y Odontología
Departamento de Estomatología

# Contributions to lip biopsy procedures for the diagnosis of Sjögren syndrome

TESIS DOCTORAL



Mariña Sánchez Sánchez

Santiago de Compostela, abril de 2014

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D. Juan Manuel Seoane Lestón, Profesor Titular de la Facultad de Medicina y Odontología de la Universidad de Santiago de Compostela y

D. Juan Manuel Seoane Romero, Doctor en Estomatología por la Universidad de Santiago de Compostela

HACEN CONSTAR:

Que el trabajo de investigación que presenta Dña. Mariña Sánchez Sánchez, con el título de "Contributions to lip biopsy procedures for the diagnosis of Sjögren syndrome", ha sido realizado bajo nuestra dirección, supervisando en todo momento su elaboración.

Que nuestro criterio reúne las características de rigor, originalidad y mérito suficientes para optar al grado de Doctor y ser elevado al superior juicio del Tribunal designado a tal efecto.

Para que así conste, a efectos de justificar los mencionados extremos ante los órganos competentes de la Universidad de Santiago de Compostela, a 11 de Abril de 2014.

| | | |
|---|---|---|
| Fdo. Pablo I. Varela Centelles | Fdo. Juan M. Seoane Lestón | Fdo. Juan M. Seoane Romero |

Fdo. Mariña Sánchez Sánchez (doctoranda)

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*A mi familia, y en especial a mi madre*

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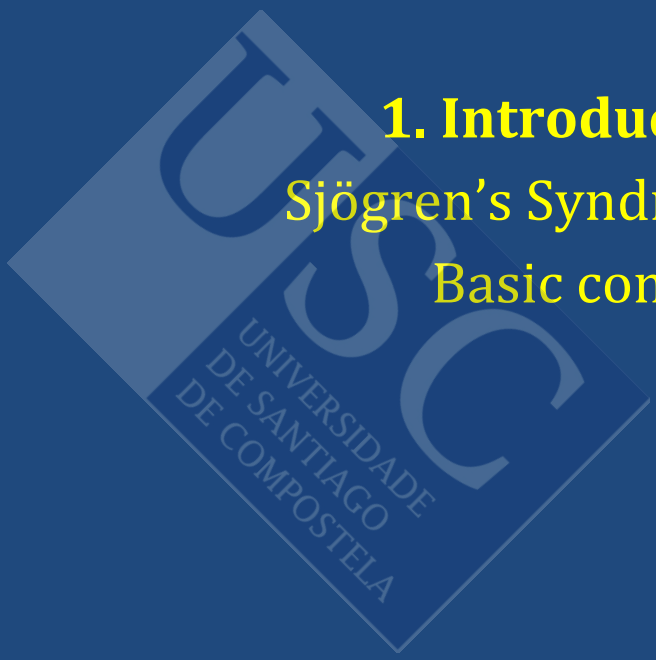


# 1. Introduction.

## Sjögren's Syndrome: Basic concepts

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# 1. Introduction. Sjögren's Syndrome: Basic concepts.

This syndrome was first described back in 1933 by Dr Henrik Samuel Conrad Sjögren as a chronic inflammation of both salivary and lachrymal glands, causing dry eye (keratoconjunctivitis sicca) and dry mouth (xerostomia). The Sjögren's syndrome (SS) alters the glandular secretion process because of a lymphocytic infiltration in the gland tissues (Brennan et al, 2014)

In those cases where the disease occurs by itself, the disorder is known as "primary Sjögren's syndrome". When the syndrome arises in relation to other autoimmune diseases (rheumatoid arthritis, erythematous lupus, scleroderma, mixed connective tissue disease, primary biliary cirrhosis, vasculitis, or active chronic hepatitis) is named "secondary Sjögren's syndrome".

The world prevalence of SS is 1.40% (95%CI 1.02 – 1-92), affecting mostly women (3.8%, 95%CI 27 – 52). The prevalence is higher for elder age groups (≥55 years-old) (4.6; 95%CI 34 – 61). Secondary SS is also present in up to 30% of patients with immune-related rheumatic disease. The estimated incidence of SS rages from 3 to 6 cases/100,000 inhabitants/year (Reksten et al, 2014).

## 1.1. Pathogenesis of Sjögren's syndrome

This disorder is characterised by a lymphocytic infiltration of exocrine glands in combination with an exaggerated response by B lymphocytes. Serologic tests of SS patients often show antibodies against unspecific antigens, like certain immunoglobulins (rheumatoid factors), and against extractable nuclear and cytoplasmic antigens (Ro/SS-A, La/SS-B) (Usuba et al, 2014).

The main type of cells infiltrating damaged exocrine glands are activated B- and T- lymphocytes, prevailing the latter in weaker lesions and the former in those more severe. Regulatory T-cells, macrophages, and dendritic cells have also been involved in this process.

The lymphocytes (apoptosis-resistant) send apoptosis signals to the epithelial glandular cells, and the epithelial and acinar cells contribute to this autoimmune course by expressing class-II major histocompatibility complex (MHC), co-stimulating molecules, and cell-membrane intracellular auto-antigens. These processes, in turn, send signals for lymphocytic activation. Besides, pro-inflammatory and lymphotactic cytokines are erroneously produced which extend the autoimmune lesion and induce the formation of more complex ectopic germinative centres. This phenomenon occurs in up to 20% of the patients. The extension of

a immune response is due to the expression of innate immunity receptors, mainly TLR 3, 7 and 9 (Cornec et al (a,b), 2014).

## 1.2. Autoimmunity mechanisms

The body immune system is designed for preserving and avoiding damage to its own tissues, relying on three processes devoted to ensure a selective insensitivity to autoantigens:

1. Autoantigen sequestration, rendering them not accessible for the immune system
2. Selective insensitivity of concerned B- or T- lymphocytes
3. Limitation of potential reactivity by regulatory mechanisms

Alterations in these processes may favour the development of an autoimmune response (Cornec et al (a), 2014). Thus, different exogenous triggers (bacteria, virus, smoking habit…) may act by:

Stimulation by superantigens: Substances of microbial origin able to stimulate a wide variety of T and B lymphocytes according to specific interactions with selected families of receptors, no-matter their antigenic specificity. Autoimmunity may arise if the T and B lymphocytes reactive to autoantigens express these receptors (Cornec et al (b),2014).

Molecular mimicry: Cross reactivity between a microbial product and a self-antigen that can induce activation of autoreactive lymphocytes.

Microbial adjuvants: Infectious microorganisms may overcome the self-tolerance because of their molecules with a quasi-adjuvant effect on the immune system, able to stimulate immune cells toll-like receptors. (Fujinami et al. 2006)

## 1.3. Clinical manifestations of the Sjögren's syndrome

This multiple, multi-organ exocrinopathy may arise as an ophthalmic and/or mucocutaneous alteration which can also be associated to a series of different extraglandular conditions, like musculoskeletal, digestive, pulmonary, haematological and neurological disorders (Lenopoli et ak, 2014).

### 1.3.1. Ophthalmic manifestations

As a result of keratoconjunctivitis sicca, patients may experience sensation of foreign body in the eyes, stinging, photosensitivity, eyestrain, impaired visual acuity, and also certain periods of watering. Human tears form a film over the eyes to protect them from irritation while keeping them moisturised and lubricated. A decrease in this secretion may cause, in severe cases, corneal ulcerations and –rarely- perforations. Another consequence of

xerophtalmia may also be conjunctivitis due to colonisation by Staphylococcus aureus.

### 1.3.2. Oral manifestations

As a result of xerostomia, patients may experiment halitosis, difficulties for chewing dry foods, pain, and loss of retention of their removable dentures (Lopez-Jornet J, 2004).

Dry mucosa with roughness and/or fissures is frequently seen at more advanced stages of the disease. Tooth decay is also a common finding, and these lesions progress rapidly and affect unusual tooth sites (molar cusps, incisal borders, and teeth necks). The tongue surface shows depapilated, reddish, areas with a lobulated appearance (Jensen et al, 2014).

Candida colonisation is also often linked to SS. These patients habitually experience difficulties for speaking because their tongue "sticks" to the palate due to the absence of saliva (Table 1).

Table 1. Signs and symptoms related to hyposalivation, according to the criteria by López-Jornet (2004).

| **Oral functional symptomatology** |
|---|
| **- Dry mouth sensation** |
| **- Difficulty to keep the mouth moisturised (need to drink water frequently)** |
| **- Difficulties for speaking (dysphonia), swallowing (dysphagia), and chewing (particularly dry food)** |
| **- Dysgeusia (taste changes)** |
| **- Oral discomfort (lack of oral lubrication)** |
| **- Burning mouth** |
| **- Need to get up for drinking water (difficulty for sleeping)** |
| **- Changes in the nutritional pattern** |
| **Organic oral symptomatology** |
| **- Tooth decay: number, extension, and site** |
| **- Gingivitis and periodontal disease** |
| **- Halitosis** |
| **- Prosthodontic problems (poor denture adaptation)** |
| **- Mucous problems:** |
| **- Atrophy, fissures and ulceration** |
| **- Changes in the lips and corners of the mouth** |
| **- Dryness, erythema, pain (burning mouth), loss of glow** |
| **- Traumatic lesions** |
| **- Increased sensitivity to irritating factors: tobacco, alcohol, etc** |
| **Related extra-oral lesions** |
| **- Eyes: blurred vision, gritty feeling, need to use artificial tears** |
| **- Genitals: dryness, itching, burning sensation, recurrent vaginitis** |
| **- Skin: peeling, cracking** |
| **- ENT: nasal dryness** |

### 1.3.3. Systemic disorders in Sjögren's syndrome

The Sjögren's syndrome is a systemic disease whose main and more suggestive symptoms are those related to the sicca syndrome. However, and taking into account the whole pathochronia of the disease, a number of organs and tissues can also be affected resulting in clinical manifestations in these patients (Lenopoli et al, 2014; Kramer et al, 2014).

#### 1.3.3.1. Musculoskeletal alterations

Despite that about 20% of patients with rheumatoid arthritis elicit a secondary SS, a high proportion of patients suffering from primary Sjögren's syndrome experience musculoskeletal alterations, typically in small joints (Carsons, 2001; Kassan et al. 2004). Approximately half of these patients suffer arthralgias and, less frequently, myalgias. Deformity of small joints and non-erosive arthritis, close to that in erythematous systemic lupus, are less frequent findings.

#### 1.3.3.2. Dermatologic alterations

The main cutaneous manifestation of this syndrome is skin dryness, found in about 50% of SS patients, sometimes accompanied by itching, excoriations, and superinfections. (Kassan et al.2004)

Another skin alterations in these patients include ulcerations, a hypothetical association to hypergammaglobulinemic purpura or to leukocytoclastic vasculitis. Vasculitis in SS patients can range from a cutaneously localised form to a systemic necrotizing vasculitis. It is also important to ensure a correct differential diagnosis with systemic erythematous lupus and with schlerodermia, because of some common findings shared with these entities. About 30% of SS patients may also present a low-intensity Raynaud phenomenon.

#### 1.3.3.2. Pulmonary alterations

The main respiratory symptom identified among patients suffering from primary Sjögren's syndrome is cough usually resulting from dryness of the bronchial tree (Carsons, 2001). Radiological examination of these patients may often display images resembling "ground glass", reticular pattern and honeycomb, affecting mostly the lower lobules.

#### 1.3.3.3. Renal alterations

Renal alterations are frequent extraglandular manifestations of primary Sjögren's syndrome. Tubular involvement may result from interstitial lymphocytic infiltrate, with interstitial fibrosis and tubular atrophy or glomerular affectation.

Tubulointerstitial nephritis is the most common renal affectation, usually characterised by distal renal tubular acidosis as a consequence of the tubulointerstitial inflammatory infiltrate. (Kassan et al.2004)

Despite the existing reports, nephrocalcinosis, nephrogenic diabetes insipidus, proximal tubular renal tubular acidosis, or Fanconi syndrome are infrequent situations. In the absence of treatment, SS may lead to the formation of stones and cause renal failure. Regarding to symptoms of tubular acidosis, early signs would be hypokalemia and hyperchloremic acidosis.

### 1.3.3.5. Gastrointestinal alterations

Esophageal dysmotility, as a consequence of xerostomia, is a common finding amongst these patients, who frequently report gastro-oesophagal and laryngopahryngeal reflux too.

The acids and the enzymatic activity in perioral tissues may cause dysphonia, chronic cough, sore throat, mucus in the throat, tooth decay, and they may even induce carcinogenic changes in the larynx. Although infrequent in SS, pancreatic involvement includes pancreatitis and pancreatic insufficiency. The symptoms reported by these patients can embrace episodes of abdominal pain and/or symptoms related to intestinal mal-absorption.

### 1.3.3.6. Liver alterations

Liver alterations caused by the Sjögren's syndrome are rarely seen, although a thorough differential diagnosis with primary biliary cirrhosis is mandatory. Both disorders share the same pathogenic autoimmune mechanisms, but SS patients show anti-Ro and anti-La antibodies whereas in primary biliary cirrhosis the main specific auto-antibody is the anti-mitochondrial antibody.

### 1.3.3.7. Gynaecologic and obstetric alterations

A number of dyspareunia cases have been reported, due to alterations in the lubrication in premenopausal women suffering from primary Sjögren's syndrome. Other described gynaecologic problems include vaginal dryness, endometriosis, and episodes of amenorrhoea and menorrhagia/metrorrhagia.

### 1.3.3.8. Neurologic alterations

Probably, these are the most frequent systemic alterations in SS patients. Despite it is mostly a peripheral neuropathy, some reports have described central nervous system disturbances such as optic neuropathy, seizures, cognitive dysfunctions, and multiple sclerosis-like manifestations. Sjögren's syndrome patients may also experience neuropathic pain involving back, extremities, or

face. One of the most frequent cranial neuropathies among SS patients is the trigeminal neuralgia.

#### 1.3.3.9. Lymphoma

Primary Sjögren's syndrome patients are at higher risk for lymphoma, and about 5% to 10% of these patients have developed this disease. The most frequent type of lymphoma is the non-Hodgkin one, partly originated from B cells, which can involve extranodal sites such as salivary glands, gastrointestinal tract, lungs, and the thyroid gland. Signs of lymphoproliferation include an increase on the size of salivary glands, lymphadenopathy, splenomegaly, and lung infiltrate. A serological follow-up is recommended. As a result of the monoclonal protein, leukopenia and anaemia, and a loss of previously present specific autoantibodies have been associated with the development of lymphoma. Moreover, the presence of signs like enlargement of the salivary glands and/or lymphadenopathy, without the typical lymphoid pathological findings is known as "pseudo-lymphoma" (Carsons, 2001; Kassan 2004).

## 1.4. Diagnosis

Different protocols for the diagnosis of Sjögren's syndrome have been agreed through the time (Goules et al, 2014; Fazaa et al, 2014; Theander et al, 2013; Shiboski, 2012 ):

### 1.4.1. Copenhagen criteria

These criteria dates back to 1976 and groups a series of diagnostic tests based upon objective signs rather than on related symptoms. (Manthorpe et al.,1986) These tests mainly relied on the diagnosis of keratoconjuctivitis and xerostomia. These criteria distinguished the primary Sjögren's syndrome (defined as the simultaneous presence of keratoconjunctivitis and xerostomia in patients without any chronic inflammatory connective tissue disorder) from the secondary SS, identified in patients with an associated chronic inflammatory alteration.

For the diagnosis of keratoconjunctivitis sicca (KCS), the following tests were suggested:

- Schimmer-I test (results ≤10mm/5min)
- Tear film break-up time (≤10 s)
- Van Bijsterverd scale (score ≥4 points in a 0-9 scale)

For the diagnosis of xerostomia:

- Non-stimulated sialometry (≤1.5 ml/15 min)
- Salivary gland scintigraphy
- Biopsy of minor salivary glands in the lower lip

### 1.4.2. Japanese committee of experts for SS diagnosis

The agreement reached by this group of experts included ophthalmologic and oral examinations (as recommended by the

Copenhagen group), together with pathological examination and specific serology. Diagnosis of Sjögren's syndrome was considered positive when the case met at least two of the four criteria. (Gomes et al (a) 2012).

### 1.4.3. Study group on diagnostic criteria for Sjögren's syndrome

In 1988, with the intention of validating and generalising a reliable diagnostic system for SS, a group of 29 experts from 12 countries (11 European nations and Israel) designed a study protocol divided into two stages:

Stage I: Questionnaires on ocular and oral manifestations of the disease (13 questionnaires on ophthalmologic manifestations, and 7 about oral ones). These forms were filled by 15 patients clinically diagnosed as primary SS, and by another 15 subjects in a control group.

Stage II: A set of diagnostic tests were proposed. These tests would be validated by later studies, as would the procedures for undertaking them. The suggested tests for ophthalmic manifestations were:

- Schirmer-I test.
- Rose Bengal scale
- Tear film break-up time
- Lactoferrin levels in lachrymal fluid

For assessing oral manifestations, the tests proposed included:

- Non-stimulated and stimulated saliva secretion levels
- Minor salivary gland biopsy
-Parotid sialography
- Salivary gland scintigraphy

Serological study would consider the levels of gamma globulin, anti-nuclear antibodies, rheumatoid factors and anti-ENA.

Every physician collaborating in the study received information about the parameters of the diagnostic tests, as well as about the possible signs and symptoms of the disease.

Each centre returned clinical and serological information from a total of 40 patients: 10 primary SS (group I), 10 secondary SS (group II), 10 patients with connective tissue diseases without SS (group III), and 10 control patients (group IV). The exclusion criteria considered were the presence of a pre-existent lymphoma, sarcoidosis, AIDS, and graft-versus-host disease.

Amongst the most remarkable results of this study was the finding that the questionnaires with 3 questions about dry mouth and 3 questions about dry eyes were the most predictive for xerophtalmia and xerostomia in primary SS.

Regarding to ophthalmological tests, the Schirmer-I test seems to keep a good relationship between sensitivity and specificity,

whereas the Rose Bengal scale was by far the most specific test. The tear film break-up time scored the highest sensitivity but a low specificity. The determination of lactoferrin levels in lachrymal fluid resulted inconclusive for SS diagnosis.

When assessing salivary tests, the stimulated salivary flow was the only test yielding irrelevant results. Salivary gland biopsy showed a good relationship between sensitivity and specificity for SS diagnosis. The anti-Ro/SS-A and anti-La/SS-B antibodies scored high specificity but low sensitivity, whereas the rheumatoid factor and the anti-nuclear antibodies (ENA) elicited an acceptable specificity.

Taking into account the results of the above mentioned investigation, the Study Group on Diagnostic Criteria for Sjögren's syndrome reached an agreement about 6 criteria for diagnosing SS, establishing that the presence of 3 criteria was required for diagnosing a probable SS, and the existence of 4 criteria and anti-Ro/SS-A and anti-La/SS-B for a definitive diagnosis of Sjögren's syndrome (Vitali et al. 1996)(Table 2).

Table 2. Classification criteria for Sjögren's syndrome by the - European Consensus Group.

| European criteria for Sjögren's síndrome) | SS criteria:<br>4/6<br>3 /4 (objective criteria) |
|---|---|
| **1. Ophthalmic symptoms** | **Dry-eye sensation for 3 months**<br>**Gritty feeling**<br>**Need for artificial tears >3 times/day** |
| **2. Oral symptoms** | **Dry-mouth sensation for 3 months**<br>**Salivary gland enlargement**<br>**Need for a drink to swallow down** |
| **3. Ophthalmic signs** | |
| **4. Glandular biopsy (Parotid echography)** | |
| **5. Objective alteration of glandular physiology** | **Sialometry**<br>**Parotid sialography**<br>**Parotid gammagraphy** |
| **6. Auto-antibodies** | **Anti Ro/SA or Anti La/SSb** |

These European criteria for SS diagnosis were widely accepted by the scientific community and used in a great number of extensively referenced clinical studies since their publication. In spite this fact, and aiming at unifying criteria and acceptation of the European classification criteria, the SS Foundation promoted a new study group integrating the European and the American working groups. As a result, a series of meetings were held between 1998 and 2000, where the criteria were agreed. It was also established that patients had to meet 4 out the 6 criteria for reaching a definitive diagnosis of SS, although two of these tests had to be the presence of inflammation in the minor salivary gland biopsy (focus score ≥1), and positive serology (anti-Ro/SS-A and/or anti-La/SS-B).

As a result of this new consensus, some modifications were made on the exclusion criteria concerning hepatitis C patients, AIDS, pre-existent lymphoma, graft-versus-host disease, patients receiving head and neck radiotherapy, and those under treatment with anticholinergic drugs.(Vitali et al., 2002)

Much more recently, and following the idea of removing subjective issues and considering only objective tests, new standards have been developed for definitive SS diagnosis, which require a positive serology (anti-SSA and/or SSB or positive rheumatic factor /ANAs), presence of focal lymphocytic sialadenitis (>1 per 4 mm2 of glandular tissue) an positive Rose Bengal staining (ocular staining score <3) (Shiboski et al. 2012) (Table 3).

**Table 3. Comparative between AESG and American criteria.**

| AESG, 2002 | American criteria, 2012 |
|---|---|
| **European Study Group, 1988** | **Serum markers:**<br>**SSA (+) and/or SSB (+)**<br>**Or**<br>**Rheumatoid factor (+) / ANAs (>1:320)** |
| **American-European Study Group, 2002** | **Focal lymphocytic sialadenitis (>1 focus)** |
| **4/6** | **Rose Bengal >3** |
| **Exclusion criteria:**<br>**Head & Neck radiotherapy**<br>**Hepatitis C**<br>**AIDS**<br>**Pre-existent lymphoma**<br>**Graft-vs.-host disease**<br>**Anticholinergic medications** | **Standardised measurements based upon objective criteria** |

## 1.5. Lip biopsy for the diagnosis of Sjögren's syndrome

Glandular biopsy is a barely cumbersome surgical procedure that has demonstrated relevant contributions to the diagnosis of SS, as well as other connective tissue disorders, amyloidosis, sarcoidosis, or neonatal hemochromatosis. The analysis of lip glandular tissue has received more support than the study of major salivary gland tissue (parotid) or glandular tissue from the palate (Ton et al, 2009).

This technique has proved high sensitivity (78.8□11.2) and specificity (88.1□11.7) together with high positive (87.6□ 9.5) and negative (79.0□16.9) predictive values. Conversely, substantial morbidity (pain, bruising, inflammation, transient difficulties for speaking and/or eating, bleeding, and alterations of cicatrisation) has been reported. Long-lasting neurological alterations (changes in lip sensitivity) may occur in up to 6% of patients. Regardless of these problems, there is a remarkable lack of standardisation of the surgical procedure: while some research groups recommend linear, circular, or elliptical incisions following parallel, oblique, or vertical directions to the fibres of the orbital muscle of the lips, other researches support the use of circular scalpels (punch) instead of the conventional one. Some different procedures for lip stabilisation have also been described to ease the biopsy technique (Risselada et al, 2014).

Anyhow, and because of the surgical nature of the procedures and its potential complications, the search for alternative, non-invasive, complementary procedures for SS diagnosis continues (eg: sialochemistry, sialography, glandular ultrasonography) (Carotti et al, 2014; Kim et al, 2014)

The chemical analysis of the saliva from SS patients has shown an increase of albumin levels due to inflammation and a high amount of inorganic components. Besides, antibodies anti-Ro/SSA and anti-La/SSB are more frequently found.

Ultrasonography has also demonstrated a good correlation with sialography. This finding seems to suggest that this procedure may be potentially useful for a non-invasive diagnosis.

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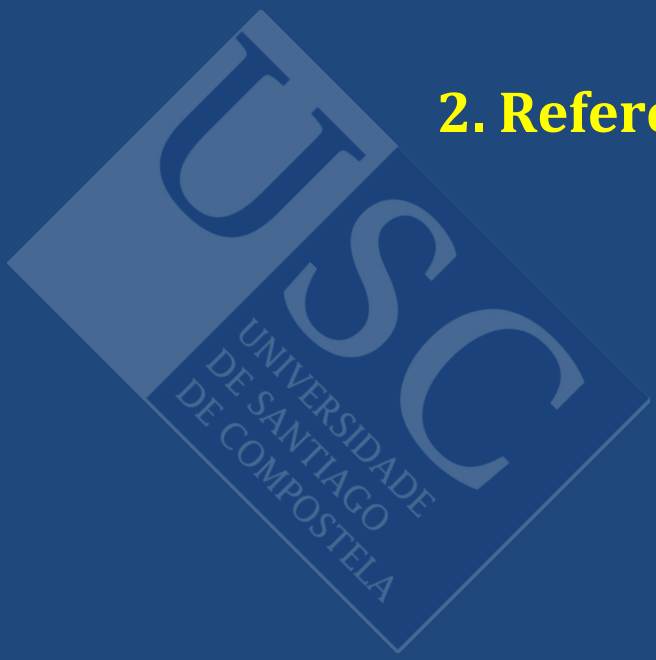


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# 3. Justification

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# 3. Justification

Lip salivary gland biopsy has proved a high diagnostic yield and great validity for the diagnosis of Sjögren's syndrome, even when dealing with unspecific clinical pictures with negative serology.

Despite these clear advantages and the morbidity associated to the procedure, such as occasional sever neurological complications, there is no standard technique for undertaking lip biopsies.

Currently, there are different surgical approaches for harvesting glandular tissue from the inner side of the lip, characterised by the size, orientation, and type of the incision as well as by the biopsy site.

Unfortunately, the number of studies comparing the existing techniques is scarce and their conclusions are limited by the frequently poor description of the surgical procedures and by the vague categorisation of the associated complications. Besides, a unique study, methodologically weak, has undertaken a narrative review of the morbidity related to the surgical procedure with equivocal results.

The aforementioned circumstances highlight the need for a quantitative, systematic study of the complications associated to

minor salivary gland biopsy, involving both the techniques and the instruments employed for this purpose.


# 4. Aims

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# 4. Aims

1. To systematically review and assess the existing literature for identifying the most suitable surgical technique for lip biopsy in terms of number and severity of neurological complications in patients suspicious for Sjögren's syndrome.

2. To undertake a pilot study on the use of circular scalpels for Sjögren's syndrome diagnostic biopsies and to analyse the features of the samples harvested, as well as to design ancillary instruments with a potential for minimising the morbidity of the procedure.

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# 5. Neurologic adverse events related to lip biopsy in patients suspicious for Sjögren's syndrome: A systematic review and prevalence meta-analysis

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# 5. Neurologic adverse events related to lip biopsy in patients suspicious for Sjögren's syndrome: A systematic review and prevalence meta-analysis.


## Abstract

Objectives: To compare the prevalence of neurological complications related to lip biopsy for Sjögren's Sindrome diagnosis using conventional versus, minimally invasive techniques.

Methods: Systematic review and prevalence meta-analysis using the search strategy: (("salivary gland biopsy" OR "labial biopsy" OR "lip biopsy") AND ("Sjögren")) in Medline, Embase and Proceedings web of science databases.

Studies were selected if they included original data for minor salivary gland biopsy, sample size, exposure of interest (tecnique description), number of complications and number of affected patients. The prevalence of total and permanent neurological adverse effects was calculated. Both fixed-effects and random-effects pooled estimates were assessed. Heterogeneity was calculated using an adaptation of the DerSimonian and Laird Q test.

Results: 16 papers were selected for the study. In the minimally invasive group (n=3), the pooled prevalence of total adverse events is almost 4 times higher than that in the linear incision group (n=12) (4.73% vs. 1.20%). On the contrary, the pooled prevalence of the permanent or potentially permanent neurological adverse events is 8.5 times lower in the minimally invasive technique group than in group of studies using linear incisions (0.17% vs. 1.45%).

Conclusions: With the limitations intrinsic to the potential biases in the studies included in this meta-analysis, we conclude that minimally invasive lip biopsy technique for Sjögren's syndrome diagnosis induces less permanent neurological complications than conventional approaches with large linear incisions in the lower lip.

**Keywords:** Sjögren's syndrome; lip biopsy; minor salivary gland; diagnosis; adverse events; complications; neural damage.

## Introduction

Since Chisholm & Mason [1] described minor salivary gland biopsy (MSGB) as a valuable investigative procedure in Sjögren's syndrome (SS) and established standardized criteria to assess inflammation, a number of surgical techniques have been suggested to harvest minor salivary glands for SS diagnosis.

Focal sialadenitis (with a focus score ≥1) in minor salivary glands is one of the six requirements established for SS diagnosis [2]. This criterion has become more important as a recent consensus limits the definition of SS case to objective criteria only, and therefore two out of this three criteria would then be required: positive serum anti-SSa and or SSB; ocular staining score >3; and presence of focal sialadenitis with a focus score >1 per 4 $mm^2$ of glandular tissue [3].

The value of MSGB for SS diagnosis is supported by high sensitivity (Mean (SD) 78.8 (11.2)) and specificity (88.1 (11.7)) values [2], reinforced by a good diagnostic confidence (positive predictive value: 87.6 (9.5); negative predictive value: 79.0 (16.9)). This diagnostic value can be augmented by evaluating the cumulative focus score using a multilevel approach, assessing different section levels of the sampled glandular tissue cut at least at 200μm apart [4]. Thus, MSGB can be particularly useful for suspected SS patients with inconclusive clinical findings and negative anti-Ro/la serology [5, 6].

A number of mediate and immediate surgical complications (pain, bruising, bleeding, wound infection) have been described for MSGB, but lip numbness has been found to be the only persistent complication and it is reported to occur in up to 6% of all MSGB [7-9]. Several techniques for lip MSGB have been proposed with a variety of incisions differing in shape (elliptical, circular, linear), orientation (vertical, oblique, parallel) and length (1mm to 3 cm), but no comparative studies on the advantages of a particular type in terms of postoperative morbidity could be retrieved [7,9-19].

The high diagnostic performance of MSGB demands an adequate surgical technique to ensure correct and sufficient sampling of glandular tissue with low related morbidity. Technique selection is hindered by the absence of comparative studies and the limited validity of the sole comprehensive review on the topic identified in the literature, due to the poor definition and vague categorization of the surgical complications [11]. These circumstances seem to justify the need for a systematic review and meta-analysis aimed at assessing the prevalence of neurological complications related to lip biopsy. The aim of this investigation was to compare the pooled prevalence of neurological adverse events induced by lip biopsy for SS diagnosis (minimally invasive technique versus linear incisions ≥5 mm).

## Methods and materials

Methods of analysis and inclusion criteria were defined in a protocol to ensure homogeneous criteria amongst all co-authors during the investigation. This protocol was initially conceived to record all post-biopsy adverse events described in the literature, but it was restricted later on during the study to permanent neurological adverse events.

A systematic search was undertaken in June 2013 at MEDLINE, Embase, and Proceedings Web of Science (Conference proceedings citation index-Science (CPCI-S)). The search strategy was : ((“salivary gland biopsy” OR “labial biopsy” OR “lip biopsy”) AND (“Sjögren”)), both in medical subject headings (MeSH) and in freetext words. This search strategy was independently reviewed and discussed by all authors.

This search was supplemented with an additional handsearch [20] performed at our Institution`s library catalogue, considering both books and relevant journals, including Annals of the Rheumatic Diseases, Arthritis & Rheumatism, Arthritis Research & Therapy, Osteoarthritis and Cartilage, Rheumatology, and the reference lists of the papers retrieved from the aforementioned databases (Fig 1).

All references identified for computerized databases were manually retrieved.

Studies were included if they fulfilled the following criteria:

I. Included original data from MSGB performed for SS diagnosis

II. Included the sample size and the exposure of interest in the study: detailed description of the technique(s) for MSGB.

III. Assessed the presence or absence of neurological complications (lip numbness) and number of affected patients.

Data were retrieved by two investigators in an unblinded standardized manner, using a custom-made extraction sheet. Disagreements between investigators were resolved by consensus.

The percentage of technique-related neurological complications was again independently recorded by the reviewers and, in case this information was not detailed in the study, lower lip numbness was considered as persistent/permanent when lasting ≥6 months after the biopsy procedure.

## Quality assessment

We assessed study quality by use of a five-point binary scale (0/1) that we specifically developed for this study. The scale is based on STROBE guidelines for reporting observational studies [21]. Throughout this assessment, when the information on a specific item was not provided by the authors, we graded this item as "0". The quality scoring was independently undertaken by two

researchers (JS & BT). The first item assessed was whether post-biopsy complications were a primary or specific objective of the reported investigation or were considered in a pre-specified hypothesis; in this case, a score of 1 was allocated. The second item assessed was the study design (one point to clearly described prospective designs). The third item assessed was the setting of the study (one point was given if the paper adequately described both the surgical scenario and data collection procedures). The fourth item assessed the follow-up time after surgery (one point if follow-up dates or dates at which the outcome events occurred or at which the outcomes were present). The last item concerned descriptive data on one important confounder: the number of glands harvested (1 point if the number of glands obtained is detailed in the paper).

## Data synthesis and analysis

The concept of "minimally invasive techniques" gathers those techniques for MSGB consisting of multiple 2-3 mm incisions on the buccal side of the lip to collect the glandular tissue using a forceps. Punch techniques are not included within this group [9,10,13,18]. Another group of studies could be defined by those reports harvesting glandular tissue from the lower lip by means of a single, linear, ≥5 cm incision.

For each study we computed the prevalence of total neurological adverse events and that of neurological permanent or potentially

permanent adverse events by dividing the number of events by the sample size of the study. We then weighted the study-specific prevalence by the inverse of their variance to compute a pooled prevalence and its 95% confidence interval.

We calculated both fixed-effects and random-effects pooled estimates but used and report the latter when heterogeneity was present, as the random-effects model is generally thought to give more reliable results than the fixed-effects model, including a more conservative (wider) CI, when the studies being considered show a considerable degree of heterogeneity. In our study, this issue is particularly relevant as, in general, the number of events is low and in some studies the resulting prevalence is 0. Therefore, instead of using the traditional asymptotic method in order to obtain an estimate of the variance, inadequate in our setting, we use the exact method proposed by Newcombe and Altman [22].

To check for heterogeneity, we used a version adapted to small samples of the DerSimonian and Laird Q test, and to quantify this heterogeneity we calculated the proportion of the total variance due to between-study variance (Ri statistic) [23]. All analyses were performed with the software HEpiMA® version 2.1.3 [24].

## Results

The aforementioned systematic searches identified 342 single papers whose abstracts were reviewed for contents relevant to

the topic of this systematic review, and 301 of them were subsequently excluded. A total of 41 papers were then retrieved and reviewed, and seven of them were also excluded because their information was not useful for this study.

The remaining 34 papers were checked according to the inclusion/exclusion criteria of the present review:

Two papers were excluded because of double publication: the same information was published in more than one paper by Caporali et al [9, 25] and López-Amado et al. [26, 27]. Thus, one reference by each research group was considered in the study [9,27].

Two papers performed MSGB for indications other than diagnosis of SS [28,29].

Three manuscripts report inadequate descriptions of the surgical technique [17,30,31].

Nine articles report on the surgical technique, but not on its related complications [18, 32-39].

A manuscript included only generic information on the surgical complication of MSGB [40].

A report did not adequately described the size of the sample studied [19].

So, 16 articles were finally selected to enter the systematic review, two of them report additional information on the same series of cases [8, 49]. (Fig 1).

Only three of these reports describe neurological complications after lip MSGB in the context of a systematized data collection on both immediate (<24h) postoperative complications (bleeding, fainting, tachycardia, and bruising) and mediate adverse events (pain, inflammation, suture dehiscence, infection, and granuloma or cheloid scarring) [9,10,12]. Three case series with larger sample sizes [9,10,13] describe minimally invasive lip biopsy techniques, and report neurological complications in a range from 0 to 11.73%, although permanent hypoesthesias account for not more than 0.22% [9]. In contrast, twelve descriptive studies performing biopsies using 5 mm to 30 mm long linear incisions using a scalpel [8] with or without a chalazion forceps, report neurological complications in a range from 0 to 5.7% which remained after six months.

Table 1 presents the characteristics of the studies included in this meta-analysis. Four studies did not provide information on permanent neurological adverse events and were excluded from this specific calculation. Their patients were not considered for calculating the pooled prevalence.

High quality studies yielded a pooled prevalence that was 3 times as high as the pooled prevalence of low quality studies. Both estimates showed large heterogeneity.

Table 2 shows the pooled prevalence for each biopsy technique. Heterogeneity between studies was low, except for total adverse events in the minimally invasive technique: in this group the random effects pooled prevalence is then a more germane measure than that of the fixed effects model. In the minimally invasive group, the pooled prevalence of total adverse events is almost 4 times higher than that in the linear incision group (4.73% vs. 1.20 %). On the contrary, the pooled prevalence of the permanent or potentially permanent neurological adverse events is 8.5 times lower in the minimally invasive technique group than in the group of studies using linear incisions (0.17% vs. 1.45%). Moreover, studies performing linear incisional biopsies report lower percentages of complications in case series with high proportion (≥50%) of chronic saladenitis (1.01 vs 1.59). When studies perfoming linear incisional techniques were stratified by quality, those papers with high scores (Qs≥3) report a higher percentage of permanent neurological adverse events (2.50 vs 0.82) (Table 2).

## Discussion

Our study shows that, apparently, minimally invasive biopsy techniques are safer than conventional incisional approaches.

No clinical trials focused on the aims of this meta-analysis could be retrieved. All reports considered in this systematic review are cases series without missing patients for follow-up, so a selection

bias is improbable. Alternatively, there is a potential for information bias as only three studies describe systematized procedures for recording information on immediate and mediate complications [9.10,12] and only one investigation employed a structured questionnaire with open and closed questions [9].

There also is an evident lack of consensus on the definition and classification of postoperative complications by severity [41] which may have facilitated inaccuracies and omissions during data collection. Very few studies provided information on confounding factors [12,42], such as corticosteroid therapy, radiotherapy, tobacco consumption, clinical setting (inpatient vs. outpatient), or chronic sialadenitis rate (table 2), which may influence post-biopsy complications [1,8,10,12,13,17,43]. In fact, only one investigation [12] made an attempt to address this issue during data analysis by stratifying the results by the use of sutures, and concluded it made no difference in terms of frequency and type of complications. This observed lack of control for confounding factors may hamper internal validity of the reports included in this meta-analysis, as high quality studies detect higher percentages of neurological complications (table 2).

Minor salivary glands in the lower lip are distributed into 1 to 3 layers of discrete gland cluster, mainly in the cuspid-premolar area [44]. The fact that minimally invasive techniques harvest only the more superficial glands would explicate the little risk of damaging the sensory nerves [13] and the lower prevalence of

permanent neurological complications. However, the need for multiple, small-sized, incisions and the proximity of nerve endings to the glands may explain the presence of postoperative transient lip hypoesthesias. Conventional techniques require larger incisions and dissection of the wound borders to frequently retrieve glands from deeper layers in the lip, where nerve fibres and glandular tissue are closely related, which may well explain the higher prevalence of permanent lip hypoesthesias in this group.

Both approaches permit the fulfilment of SS diagnostic requirements –retrieval of at least five minor salivary glands [45,46]- in a minimal operative time [18,47], and both share common shortcomings: harvest similar percentages of fatty and fibro-muscular tissues and comparable numbers of biopsies resulting in insufficient amount of glandular tissue [7,10].

Two of the studies included in the meta-analysis did not report neurological morbidity after sublingual salivary gland biopsy [15,48], whereas another two studies found neither sensory nor motor nerve loss related to parotid gland biopsy for SS diagnosis [7,47]. These comparative studies seem to suggest that both procedures –sublingual and parotid biopsy- have a diagnostic potential for SS comparable to MSGB, but they may offer minor postoperative morbidity. Alternatively, MSGB have proved to be safe, simple, and suitable to extensive routine application in outpatient –internal medicine and rheumatology- settings [7,9].

Despite techniques removing labial mucosa together with its attached glands have been discouraged because of the potential for neurological damage [16], a research group has recommended the use of a punch (4 mm diameter) for taking biopsies from the inner side of the lower lip. This paper described only 4% of transient numbness in a series of 50 patients with unknown follow-up periods [17]. This approach implies retrieval of insufficient glandular material and inherent sampling mistakes which would force a second biopsy, which would in turn increment patient's morbidity.

With the limitations intrinsic to the potential biases in the studies included in this meta-analysis, we conclude that minimally invasive lip biopsy technique for SS diagnosis induces less permanent neurological complications than conventional approaches with large linear incisions in the lower lip. Moreover, and due to the absence of relevant scientific evidences supporting the selection of a particular technique for salivary gland biopsy in SS diagnosis, it is recommended to undertake clinical trials to assess the existing approaches for lip MSG (minimally invasive vs. large linear incisions), together with sublingual and parotid biopsy, and ensuring adequate follow-up periods.

## Key messages

1. Minor salivary gland biopsy in the lip has proved useful for Sjögren's syndrome diagnosis.
2. Minimally invasive biopsy techniques induce less permanent neurological adverse events than conventional incisional approaches.

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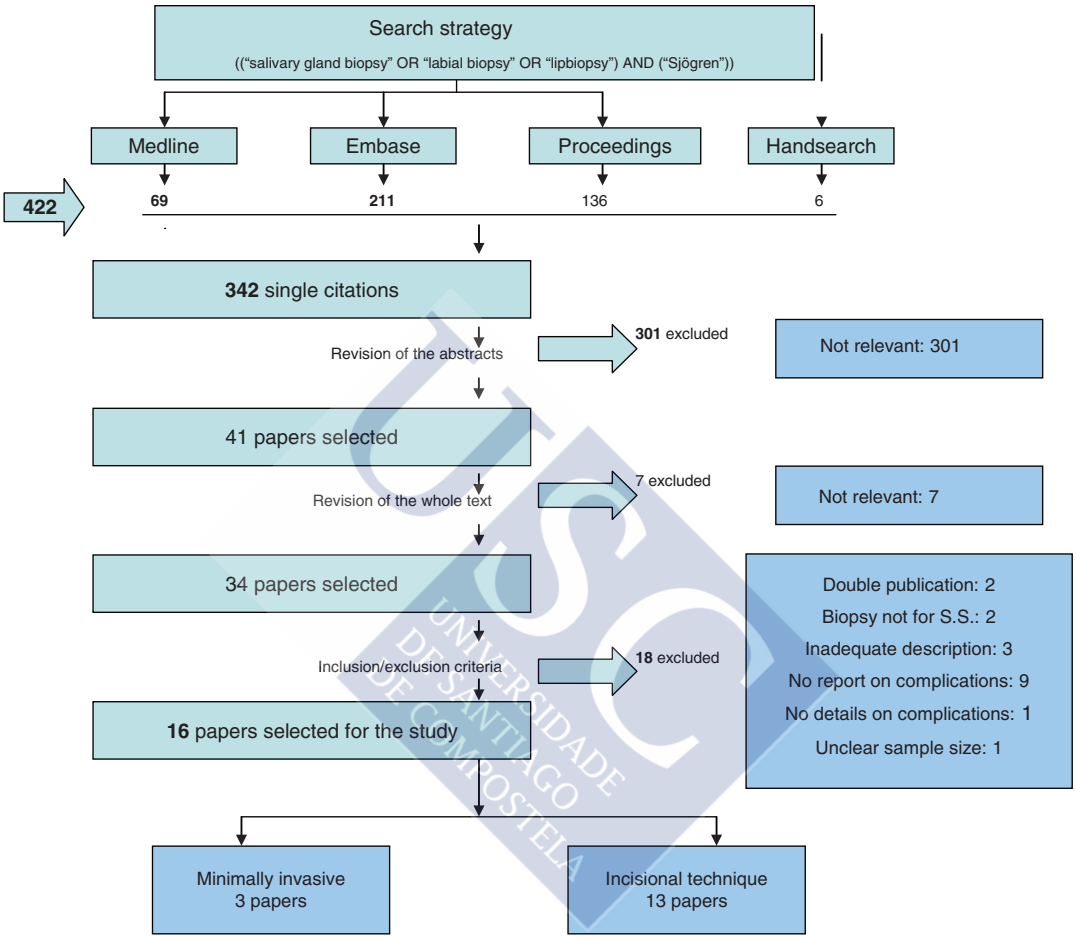
## Fig 1. Flow chart of the study.



## Table 1 Characteristics of the studies included in this meta-analysis

| Author (year) | Country | n (M/F) | Approach | Instrument | Incision | Glands,*n* | Suture | Chronic Sialadenitis n(%) | Follow up | Total neurological complications *n* (prevalence %) | Permanent neurological complications *n* (prevalence %) | Quality score (Q) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chisholm and Mason [1] | UK | 40 (15/25) | Incisional | Scalpel | Ellipse central 3x0.75cm | | Y | 10 (25) | No information | 0 (0) | 0 (0) | 2 |
| Greenspan *et al.* [43] | USA | 75 | Incisional | Scalpel | 1.5 – 2cm linear parallel vermillion lateral to the midline | 4-7 | Y | 43 (57.3) | Several months | 1 (1.33) | 1 (1.33) | 4 |
| Daniels [16] | USA | 362 | Incisional | Scalpel | Lateral 1.5-2cm | 1-16 (6 average) | Y | 225 (62.1) | No information | 3 (0.83) | No information | 1 |
| Marx *et al.* [47] | USA | 79 | Incisional | Scalpel | 3x0.75cm linear parallel vermillion | 7 | Y | 32 (40.5) | 2 years | 3 (3.80) | 1 (1.27) | 5 |
| Pennec *et al.* [48] | France | 50 (7/43) | Incisional | Scalpel | Lateral 1.5-cm horizontal | | Y | 14 (28) | No information | 0 (0) | 0 (0) | 1 |
| Richards *et al.* [14] | UK | 58 (5/53) | Incisional | Scalpel | Horizontal | 6-8 | Y | - | 1 year | 2 (3.45) | 2 (3.45) | 3 |
| Lopez-Amado *et al.* [27] | Spain | 35 | Incisional | Scalpel | Linear (2 cm)<br>Ellipse | 7<br>5 | Y<br>Y | 32 (91.4) | No information | 0 (0)<br>0 (0) | 0 (0)<br>0 (0) | 2 |
| Friedman *et al.* [12] | Israel | 118 | Incisional | Scalpel | 5-7mm | >5 | Y<br>N | - | 3 weeks | 2 (1.69) | No information | 5 |
| Gorson and Ropper [46] | USA | 54 (19/35) | Incisional | Scalpel | Vertical (1 cm) | ≥4 | - | 20 (37) | No information | 1 (1.85) | 1 (1.85) | 1 |
| Berquin *et al.* [15] | Belgium | 24 | Incisional | Scalpel | Oblique 1.5cm from midline | | Y | 12 (50) | No information | 1 (4.17) | 1 (4.17) | 2 |
| Teppo and Revonta [13] | Finland | 191 (36/155) | Minimally invasive | Scalpel Cup forceps | 2-3mm multiple horizontal incisions | | N | 154 (80.6) | 1.5 -19 years | 0 (0) | 0 (0) | 2 |
| Pijpe *et al.* [7] | Holland | 35 | Incisional | Scalpel | 3cm linear parallel vermillion | 7 | Y | 19 (54.2) | 1 year | 2 (5.71) | 2 (5.71) | 5 |
| Langerman and Blair[8], Langerman[49] | USA | 47 | Incisional | Scalpel Chalazion forceps | 5-10 mm horizontal elliptical incision | 3-7 | Y | 39 (82.9) | 1 month | 2 (4.26) | No information | 3 |
| Caporali *et al.* [9] | Italy | 452 | Minimally invasive | Scalpel forceps | 2-3mm | 4-5 | Y | 93 (20.5) | 6 months | 53 (11.73) | 1 (0.22) | 3 |
| Lida *et al.* (2012) [10] | Argentina | 186 (9/177) | Minimally invasive | Scalpel forceps | 2-3mm. | 4-5 | Y | - | 1 month | 5 (2.69) | No information | 5 |

**Table 2. Pooled prevalence and 95% confidence intervals (CI) of total and permanent neurological adverse events.**

| | Number of studies | Pooled prevalence (95% CI) Fixed effects | Pooled prevalence (95% CI) Random effects | Ri* | Q test p-value |
|---|---|---|---|---|---|
| Incision (all) | 12 | 1.20 (0.00-2.49) | 1.20 (0.00-2.49) | 0.00 | 0.99 |
| Incision (permanent) | 9 | 1.45 (0.00-4.08) | 1.45 (0.00-4.08) | 0.00 | 0.99 |
| Incision (high quality) | 6 | 2.50 (0.00-5.21) | 2.50 (0.00-5.21) | 0.00 | 0.97 |
| Incision (low quality) | 6 | 0.82 (0.00-2.28) | 0.82 (0.00-2.28) | 0.00 | 0.99 |
| Incision (high sialadenitis rate) | 6 | 1.01(0.00-2.48) | 1.01(0.00-2.48) | 0.00 | 0.94 |
| Incision (low sialadenitis rate) | 4 | 1.59 (0.00-5.36) | 1.59 (0.00-5.36) | 0.00 | 0.86 |
| Minimally invasive (all) | 3 | 3.01 (1.50-4.53) | 4.73 (0.00-11.74) | 0.95 | 0.00001 |
| Minimally invasive (permanent) | 2 | 0.17 (0.00-1.08) | 0.17 (0.00-1.08) | 0.00 | 0.85 |

* Proportion of total variance due to between-study variance.

# 6. Lip biopsy for the diagnosis of Sjögren's Syndrome: Beware of the punch!

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# 6. Lip biopsy for the diagnosis of Sjögren's Syndrome: Beware of the punch !


## Abstract

A pilot study aimed at examining the presence of nerve fibres in minor salivary glands tissue samples obtained by two procedures (punch *vs*. linear incisional technique). The study was undertaken on a convenience sample of five cryopreserved corpses (mean age 74±3.5; 3 males and 2 females), and biopsies performed on the buccal side of the lower lip, between the mid-line and the corner of the mouth. Each corpse had one side of its lower lip biopsied by punch and the contra-lateral side using a linear incision. All punch samples (100%) displayed severed nerve fibres whereas no nerve fibres (0%) could be identified in the samples obtained by means of the linear incision technique. Within the limitations the study, our results strongly discourage punch techniques for minor salivary gland lip biopsies and provide information on the superiority of linear incisional biopsies in terms of neural damage. These results may also recommend undertaking clinical trials on patients suspicious for SS comparing the morbidity associated to linear incisional technique *vs*. minimally invasive biopsies.

## Introduction

Minor salivary gland biopsy (MSGB) has been used for the diagnosis of systemic disorders, like amyloidosis, sarcoidosis, Sjögren's syndrome (SS) and for the confirmation of neonatal hemochromatosis.

The presence of focal lymphocytic sialadenitis with a focus score >1 per 4 $mm^2$ of glandular tissue is an objective criterion to consider when diagnosing Sjögren's syndrome. MSGB has proved validity and diagnostic confidence with a high specificity and positive predictive value, and an average sensitivity of 79% [1]. These features make MSGB particularly useful for patients with inconclusive clinical findings, incipient forms of the syndrome, SS with negative anti Ro/la serology and extra-glandular involvement.

A wide range of surgical approaches have been described for harvesting at least five accessory glands from the lower lip using different instruments (scalpel, punch, or cup forceps), and for producing different incisions (circular, linear or elliptical) with a variety of sizes (from 2 mm to 3 cm) [1-5] and orientations (parallel to the lip, oblique, or even vertical). The use of a forceps with a fenestrated active end (chalazion forceps) to stabilize the lip has also been suggested.

The selection of the best surgical approach in terms of related morbidity is hampered by the absence of comparative studies and the proliferation of descriptive papers that do not state negative

outcomes associated to the technique performed [4]. Moreover, those reports describing percentages of surgical complications have a limited validity due to the lack of standardization when defining and categorizing the complications according to their severity [4,5]. Anyhow, persistent lip numbness is the most frequently published surgical complication [4], occurring in up to 6% of MSGBs performed in the lower lip.

Despite the existing investigations discouraging the removal of labial mucosa with attached glands when performing MSGB because of the potential for neurological damage, punch use has been widely recommended because of safety and handling simplicity reasons, as this procedure is not technically demanding and can be undertaken at an outpatient setting [2,3]. However, and to the best of our knowledge, there are no quality comparative studies assessing neurological damages induced by different techniques for MSGB. Thus, the aim of this pilot study was to examine the presence of nerve fibres in minor salivary gland tissue samples obtained by means of two different procedures (punch technique *vs*. linear incisional technique).

## Methods and materials

On the basis of the feasibility of the investigation and to minimize potential ethical conflicts, an observational, descriptive pilot study designed to replicate the techniques for minor salivary gland

biopsy for SS diagnosis [2-4] was undertaken on a convenience sample of five not formolized, frozen corpses (mean age 74±3.5; 3 males and 2 females). All subjects had bequeathed their bodies to the Department of Morphological Sciences for medical-scientific research and training purposes and all procedures were undertaken according to our University Ethics Committee (14/2007) recommendations.

Biopsies were performed in the inner side of the lower lip, between the mid-line and the corner of the mouth. Each corpse had one side of its lower lip biopsied by punch and the contra-lateral side using a linear incision. The biopsy site was randomly allocated to each technique using a computer-generated list of random numbers.

The punch biopsy technique (MSGPB) was undertaken following previously established protocols [2,3] by everting the lip, perpendicularly positioning a 4 mm diameter punch (Stiefel laboratories, Madrid, Spain) and performing simultaneous rotational movements under gentle pressure to reach 8 mm deep into the lip. The cylinder of tissue was removed from its base using a scalpel with a No. 15 blade and placed onto a filter paper to avoid curling or twisted artefacts.

For the incisional biopsy, the lip was stabilized with a forceps (OEPM nº 201200158) and the incision performed away from the mid-line using a No 15 scalpel blade. This incision was directed horizontally for about 1.5 cm, just penetrating the epithelium and

combined with a blunt dissection of the borders or the wound. Five minor salivary glands were harvested from each corpse [6].

All specimens were immediately introduced in a wide-mouthed container, coded, and fixed in a generous amount of 10% formalin buffered saline for 24 hours.

A single pathologist longitudinally cut all specimens with a new disposable scalpel for every section to obtain 3 slices 200 μm apart from each specimen and orientated them before paraffin embedding. Samples were prepared in 4 μm sections, stained with hematoxylin and eosin and processed by the same technician. All specimens were examined using an Optiphot-2 microscope (Nikon, Tokyo, Japan) equipped with a millimetre-calibrated eyepiece graticule (Graticules Town Bridge, Kent, UK) in order to measure the length of the core tissue obtained by punch procedures. Pathological analysis also assessed the presence of severed nerve fibres within the tissue samples and the number of glands obtained by each technique.

The scores obtained for each variable were recorded and the confidence intervals for the differences between techniques calculated by means of the Epidat 3.1 statistical package (Xunta de Galicia, Santiago de Compostela, Spain).

## Results

The punch technique for minor salivary gland biopsy produced specimens of 7.2±1.1 mm long. The procedure harvested one minor salivary gland per sample in three cases; another case showed 2 glands located at the same depth in the tissue sample and the last case showed no glandular tissue in the specimen.

All MSGPB samples (100%) displayed severed nerve fibres, located deeper in the tissue than the minor salivary glands. Only one sample showed nerve fibres close to the glandular tissue, at a more superficial level (Fig 1). No nerve fibres (0%) could be identified in the samples obtained by means of the linear incision technique (Fig 2). The results are summarized in table 1.

Pathological analysis revealed no handling-related artefacts (pseudo-cysts, crushing, fragmentation, haemorrhage or fissures), although fixing alterations could be recognized (more appealing at the lip mucosa epithelium and the minor salivary glands), probably due to the use of frozen cadaveric material.

## Discussion

The selection of a particular technique for MSGB is limited by the absence of clinical trials and standardization of surgical complications, which seriously compromise the validity of the information available from the literature on this topic. Existing case series display a wide range of sample sizes (6 to 502

patients) with different frequencies of complications associated to the variety of techniques employed [1,4,5]: studies performing incisions shorter than 2 cm with a scalpel report complications ranging from none to 9.3% [4] , whereas larger incisions (2 - 3 cm) are described to cause complications in a range from 3.7% to 31% [7]. Transient disorders of lip sensitivity occurred in up to 11.7% of the procedures [4], and those studies with follow-up periods beyond one year report persistent lower lip hypoaesthesia in about 3.4% to 4% of the cases [4]. Only minimally invasive techniques [5,6] (excluding punch), based upon multiple 2 to 3 mm scalpel incisions on the inner side of the lower lip combined with a cup forceps to retrieve the tissue samples have proved absence of neurological morbidity after a 1.5 years follow-up period [4].

However, minor salivary gland punch biopsy has been suggested as an alternative to incisional biopsy techniques precisely because of the absence of risk for the patient, its simplicity (can be performed by a single operator) and also because it is less expensive [2,3]. This technique consists on biopsy taking from the buccal side of the lower lip -which is stabilized by the patient him/herself- using a 4 to 5 mm punch which permits the retrieval of a tissue cylinder up to 8mm long [2,3]; no complications have been reported using this protocol in a series of 14 patients [3] and only 4% of transient numbness in a series of 50 cases with unspecified follow-up period [2].

Although this approach may seem interesting, the percentages of undesired events reported in these investigations [2,3] should be considered with caution as they are unexpectedly well under those described for incisional techniques, where sensory nerves can be clearly seen and subsequently avoided, and also because they strongly disagree with our results obtained under controlled experimental conditions.

In general, sample size calculations may not be required for pilot studies, as they are focused mainly on feasibility rather than on statistical signification. However, it has to be kept in mind that studies with small sample sizes may not disclose differences between groups. Despite our study was performed on a small series of cadaveric material, the massive differences in terms of neural damage between techniques disclosed by this study seem to suggest that chances for error are remote, irrespective of sample size issues. Moreover, post-mortem studies have been frequently employed when validating glandular biopsy for SS diagnosis [8]. Fresh, cryopreserved (frozen) corpses are the best model in terms of similarity of tissue quality and surgical handling. The age of the patients in the series (>50) may also be and advantage, because as persons grow older fibrosis and reductions of the acinar volume occurs which is a similar situation to that found in SS patients [8].

Previous reports have described collection of an average of six MSG using incisional techniques 1.5 y 2cm [1,4,5,7]. Additionally,

our study on MSGB using a 1.5 cm linear incision permitted retrieval of at least 5 glands, whereas punch-biopsy did not provide enough gland material for diagnosis of SS in any situation. Incisional techniques (including those minimally invasive) have enabled the retrieval of an average of more than 5 glands accompanied by only a 1.6% of undesired material, mostly fatty or fibromuscular in nature [5]. In contrast, sampling errors are an inherent handicap for punch techniques, which sometimes require an additional biopsy on the other side of the lip [2] and increases morbidity. The reduction of surgical time to a "few minutes" [2] does not seem to be an advantage for punch biopsy either, as incisional biopsies using linear incisions take from 5 minutes to 9 [7]. Minimally invasive techniques can also be performed within a minimal operative time.

Alternative procedures to MSGB for SS diagnosis, such as sublingual salivary gland biopsy [9] or parotid biopsy [10] caused no neural morbidity. These comparative studies seem to suggest that both procedures -sublingual and parotid biopsy- retain a diagnostic potential comparable to that of lip biopsy and may be related to lower postoperative morbidity [9-10].

Within the limitations of this kind of studies, our results discourage the use of punch techniques for biopsies of minor salivary glands in the lip and provide information on the superiority of linear incisional biopsies in terms of neural damage. The results of this pilot study may permit the recommendation of

undertaking clinical trials on patients suspicious for SS comparing the morbidity associated to MSGB (linear incisional technique vs. minimally invasive) and major salivary gland biopsy .

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## Figure legends.

Figure 1: H&E 4x. Deep portion of a lower lip sample obtained by punch biopsy: sectioned nerve fibres can be seen close to a minor salivary gland.

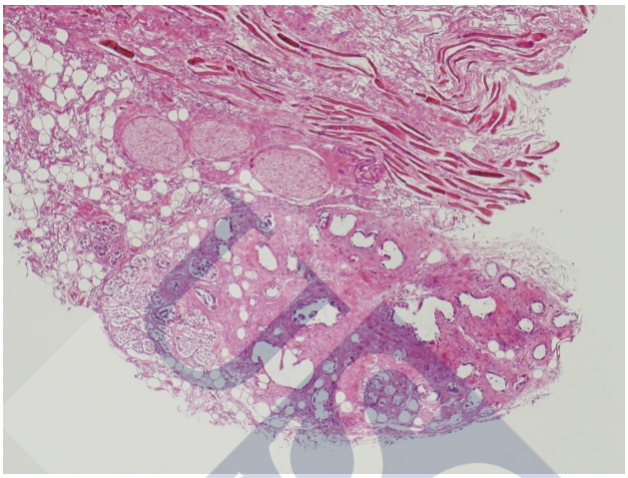
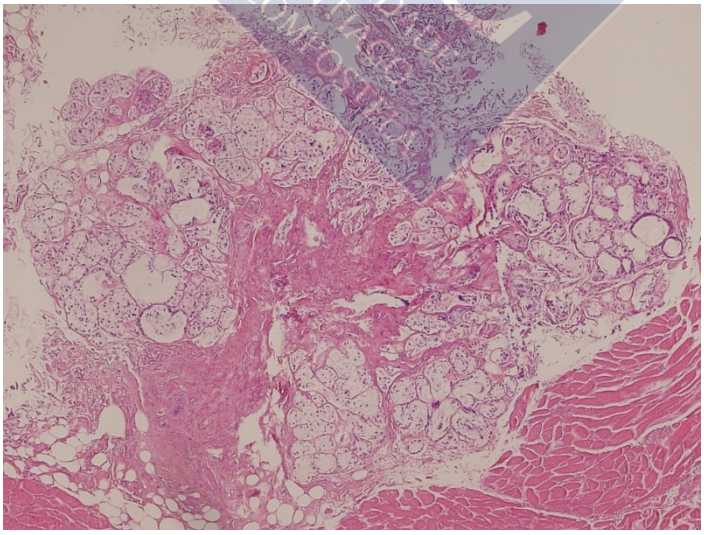


Figure 2. H&E 4x. Minor salivary gland obtained by linear incisional technique.



## Table legend.

| Case | Age | Gender | Specimen length (mm) | No. of accessory glands | | Presence of nerve tissue | |
|---|---|---|---|---|---|---|---|
| | | | | Punch | Linear incision | Punch | Linear incision |
| 1 | 76 | F | 5.5 | - | 5 | + | - |
| 2 | 72 | M | 7.0 | 1 | 5 | + | - |
| 3 | 79 | M | 7.0 | 1 | 5 | + | - |
| 4 | 70 | F | 8.5 | 1 | 5 | + | - |
| 5 | 73 | M | 8.2 | 2 | 5 | + | - |

Table 1. Number of glands and presence of nerve tissue in the specimens.

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# 7. Minor salivary gland biopsy in Sjögren's syndrome: a review and introduction of a new tool to ease the procedure.


## Summary

Objectives: To review the existing techniques for minor salivary gland biopsy (MSGB) in the lip and to suggest a new approach to ease the procedure and reduce post-operative complications.

Study design: A comprehensive literature review and a descriptive study of a new surgical technique

Results: Diverse incisions have been suggested for MSGB with different designs (ellipse, circular, linear), different directions (parallel, oblique, vertical) and a wide range of lengths (from 1 mm up to 3 cm), but no comparative studies supporting the advantages of a particular type of incision over the others could be retrieved. A variety of features of the existing techniques for MSGB are linked to undesired events and surgical complications which could be minimized by modifying certain aspects of these procedures. The technique described, together with the use of the S forceps, represents a significant improvement over the already described chalazion forceps because it allows for a better access and positioning of the lower lip, improves the ergonomic

conditions of the assistant, and facilitates the identification of lip areas with more superficial gland lobules.

Conclusion: The suggested approach for lip MSGB includes a specifically designed instrument whose performance during lip biopsy may contribute to minimize post-operative complications.

## Keywords

Sjögren's syndrome; diagnosis; minor salivary gland biopsy; surgical technique; lower lip.

## Introduction

The Sjögren's syndrome (SS) is an autoimmune exocrine disorder with signs and symptoms of dry mouth and keratoconjunctivitis sicca, which may sometimes display a wide range of systemic, non-glandular alterations (1,2). The prevalence of this syndrome has been estimated to range between 0.5% and 1% (3), with a female:male ratio of about 9:1 (1-3).

Histopathology in minor salivary gland (presence of focal lymphocytic sialadenitis with a focus score ≥1) is one out of the six diagnostic criteria set in the revised international classification for Sjögren's Syndrome (2) for diagnosis of SS. It has recently become more important because of the consensus in considering only objective criteria to define a SS case, which has to meet at least 2 of the following 3 findings: 1. Positivity serum anti-SSA

and/or SSB; 2. Ocular staining score >3; and 3. Presence of focal lymphocytic sialadenitis with a focus score >1 per 4 $mm^2$ of glandular tissue (2).

A systematic review on minor salivary gland biopsy (MSGB) has proved diagnostic value for SS with high specificity (X±SD= 88.1±11.7) and sensitivity (X±SD= 78.8±11.2), as well as diagnostic confidence in terms of positive (X±SD= 87.6±9.5) and negative (X±SD= 79.0±16.9) predictive values (3). These results make this technique particularly useful for patients suspicious for SS with inconclusive clinical findings (4). MSGB may also contribute to diagnosis of amyloidosis, sarcoidosis, and confirmation of neonatal hemochromatosis (3,5,6).

Despite the different surgical approaches suggested for MSGB (use of chalazion forceps for tissue stabilization, usage of scalpel vs. punch, different incision sizes, and need or not for suturing), both immediate and mediate complications are continuously described in the literature, being the most relevant a long-lasting lower lip numbness occurring in up to 6% of MSGB procedures (7). These events support the need for a review of the technique to reduce morbidity. In this sense, we suggest the use of a specifically designed forceps for lip biopsy in SS patients that improves tissue stabilization, eases the procedure, and reduces complications.

## Materials and methods

The materials required for this technique include a syringe for intraoral local anaesthesia, scalpel with a No. 15 blade, non-toothed Adson forceps, 4/0 braided silk suture, and the "S" forceps for biopsy (OEPM nº 201200158) (Fig.1). This is a 18.5 cm long forceps with a fenestrated active end (5 $cm^2$). Both the fenestrated area (longitudinal to the axis of the forceps) and its wide size are conceived to provide an ample surgical field. The non-fenestrated blade of the forceps is slightly convex in shape to facilitate herniation of minor salivary gland lobules. There is a screw in the shank for adjustment of the space between the blades, thus permitting a variable and controlled pressure over the soft tissues during the surgical procedure. The handles of the forceps are at an angle with the blades to help traction and visibility of the surgical field. This angle also permits the forceps to work as a surgical separator improving accessibility by means of a traction-separation movement.

## Technique

The biopsy site should be selected from the inner side of the lower lip, rich in minor salivary glands, avoiding the midline area due to its lesser content of glandular component (Fig.2).

Local anesthesia is performed by perilesional infiltration or blockage of the mental nerve. Once anesthesia is achieved, the whole lower lip is stabilized using the S forceps, and the biopsy

site selected taking advantage of the forceps design which forces the gland lobules to protrude through the fenestrated blade.

A horizontal linear incision of about 1 cm to 1.5 cm is performed away from the midline, combined with a blunt dissection of the borders of the wound. At this stage, the lobules are herniated towards the surface of the wound pushed by the non-fenestrared, convex, blade of the forceps (Fig. 3). Five to seven lobules can now be gently removed using the Adson tweezers and introduced into an abundant fixing solution (at least ten fold the volume of the tissue sampled). The wound is then sutured with interrupted single sutures. Use of magnification is recommended when performing the technique in order to identify superficial nerves and vessels and to diminish surgical morbidity.

## Observations about the technique

MSGB of the lip is a key diagnostic tool for the diagnosis of systemic disorders and particularly of SS.

The technique described above, together with the use of the S forceps, represents a significant improvement over the already described chalazion forceps because it allows for a better access and positioning of the lower lip, improves the ergonomic conditions of the assistant, and facilitates the identification of lip areas with more superficial gland lobules. It also permits a better bleeding control during surgery, an enhanced visualization of

vessel and nerve endings, reduces the surgical time, and provides non-artefacted lobules for pathological analysis (Fig. 4).

## Discussion

*Techniques and complications in MSGB*

Despite there is a wide agreement on avoiding the glandular-free zone in the centre of the lower lip, it seems to exist a remarkable lack of standardisation of the MSGB technique when aimed at obtaining at least five glandular lobules for the diagnosis of SS (8). Different incisions have been suggested with different designs (ellipse, circular, linear), different directions (parallel, oblique, vertical) and a wide range of lengths (from 1 mm up to 3 cm), but no comparative studies supporting the advantages of a particular type of incision over the others could be retrieved (9-19).

Most frequent immediate surgical complications include intra- and post-operative bleeding (9,11). Pain, inflammation, wound infection, suture dehiscence, and cheloid scars are described as mediate complications of glandular biopsy (7,9-15), but the so-called "disorders of lip sensitivity" are the most frequently reported complication (18, 19), occurring in up to 11% of cases in large series (12). This finding has discouraged the use of a punch for MSGB because it removes lip mucosa together with the attached gland, and favoured techniques that permit identification

and avoidance of sensory nerve endings (16). These complications may well justify that only patients in a community setting with negative results for anti-RO/la antibodies would be referred for MSGB (20).

*Lip stabilization devices*

In this sense, some authors have suggested the use of chalazion forceps, employed by ophtalmologists during chalazion exeresis, to ease biopsy of minor salivary gland from mobile lip tissue, as it permits tissue stabilization and to work under ischemic conditions (6,7). However, this instrument was originally designed for ophthalmology and has a number of shortcomings for oral use: the handles of the chalazion forceps are small-sized to allow finger control and are placed perpendicular to the main axis of the blades; this forces the assistant's hand to work on an uncomfortable position, too near to the surgical field. The size of the fenestration also limits the incision design, particularly when undertaking minimally invasive techniques with multiple 2 mm incisions along the inner face of the lower lip (13,18,19). An improved chalazion forceps was introduced by López-Jornet & Bermejo-Fenoll (21): this forceps was larger than the original (20 cm.) and its active end provided a constant pressure of 1Kg/cm2 on the tissues exerted by means of two flat plates (one of them with a round opening, sized 1.7 cm diameter). This design eases lip stabilization by the assistant, but it is impossible to graduate

the pressure on the lip tissue and the fenestrated blade provides a reduced surgical field.

On the other hand, the forceps we suggest for MSGB, besides permitting a controlled pressure adapted to the surgical time and to the features of the lip of the patient (macrochelia, etc.), allows a more ergonomic hand grasp in such a way that keeps the assistant's hand away from the working area without disturbing the surgeon. Moreover, the width of the fenestrated blade in this forceps conditions neither the design nor the size of the incisions as well as permits minimally invasive techniques, where a wide surgical field is required to harvest glandular tissue all over th inner side of the lower lip (18).

## Conclusion

This forceps stabilizes lip tissues, avoids excessive intra-operative bleeding, permits better visibility of the surgical field, allows improved selection of tissue samples for pathological analysis and has a potential to minimize the morbidity related to iatrogenic nerve lesions.

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## Figure legends:

Fig 1. “S” forceps.

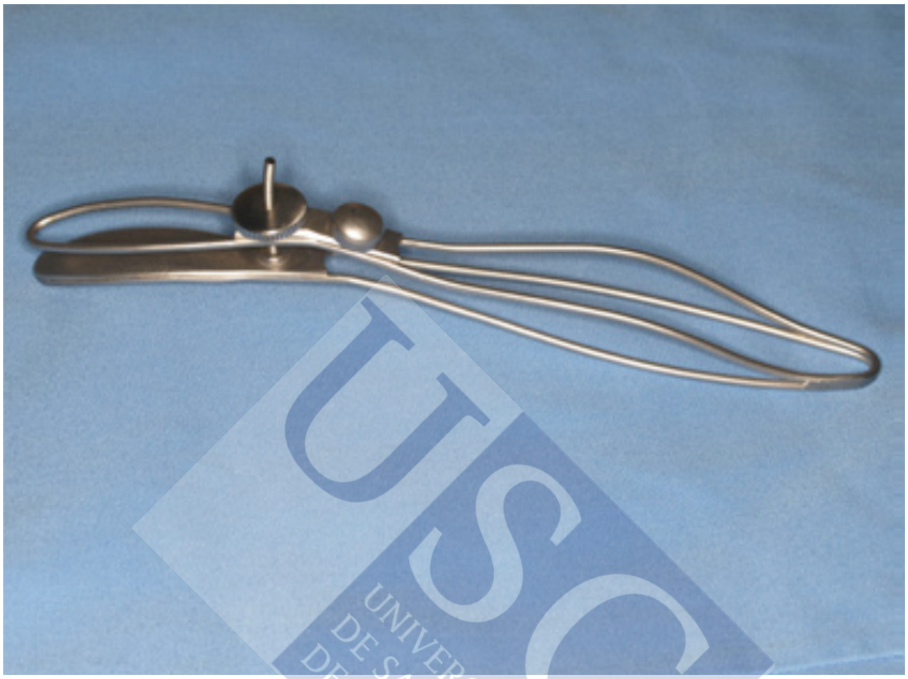


Fig 2. “S” forceps in use

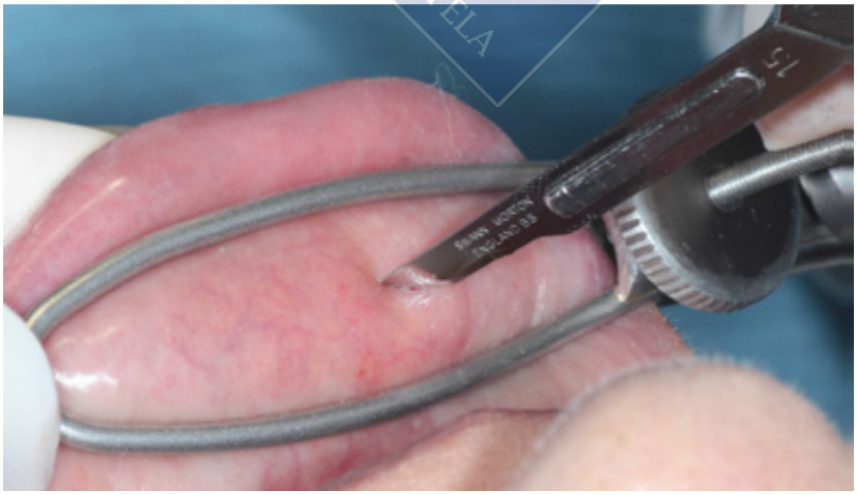


Fig 3. Excision of a minor salivary gland

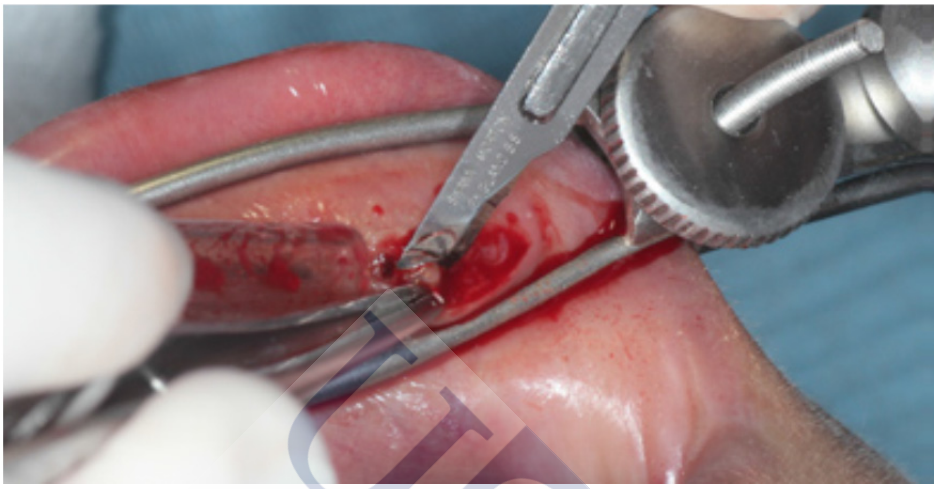
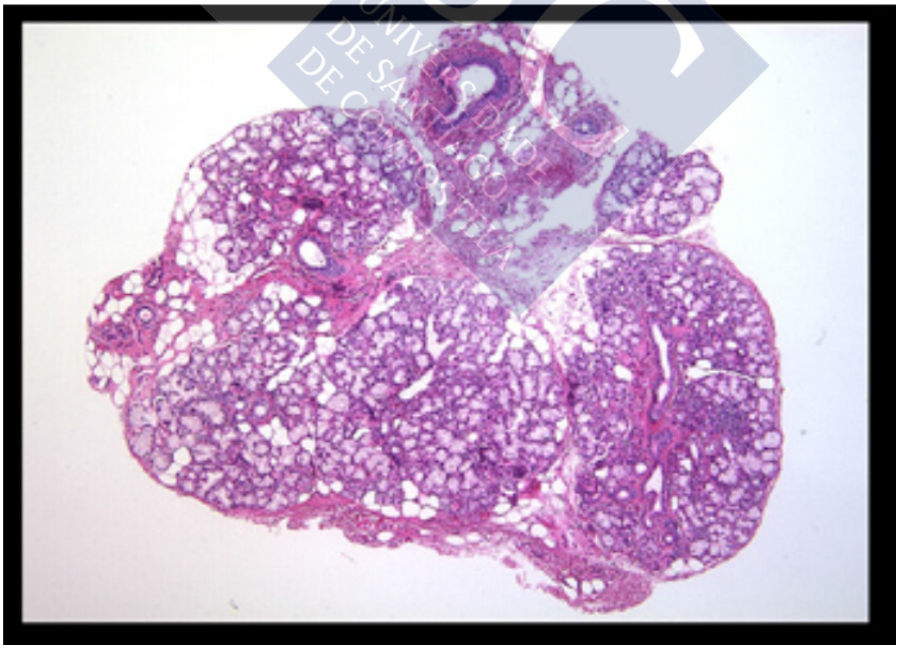


Fig 4. Minor salivary gland (H&E x10)




# 8. Discussion

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# 8. Discussion

Essentially, all studies considered in our meta-analysis are descriptive case-series with a low risk for selection bias, use heterogeneous criteria for defining complications, and employ different follow-up periods. Data collection procedures are also insufficiently described, and only one study reported the use of a structured questionnaire for this purpose (Caporali et al, 2008); another three studies displayed their results as mediate and immediate complications (Friedman et al, 2002; Caporali et al, 2008; Lida-Santiago et al, 2012).

Unfortunately, most studies on this topic show potential confounding variables related to the surgical setting, the clinical features of the patient, and even to toxic habits such as tobacco consumption, etc. which had not been adequately controlled; and any of these factors may compromise the validity of the investigations. Anyhow, our meta-analysis revealed a higher proportion of postoperative complications in those reports with higher methodological quality.

Actually, all approaches for minor salivary gland biopsy may be grouped under two headings: techniques using small incisions

(minimally invasive) vs. approaches using wide incisions aimed at harvesting at least five glandular lobules. Another key point that remains unclear is about which gland is the most suitable one for obtaining the diagnostic sample: some reports have described an absence of neurological morbidity after sublingual (Pennec et al, 1990; Berkin et al, 2006) or parotid (Marx et al, 1998; Pijpe et al, 2007) gland biopsy for SS diagnostic purposes.

Bearing in mind the potential limitations of the studies included in this meta-analysis, it appears that minimally invasive approaches using small (even multiple) incisions cause less morbidity in terms neurological damage than techniques using wide incisions. Nonetheless, the scientific community should undertake methodologically sound clinical trials for testing this hypothesis without any risk for biases.

It is also worth mentioning the paper by Gevara-Gutiérrez et al (2011) supporting the use of a punch for minor salivary gland biopsies in the lower lip. This recommendation is based upon their results obtained from a large sample of patients suspicious for SS, where only minor transient neurological complications in a small number of patients were found. The lack of biological plausibility of the results reported by this group made us assess this approach on an experimental model.

Experienced clinicians have described the inconvenience of removing lip mucosa together with the glandular material due to the higher risk for neurological damage during the surgical procedure because of the poor identification of anatomical structures. This circumstance always occurs when a circular scalpel (punch) is employed. Despite this fact, some research groups still consider this approach a convenient, minimally invasive, simple, and cheap procedure for lip biopsy (Bertram et al, 1970; Guevara-Gutierrez et al, 2001). These latter reasons, along with the absence of complications (Bertram et al, 1970) and the possibility for a single operator to undertake the procedure, the use of a 5mm–diameter and 8 mm-length punch has been proposed as an alternative to conventional biopsy for SS diagnosis, although no further investigations for endorsing or discarding this recommendation has been undertaken.

Because of ethical issues, we have carried out a pilot study on an experimental model (cryopreserved corpses) aimed at assessing both the potential damage to lip nerve tissue and the amount of glandular tissue obtained under this biopsy protocol with punch. This model has been widely employed for validating this kind of techniques, as the particularities of this particular model in terms of surgical handling and condition of the glandular content closely resemble those of SS patients (Chisholm et al, 1968; Chisholm et al, 1970).

This pilot study, performed on a reduced sample, has disclosed sectioning of sensitive nervous fibres in all cases; and this finding makes us discourage the use of circular scalpel for lip biopsy.

A number of devices aimed at easing this surgical technique, particularly for stabilizing the lip, have been previously described (Seoane et al, 2000; Lopez-Jornet et al, 2005). The first reports on this issue used ophthalmologic devices (Seoane et al, 2000) for this sake, although limitations like the size and the orientation of the fenestration of the forceps and the poor access to posterior areas in the mouth, mainly due to the presence of the pressure-regulating screw too close to the active end of the forceps. This limitation was solved in successive prototypes (López-Jornet et al, 2005), although some drawbacks still remained, such as the impossibility for maintaining a controlled pressure, and the existence of a small, circular, fenestrated area. These features made these devices unsuitable for both approaches requiring wide incisions, and also for minimally invasive techniques involving multiple incisions in both sides of the lip.

In order to solve these shortcomings, we have developed a new instrument with improved ergonomics that permits a better access to the whole of the lower lip while allowing a controlled pressure capable of producing surgical ischemia for an adequate visibility of the nerve fibres. We have also described the technique

for lip biopsy in this clinical context (SS), although clinical trials are needed for assessing the suggested approach.

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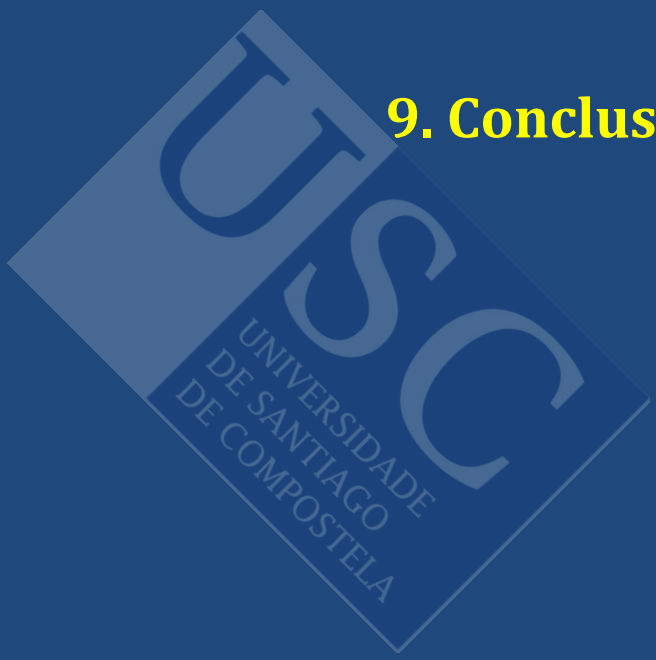


# 9. Conclussions

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# 9. Conclussions

1. Glandular biopsy is a key criterion for the diagnosis of Sjögren's syndrome because of its high diagnostic validity. Despite the absence of comparative studies, minimally invasive techniques using small, multiple incisions seem to induce fewer permanent neurological complications than conventional approaches using longitudinal incisions in the lower lip. However, and to optimize the process of making surgical decisions, clinical trials comparing the lip with alternative sites for salivary gland biopsy -such sublingual or parotid glands- are needed.

2. The use of circular scalpels are strongly discouraged for glandular biopsy in the lip, due to the impossibility of harvesting glandular tissue enough for diagnostic purposes, the lack of specificity of the sample obtained, and also to the high probability for neurological damage during the procedure. However, lip stabilization forceps may become a useful ancillary instrument for lip biopsy, whose contribution should be analyzed in future clinical trials.

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# 10. Resumen

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# 10. Resumen

Los criterios de síndrome de Sjögren consideran la biopsia de glándula salival menor (≥1 foco por 4mm$^2$), junto con la tinción ocular del Rosa de Bengala (>3) y la positividad de la serología (SSa/SSB) entre los requerimientos para establecer un diagnóstico.

La biopsia glandular ha proporcionado en estudios previos un alto valor predictivo, así como una considerable sensibilidad (78.8%) y especificidad diagnóstica (88.1%). Además esta seguridad diagnóstica puede ser incrementada, utilizando índices acumulados, con el estudio a diferentes niveles de la muestra.

## 10.1. Reacciones adversas neurológicas descritas tras biopsia labial en pacientes sospechosos de Síndrome de Sjögren: Una revisión sistemática y meta-análisis.

La biopsia labial de glándulas salivares menores como método diagnóstico no está exento de morbilidad (dolor, inflamación, hematomas, sangrado, infección…etc), incluso de complicaciones neurológicas potencialmente severas y permanentes (anestesia, hipoestesia y/o disestesia labial). En cualquier caso, existen un

gran número de diferentes técnicas de biopsia labial glandular con diseños elípticos, circulares o lineales, de diferentes longitudes (2mm a 30 mm) y con diferentes direcciones (paralelas, oblicuas o transversales), respecto a la dirección de las fibras del orbicular del labio. A pesar de esta circunstancia no existen estudios comparativos o ensayos clínicos que permitan elegir al clínico el procedimiento con menor morbilidad.

Estas circunstancias parecen justificar la necesidad de un meta-análisis encaminado a responder qué técnicas quirúrgicas (mínimamente invasivas *vs* convencionales –incisiones lineales de ≥5mm-) generan menos efectos adversos en términos de morbilidad neurológica.

En Junio de 2013, se llevó a cabo una revisión sistemática con metanálisis que incluyó las bases MEDLINE (1966-2013), EMBASE (1980-2013) y Proceedings Citation Index Science (CPCI-S), así como mediante búsqueda manual en revistas especializadas para artículos publicados en cualquier idioma. En la estrategia de búsqueda se utilizaron los términos [(salivary gland biopsy OR labial biopsy OR lip biopsy) AND (Sjögren)]. Está búsqueda fue realizada independientemente por cada autor.

Para efectuar este metanálisis se siguió el consenso PRISMA para metodología de revisiones sistemáticas y metaanálisis, considerando como criterios de inclusión:

I. Los estudios que incluyan datos originales sobre biopsia glandular labial con intenciones diagnósticas de síndrome de Sjögren.

II. Incluir el tamaño muestral y la exposición de interés (descripción detallada de la técnica quirúrgica).

III. La presencia o ausencia de complicaciones neurológicas y el número de pacientes afectados.

Para la asignación de calidad se utilizó una escala binaria de 5 puntos para estudios observacionales (STROBE) y tanto la aplicación de los criterios de elegibilidad como la asignación de la calidad a los estudios fue llevada a cabo por dos observadores de forma independiente. Todos los análisis se hicieron mediante el software HEPIMA, versión 2.1.3.

Mediante la anteriormente citada búsqueda sistemática se identificaron 342 estudios potencialmente elegibles y finalmente se consideraron 3 estudios sobre las técnicas mínimamente invasivas (TMI) y 13 estudios con información relevante sobre técnicas de incisión lineal convencional. Las medidas de los efectos presentaron una baja heterogeneidad, excepto para los estudios con TMI, para los que se seleccionó la medida de efectos aleatorios. Estos resultados muestran una mayor morbilidad neurológica asociada a incisiones lineales de más de 5 mm, frente a incisiones labiales mínimamente invasivas. Sin embargo, la interpretación de los mismos habrá de ser cautelosa debido a

potenciales sesgos metodológicos, especialmente ligados al sesgo de selección, falta de representatividad de la muestra, variabilidad de las habilidades quirúrgicas, débil descripción de las muestras, el empleo de criterios heterogéneos en la definición de las complicaciones quirúrgicas…etc.

Por otra parte, la biopsia labial ha demostrado seguridad diagnóstica en formas de SS poco definidas, con serología negativa (SSa/SSB) y específicamente formas extraglandulares del síndrome. Para ello, se ha utilizado diferente armamentario quirúrgico, como el bisturí frío, el bisturí circular, las pinzas en sacabocados…etc.

## 10.2. Biopsia labial en el diagnóstico de síndrome de Sjögren: Cuidado con el bisturí circular

A pesar de que mayoritariamente los clínicos evitan la toma de tejidos labiales en bloque (mucosa y submucosa con tejido glandular), algunos autores han propuesto el empleo de bisturí circular (punch), en base a criterios ergonómicos, y a que parecen generar escasa morbilidad intra y postquirúrgica. Debido a que estos resultados parecen poco plausibles, decidimos reproducir los protocolos de biopsia glandular labial (BGL), utilizando un punch de 4 milímetros de diámetro , a una profundidad de 8 milímetros, sobre un modelo experimental.

Con esta finalidad se llevó a cabo un estudio piloto, con un diseño a boca partida, utilizando 5 cadáveres crio-preservados. Las intervenciones (incisión labial horizontal de 10 milímetros frente a punch) fueron asignadas de forma aleatoria, a cada lado del labio. Posteriormente Se efectuó un estudio histopatológico multinivel de las muestras mediante hematoxilina-eosina.
En todos los casos se registró, la edad y sexo del cadáver, así como, la longitud del cilindro obtenido mediante el bisturí circular, la presencia y número de lobulillos glandulares en el espécimen y la presencia de fibras nerviosas seccionadas en la muestra obtenida. En todas las biopsias con punch pudo evidenciarse la presencia de fibras seccionadas, en tanto no se pudo demostrar este hallazgo en ninguna de las muestras obtenidas con bisturí frío.

Estos hallazgos nos permiten sugerir la inconveniencia de utilizar una técnica ciega, en la que no se efectúa una disección quirúrgica, y en la que tan solo se obtienen un máximo de 1 ó 2 lobulillos glandulares. A pesar de que estos hallazgos dimanan de un estudio piloto, con un escaso tamaño muestral, la propia esencia de este tipo de estudios esta focalizada más en la factibilidad y en generar hipótesis para ensayos clínicos, que en buscar significación en el estudio. En base a los resultados obtenidos, se percibe la necesidad de diseñar ensayos clínicos que enfrenten técnicas mínimamente invasivas de BGL a biopsias

labiales incisionales lineales y a técnicas de biopsia sobre glándulas mayores (parótida y sublingual).

## 10.3. Biopsia de glándula salival menor en el diagnóstico de síndrome de Sjögren: Revisión y propuesta de una nueva herramienta para facilitar el procedimiento

Con independencia del tipo de bisturí empleado, también se han utilizado pinzas fenestradas (pinzas de chalazion) con la finalidad de estabilizar el labio y conseguir isquemia del campo operatorio. Además, un gran número de publicaciones continúan informando de la alta frecuencia de complicaciones inmediatas y postoperatorias, de diversa severidad, asociadas a la BGL. A pesar de ello, se han llevado a cabo muy pocos intentos de mejorar los dispositivos y la técnica quirúrgica. Específicamente, las pinzas de chalazion (Desmarres) presentan limitaciones a su empleo en biopsias labiales, básicamente debido a la forma y al área de la fenestración y a la falta de accesibilidad a partes posteriores/profundas de los tejidos orales.

Posteriormente y con la misma finalidad se han diseñado las pinzas B, con una longitud de 20 centímetros, una fenestración de 1,7 centímetros de diámetro y una presión estable sobre los tejidos de 1 Kg/cm$^2$. Este diseño solucionó parcialmente los déficits atribuidos a la pinza de chalazion. Sin embargo, la

fenestración de este dispositivo no permite llevar a cabo incisiones lineales amplias, ni TMI con incisiones múltiples. Otro inconveniente reside en la incapacidad de controlar la forcipresión e individualizarla de acuerdo a las características del paciente.

Con el objetivo de evitar los inconvenientes anteriormente citados y de facilitar la técnica de biopsia labial, se planteó diseñar unas pinzas de estabilización labial y describir una técnica quirúrgica para la BLG en pacientes con sospecha de síndrome de Sjögren. Se llevaron a cabo diversos prototipos que permitiesen incisiones lineales amplias, incluso mayores a 3 centímetros, el acceso a zonas profundas del labio, que pudiesen estabilizar el labio completo en caso de técnicas de incisiones múltiples bilaterales y que permitiese así mismo, individualizar la presión de acuerdo a las características del paciente.

Fruto de ello ha sido la elaboración de las pinzas S, con un mango ergonómico y una longitud total de 18,5 centímetros y un área fenestrada de 5 $cm^2$. La parte activa fenestrada presenta una dirección axial respecto al mango y facilita el acceso del tejido a biopsiar. Además se realizó una descripción de la técnica quirúrgica de BLG paso a paso.

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# 11. Publicaciones derivadas de la tesis doctoral

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# 11. Publicaciones derivadas de la tesis doctoral

I. Varela Centelles P, Sánchez-Sánchez M, Costa-Bouzas J, Seoane-Romero JM, Seoane J, Takkouche B. Neurological adverse events related to lip biopsy in patients suspicious for Sjogren's syndrome: a systematic review and prevalence meta-analysis. Rheumatology (Oxford). 2014 Mar 4. [Epub ahead of print]
JCR Impact Factor: 4.212

II. Varela-Centelles P, Sanchez-Sanchez M, Seoane J. Lip biopsy for the diagnosis of Sjögren's syndrome: beware of the punch. Int J Oral Maxillofac Surg. 2014 Jan;43(1):127-30. doi: 10.1016/j.ijom.2013.05.014. Epub 2013 Jul 6.
JCR Impact Factor: 1.521

III. Varela-Centelles P, Seoane-Romero JM, Sánchez-Sánchez M, González-Mosquera A, Diz-Dios P, Seoane J. Minor salivary gland biopsy in Sjögren's syndrome: a review and introduction of a new tool to ease the procedure. Med Oral Patol Oral Cir Bucal. 2014 Jan 1;19(1):e20-3.
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# Neurological adverse events related to lip biopsy in patients suspicious for Sjögren's syndrome: a systematic review and prevalence meta-analysis

**Pablo Varela Centelles[1], Mariña Sánchez-Sánchez[1], Julián Costa-Bouzas[2], Juan Manuel Seoane-Romero[1], Juan Seoane[1] and Bahi Takkouche[3]**

## Abstract

**Objective.** The aim of this study was to compare the prevalence of neurological complications related to lip biopsy for SS diagnosis using conventional *vs* minimally invasive techniques.

**Methods.** We performed a systematic review and prevalence meta-analysis using the search strategy [(salivary gland biopsy OR labial biopsy OR lip biopsy) AND (Sjögren)] in the MEDLINE, EMBASE and Web of Science Conference Proceedings Citation Index databases. Studies were selected if they included original data for minor salivary gland biopsy, sample size, exposure of interest (technique description), number of complications and number of affected patients. The prevalence of total and permanent neurological adverse effects was calculated. Both fixed-effects and random-effects pooled estimates were assessed. Heterogeneity was calculated using an adaptation of the DerSimonian and Laird *Q* test.

**Results.** Sixteen articles were selected for the study. In the minimally invasive group ($n = 3$), the pooled prevalence of total adverse events is almost four times higher than that in the linear incision group ($n = 12$) (4.73% *vs* 1.20%). In contrast, the pooled prevalence of the permanent or potentially permanent neurological adverse events is 8.5 times lower in the minimally invasive technique group than in the studies using linear incisions (0.17% *vs* 1.45%).

**Conclusion.** With the limitations intrinsic to the potential biases in the studies included in this meta-analysis, we conclude that the minimally invasive lip biopsy technique for SS diagnosis induces fewer permanent neurological complications than conventional approaches with large linear incisions in the lower lip.

**Key words:** Sjögren's syndrome, lip biopsy, minor salivary gland, diagnosis, adverse events, complications, neural damage.

CLINICAL SCIENCE

## Introduction

Since Chisholm and Mason [1] described minor salivary gland biopsy (MSGB) as a valuable investigative procedure in SS and established standardized criteria to assess inflammation, a number of surgical techniques have been suggested to harvest MSG for SS diagnosis. Focal sialadenitis (with a focus score $\geqslant 1$) in MSG is one of the six requirements established for the diagnosis of SS [2]. This criterion has become more important as a recent consensus limits the definition of SS to objective criteria only and therefore two of the three criteria would then be required: positive serum anti-SSa and/or SSB, ocular staining score $>3$ or the presence of focal sialadenitis with a focus score $>1$ per 4 mm$^2$ of glandular tissue [3].

The value of MSGB for the diagnosis of SS is supported by high sensitivity [mean 78.8 (S.D. 11.2)] and specificity [88.1 (11.7)] values [2], reinforced by good diagnostic confidence [positive predictive value 87.6 (9.5); negative predictive value 79.0 (16.9)]. This diagnostic value can be

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augmented by evaluating the cumulative focus score using a multilevel approach, assessing different section levels of the sampled glandular tissue cut at least at 200 μm apart [4]. Thus MSGB can be particularly useful for suspected SS patients with inconclusive clinical findings and negative anti-Ro/la serology [5, 6].

A number of mediate and immediate surgical complications (pain, bruising, bleeding, wound infection) have been described for MSGB, but lip numbness has been found to be the only persistent complication and it is reported to occur in up to 6% of all MSGBs [7–9]. Several techniques for lip MSGB have been proposed with a variety of incisions differing in shape (elliptical, circular, linear), orientation (vertical, oblique, parallel) and length (1 mm–3 cm), but no comparative studies on the advantages of a particular type in terms of postoperative morbidity could be retrieved [7, 9–19].

The high diagnostic performance of MSGB demands an adequate surgical technique to ensure correct and sufficient sampling of glandular tissue with low related morbidity. Technique selection is hindered by the absence of comparative studies and the limited validity of the sole comprehensive review on the topic identified in the literature, due to the poor definition and vague categorization of the surgical complications [11]. These circumstances seem to justify the need for a systematic review and meta-analysis aimed at assessing the prevalence of neurological complications related to lip biopsy. The aim of this investigation was to compare the pooled prevalence of neurological adverse events induced by lip biopsy for the diagnosis of SS (minimally invasive technique *vs* linear incisions $\geqslant$5 mm).

## Methods

Methods of analysis and inclusion criteria were defined in a protocol to ensure homogeneous criteria among all co-authors during the investigation. This protocol was initially conceived to record all post-biopsy adverse events described in the literature, but it was restricted later on during the study to permanent neurological adverse events.

A systematic search was undertaken in June 2013 of the MEDLINE, EMBASE and Web of Science Conference Proceedings Citation Index–Science (CPCI-S) databases. The search strategy was [(salivary gland biopsy OR labial biopsy OR lip biopsy) AND (Sjögren)], both in medical subject headings (MeSH) and in free text words. This search strategy was independently reviewed and discussed by all authors.

This search was supplemented with an additional manual search [20] performed on our institution's library catalogue, considering both books and relevant journals, including *Annals of the Rheumatic Diseases*, *Arthritis & Rheumatism*, *Arthritis Research & Therapy*, *Osteoarthritis and Cartilage*, *Rheumatology* and the reference lists of the articles retrieved from the aforementioned databases (Fig. 1). All references identified for computerized databases were manually retrieved. Studies were included if they fulfilled the following criteria: (i) included original data from an MSGB performed for the diagnosis of SS; (ii) included the sample size and the exposure of interest in the study, i.e. a detailed description of the techniques for MSGB; and (iii) assessed the presence or absence of neurological complications (lip numbness) and number of affected patients.

Data were retrieved by two investigators in an unblinded standardized manner using a custom-made extraction sheet. Disagreements between investigators were resolved by consensus. The percentage of technique-related neurological complications was again independently recorded by the reviewers; in case this information was not detailed in the study, lower lip numbness was considered as persistent/permanent when lasting $\geqslant$6 months after the biopsy procedure.

### Quality assessment

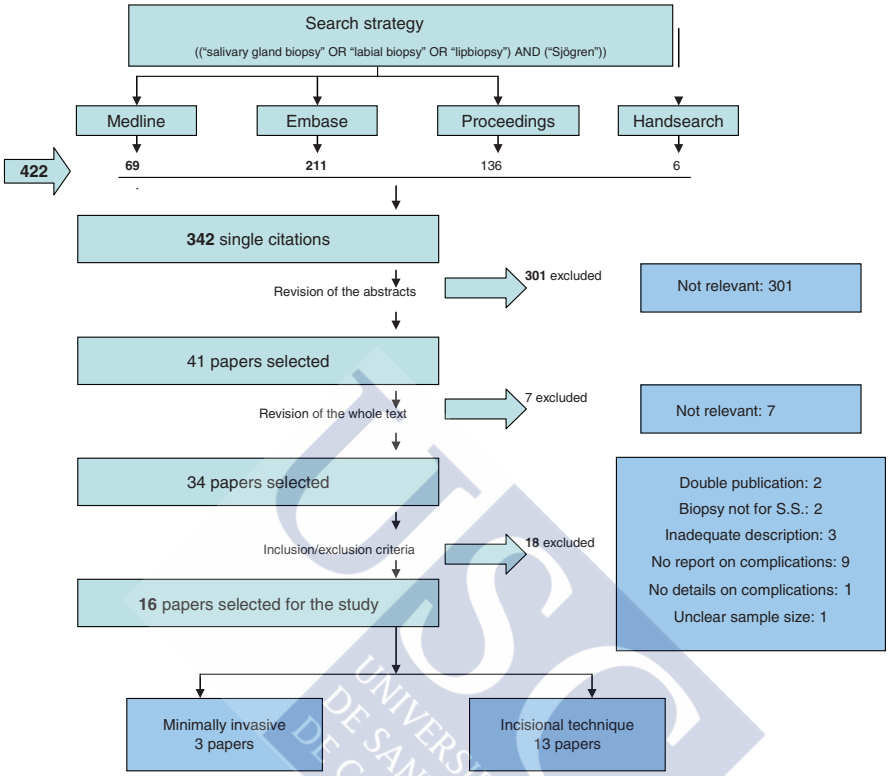
We assessed study quality using a 5-point binary scale (0/1) that we specifically developed for this study. The scale is based on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [21]. Throughout this assessment, when the information on a specific item was not provided by the authors, we graded this item as 0. The quality scoring was independently undertaken by two researchers (J.S. and B.T.). The first item assessed was whether post-biopsy complications were a primary or specific objective of the reported investigation or were considered in a prespecified hypothesis; in this case, a score of 1 was allocated. The second item assessed was the study design (1 point to clearly described prospective designs). The third item assessed was the setting of the study (1 point was given if the article adequately described both the surgical scenario and data collection procedures). The fourth item assessed the follow-up time after surgery (1 point if follow-up dates or dates at which the outcome events occurred were present). The last item concerned descriptive data on one important confounder: the number of glands harvested (1 point if the number of glands obtained was detailed in the article).

### Data synthesis and analysis

The concept of minimally invasive techniques included those techniques for MSGB consisting of multiple 2–3 mm incisions on the buccal side of the lip to collect the glandular tissue using a forceps. Punch techniques are not included within this group [9, 10, 13, 18]. Another group of studies was defined as those reports harvesting glandular tissue from the lower lip by means of a single, linear, $\geqslant$5 cm incision. For each study we computed the prevalence of total neurological adverse events and that of neurological permanent or potentially permanent adverse events by dividing the number of events by the sample size of the study. We then weighted the study-specific prevalence by the inverse of their variance to compute a pooled prevalence and its 95% CI.

We calculated both fixed-effects and random-effects pooled estimates, but used and reported the latter when heterogeneity was present, as the random-effects model

FIG. 1 Flow chart of the study




is generally thought to give more reliable results than the fixed-effects model, including a more conservative (wider) CI, when the studies being considered show a considerable degree of heterogeneity. In our study, this issue is particularly relevant as, in general, the number of events is small and in some studies the resulting prevalence is zero. Therefore, instead of using the traditional asymptotic method in order to obtain an estimate of the variance, which was inadequate in our setting, we used the exact method proposed by Newcombe and Altman [22].

To check for heterogeneity we used a version adapted to small samples of the DerSimonian and Laird *Q* test, and to quantify this heterogeneity we calculated the proportion of the total variance due to between-study variance [R(I)] [23]. All analyses were performed with the software HEpiMA version 2.1.3 [24].

## Results

The aforementioned systematic searches identified 342 articles whose abstracts were reviewed for contents relevant to the topic of this systematic review; 301 of these were subsequently excluded. A total of 41 articles were then retrieved and reviewed and 7 of them were also excluded because their information was not useful for this study. The remaining 34 articles were checked according to the inclusion/exclusion criteria of the present review. Two articles were excluded because of double publication, i.e. the same information was published in more than one article by Caporali *et al.* [9, 25] and López-Amado *et al.* [26, 27]. Thus only one reference by each research group was considered in the study [9, 27]. Two articles performed MSGB for indications other than the diagnosis of SS [28, 29]. Three articles reported inadequate descriptions of the surgical technique [17, 30, 31]. Nine articles reported on the surgical technique, but not on its related complications [18, 32–39]. One article included only generic information on the surgical complication of MSGB [40], and one article did not adequately describe the size of the sample studied [19].

Sixteen articles were finally selected to enter the systematic review; two of them report additional information

on the same series of cases [8, 49] (Fig. 1). Only three of these articles describes neurological complications after lip MSGB in the context of systematized data collection on both immediate (<24 h) postoperative complications (bleeding, fainting, tachycardia and bruising) and mediate adverse events (pain, inflammation, suture dehiscence, infection and granuloma or keloid scarring) [9, 10, 12]. Three case series with larger sample sizes [9, 10, 13] describe minimally invasive lip biopsy techniques and report neurological complications in a range from 0 to 11.73%, although permanent hypoaesthesias account for not more than 0.22% [9]. In contrast, 12 descriptive studies performing biopsies using 5- to 30-mm long linear incisions using a scalpel [8] with or without a chalazion forceps report neurological complications in a range from 0 to 5.7% that remained after 6 months.

Table 1 presents the characteristics of the studies included in this meta-analysis. Four studies did not provide information on permanent neurological adverse events and were excluded from this specific calculation. Their patients were not considered for calculating the pooled prevalence. High-quality studies yielded a pooled prevalence that was three times as high as the pooled prevalence of low-quality studies. Both estimates showed large heterogeneity.

Table 2 shows the pooled prevalence for each biopsy technique. Heterogeneity between studies was low, except for total adverse events in the minimally invasive technique: in this group the random effects pooled prevalence is thus a more germane measure than that of the fixed-effects model. In the minimally invasive group, the pooled prevalence of total adverse events is almost four times higher than that in the linear incision group (4.73% *vs* 1.20%). In contrast, the pooled prevalence of the permanent or potentially permanent neurological adverse events is 8.5 times lower in the minimally invasive technique group than in the group of studies using linear incisions (0.17% *vs* 1.45%). Moreover, studies performing linear incisional biopsies report lower percentages of complications in case series with a high proportion ($\geqslant$50%) of chronic saladenitis (1.01% *vs* 1.59%). When studies performing linear incisional techniques were stratified by quality, those studies with high scores ($Q \geqslant 3$) report a higher percentage of permanent neurological adverse events (2.50% *vs* 0.82%) (Table 2).

## Discussion

Our study shows that minimally invasive biopsy techniques are apparently safer than conventional incisional approaches. No clinical trials focused on the aims of this meta-analysis could be retrieved. All reports considered in this systematic review are cases series without missing patients for follow-up, so a selection bias is improbable. Alternatively, there is a potential for information bias, as only three studies describe systematized procedures for recording information on immediate and mediate complications [9, 10, 12] and only one investigation employed a structured questionnaire with open and closed questions [9].

There also is an evident lack of consensus on the definition and classification of postoperative complications by severity [41], which may have facilitated inaccuracies and omissions during data collection. Very few studies provided information on confounding factors [12, 42], such as corticosteroid therapy, radiotherapy, tobacco consumption, clinical setting (inpatient *vs* outpatient) or chronic sialadenitis rate (Table 2), which may influence post-biopsy complications [1, 8, 10, 12, 13, 17, 43]. In fact, only one investigation [12] made an attempt to address this issue during data analysis by stratifying the results by the use of sutures, and concluded it made no difference in terms of frequency and type of complications. This observed lack of control for confounding factors may hamper the internal validity of the reports included in this meta-analysis, as high-quality studies detect higher percentages of neurological complications (Table 2).

MSGs in the lower lip are distributed into one to three layers of discrete gland clusters, mainly in the cuspid-premolar area [44]. The fact that minimally invasive techniques harvest only the more superficial glands would explain the reduced risk of damaging sensory nerves [13] and the lower prevalence of permanent neurological complications. However, the need for multiple, small incisions and the proximity of nerve endings to the glands may explain the presence of postoperative transient lip hypoaesthesias. Conventional techniques require larger incisions and dissection of the wound borders to retrieve glands from deeper layers in the lip, where nerve fibres and glandular tissue are in close proximity, which may well explain the higher prevalence of permanent lip hypoaesthesias in this group.

Both approaches permit the fulfilment of SS diagnostic requirements, i.e. retrieval of at least five MSGs [45, 46], in a minimal operative time [18, 47], and both share common shortcomings, i.e. harvesting similar percentages of fatty and fibromuscular tissues and a comparable number of biopsies resulting in insufficient amounts of glandular tissue [7, 10].

Two of the studies included in the meta-analysis did not report neurological morbidity after sublingual salivary gland biopsy [15, 48], whereas two studies found neither sensory nor motor nerve loss related to parotid gland biopsy for the diagnosis of SS [7, 47]. These comparative studies seem to suggest that both procedures—sublingual and parotid biopsy—have a diagnostic potential for SS comparable to MSGB, but they may offer minor postoperative morbidity. Alternatively, MSGB has been proved safe, simple and suitable for extensive routine application in outpatient (internal medicine and rheumatology) settings [7, 9].

Despite the fact that techniques removing labial mucosa together with its attached glands have been discouraged because of the potential for neurological damage [16], a research group has recommended the use of a punch (4 mm diameter) for taking biopsies from the inner side of the lower lip. This article described rate of transient numbness of only 4% in a series of 50 patients

**TABLE 1** Summary of the 16 articles selected for the meta-analysis

| Author [reference] | Country | *n* (M/F) | Approach | Instrument | Incision | Glands, *n* | Suture | Chronic sialadenitis, *n* (%) | Follow-up | Total neurological complications, *n* (prevalence %) | Permanent neurological complications, *n* (prevalence %) | Quality score (*Q*) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chisholm and Mason [1] | UK | 40 (15/25) | Incisional | Scalpel | Ellipse, central, 3 × 0.75 cm | | Y | 10 (25) | No information | 0 (0) | 0 (0) | 2 |
| Greenspan *et al.* [43] | USA | 75 | Incisional | Scalpel | 1.5–2 cm, linear, parallel vermillion, lateral to the midline | 4–7 | Y | 43 (57.3) | Several months | 1 (1.33) | 1 (1.33) | 4 |
| Daniels [16] | USA | 362 | Incisional | Scalpel | Lateral, 1.5–2 cm | 1–16 (6 average) | Y | 225 (62.1) | No information | 3 (0.83) | No information | 1 |
| Marx *et al.* [47] | USA | 79 | Incisional | Scalpel | 3 × 0.75 cm, linear, parallel vermillion | 7 | Y | 32 (40.5) | 2 years | 3 (3.80) | 1 (1.27) | 5 |
| Pennec *et al.* [48] | France | 50 (7/43) | Incisional | Scalpel | Lateral, 1.5 cm, horizontal | | Y | 14 (28) | No information | 0 (0) | 0 (0) | 1 |
| Richards *et al.* [14] | UK | 58 (5/53) | Incisional | Scalpel | Horizontal | 6–8 | Y | — | 1 year | 2 (3.45) | 2 (3.45) | 3 |
| Lopez-Amado *et al.* [27] | Spain | 35 | Incisional | Scalpel | Linear, 2 cm<br>Ellipse | 7<br>5 | Y<br>Y | 32 (91.4) | No information | 0 (0)0 (0) | 0 (0)0 (0) | 2 |
| Friedman *et al.* [12] | Israel | 118 | Incisional | Scalpel | 5–7 mm | >5 | Y<br>N | — | 3 weeks | 2 (1.69) | No information | 5 |
| Gorson and Ropper [46] | USA | 54 (19/35) | Incisional | Scalpel | Vertical, 1 cm | ⩾4 | — | 20 (37) | No information | 1 (1.85) | 1 (1.85) | 1 |
| Berquin *et al.* [15] | Belgium | 24 | Incisional | Scalpel | Oblique, 1.5 cm from midline | | Y | 12 (50) | No information | 1 (4.17) | 1 (4.17) | 2 |
| Teppo and Revonta [13] | Finland | 191 (36/155) | Minimally invasive | Scalpel, cup forceps | 2–3 mm multiple horizontal incisions | | N | 154 (80.6) | 1.5 –19 years | 0 (0) | 0 (0) | 2 |
| Pijpe *et al.* [7] | Holland | 35 | Incisional | Scalpel | 3 cm, linear, parallel vermillion | 7 | Y | 19 (54.2) | 1 year | 2 (5.71) | 2 (5.71) | 5 |
| Langerman and Blair [8], Langerman [49] | USA | 47 | Incisional | Scalpel, chalazion forceps | 5–10 mm horizontal elliptical incision | 3–7 | Y | 39 (82.9) | 1 month | 2 (4.26) | No information | 3 |
| Caporali *et al.* [9] | Italy | 452 | Minimally invasive | Scalpel, forceps | 2–3 mm | 4–5 | Y | 93 (20.5) | 6 months | 53 (11.73) | 1 (0.22) | 3 |
| Lida *et al.* (2012) [10] | Argentina | 186 (9/177) | Minimally invasive | Scalpel, forceps | 2–3 mm | 4–5 | Y | — | 1 month | 5 (2.69) | No information | 5 |

**Table 2** Pooled prevalence and 95% CI of total and permanent neurological adverse events

| | Number of studies | Pooled prevalence, % (95% CI), fixed effects | Pooled prevalence, % (95% CI), random effects | R(I)[a] | *Q* test *P*-value |
|---|---|---|---|---|---|
| Incision (all) | 12 | 1.20 (0.00, 2.49) | 1.20 (0.00, 2.49) | 0.00 | 0.99 |
| Incision (permanent) | 9 | 1.45 (0.00, 4.08) | 1.45 (0.00, 4.08) | 0.00 | 0.99 |
| Incision (high quality) | 6 | 2.50 (0.00, 5.21) | 2.50 (0.00, 5.21) | 0.00 | 0.97 |
| Incision (low quality) | 6 | 0.82 (0.00, 2.28) | 0.82 (0.00, 2.28) | 0.00 | 0.99 |
| Incision (high sialadenitis rate) | 6 | 1.01(0.00, 2.48) | 1.01 (0.00, 2.48) | 0.00 | 0.94 |
| Incision (low sialadenitis rate) | 4 | 1.59 (0.00, 5.36) | 1.59 (0.00, 5.36) | 0.00 | 0.86 |
| Minimally invasive (all) | 3 | 3.01 (1.50, 4.53) | 4.73 (0.00, 11.74) | 0.95 | 0.00001 |
| Minimally invasive (permanent) | 2 | 0.17 (0.00, 1.08) | 0.17 (0.00, 1.08) | 0.00 | 0.85 |

[a]Proportion of total variance due to between-study variance.

with unknown follow-up periods [17]. This approach has the drawbacks of retrieval of insufficient glandular material and inherent sampling mistakes, which would force a second biopsy, which would in turn increase the patient's morbidity.

With the limitations intrinsic to the potential biases in the studies included in this meta-analysis, we conclude that the minimally invasive lip biopsy technique for the diagnosis of SS induces fewer permanent neurological complications than conventional approaches with large linear incisions in the lower lip. Moreover, and due to the absence of relevant scientific evidence supporting the selection of a particular technique for salivary gland biopsy in the diagnosis of SS, it is recommended that clinical trials be undertaken to assess the existing approaches for lip MSGB (minimally invasive *vs* large linear incisions), together with sublingual and parotid biopsies, and ensuring adequate follow-up periods.

**Rheumatology key messages**

- Minor salivary gland biopsy in the lip has proved useful for the diagnosis of SS.
- Minimally invasive biopsy techniques induce fewer permanent neurological adverse events than conventional incisional approaches for the diagnosis of SS.

*Disclosure statement*: The authors have declared no conflicts of interest.

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Technical Note
Oral Medicine

# Lip biopsy for the diagnosis of Sjögren's syndrome: beware of the punch

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*P. Varela-Centelles: Lip biopsy for the diagnosis of Sjögren's syndrome: beware of the punch. Int. J. Oral Maxillofac. Surg. 2014; 43: 127–130.* 

*Abstract.* A pilot study was performed to examine the presence of nerve fibres in minor salivary gland tissue samples obtained by two procedures: punch and linear incisional techniques. The study was undertaken on a convenience sample of five cryopreserved corpses (mean age 74 ± 3.5 years; three males and two females). Biopsies were performed on the buccal side of the lower lip, between the mid-line and the corner of the mouth. Each corpse had one side of the lower lip biopsied by punch and the contralateral side using a linear incision. All punch samples (100%) displayed severed nerve fibres, whereas no nerve fibres (0%) could be identified in the samples obtained by means of the linear incision technique. While the linear incision approach permitted retrieval of at least five glands, punch biopsies did not provide enough material for the diagnosis of Sjögren's syndrome. Within the limitations of the study, our results strongly discourage the punch technique for minor salivary gland lip biopsy and provide information on the superiority of the linear incisional biopsy in terms of neural damage. These results may also promote the undertaking of clinical trials on patients in whom Sjögren's syndrome is suspected, comparing the morbidity associated with the linear incisional technique vs. minimally invasive biopsies.

Key words: punch; biopsy; minor salivary gland; lower lip; Sjögren's syndrome; diagnosis.

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The minor salivary gland biopsy (MSGB) has been used for the diagnosis of systemic disorders, such as amyloidosis, sarcoidosis, and Sjögren's syndrome (SS), and for the confirmation of neonatal haemochromatosis.

The presence of focal lymphocytic sialadenitis with a focus score >1 per 4 $mm^2$ of glandular tissue is an objective criterion for consideration when diagnosing Sjögren's syndrome. MSGB has proven validity and diagnostic confidence, with a high specificity and positive predictive value, and an average sensitivity of 79%.[1] These features make MSGB particularly useful for patients with inconclusive clinical findings, incipient forms of the syndrome, SS with negative anti-Ro/la serology, and extra-glandular involvement.

A wide range of surgical approaches have been described for harvesting at least five accessory glands from the lower lip using different instruments (scalpel, punch, or cup forceps) and for producing different incisions (circular, linear, or elliptical) in a variety of sizes (from 2 mm to 3 cm)[1–5] and orientations (parallel to the lip, oblique, or even vertical). The use of a forceps with a fenestrated active end (chalazion forceps) to stabilize the lip has also been suggested.

The selection of the best surgical approach in terms of related morbidity is hampered by the absence of comparative studies and the proliferation of descriptive papers that do not state negative outcomes associated with the technique performed.[4] Moreover, those

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reports describing percentages of surgical complications have a limited validity due to the lack of standardization when defining and categorizing the complications according to their severity.[4,5] Nonetheless, persistent lip numbness is the most frequently published surgical complication,[4] occurring in up to 6% of MSGBs performed in the lower lip.

Despite the existing investigations discouraging the removal of labial mucosa with attached glands when performing MSGB because of the potential for neurological damage, punch use has been widely recommended because of safety and handling simplicity reasons, as this procedure is not technically demanding and can be undertaken in an outpatient setting.[2,3] However, and to the best of our knowledge, there are no quality comparative studies assessing neurological damage induced by the different techniques for MSGB. Thus, the aim of this pilot study was to examine the presence of nerve fibres in minor salivary gland tissue samples obtained by means of two different procedures: punch technique and linear incisional technique.

## Materials and methods

On the basis of the feasibility of the investigation and to minimize potential ethical conflicts, an observational, descriptive pilot study designed to replicate the techniques for minor salivary gland biopsy for SS diagnosis[2–4] was undertaken on a convenience sample of five non-formolized, frozen corpses (mean age 74 ± 3.5 years; three males and two females). All subjects had bequeathed their bodies for medical – scientific research and training purposes and all procedures were undertaken in accordance with the university ethics committee recommendations (14/2007).

Biopsies were performed at the inner side of the lower lip, between the mid-line and the corner of the mouth. Each corpse had one side of the lower lip biopsied by punch and the contralateral side using a linear incision. The biopsy site was randomly allocated to each technique using a computer-generated list of random numbers.

The punch biopsy technique was undertaken following previously established protocols[2,3] by everting the lip, perpendicularly positioning a 4-mm diameter punch (Stiefel Laboratories, Madrid, Spain), and performing simultaneous rotational movements under gentle pressure to reach 8 mm deep into the lip. The cylinder of tissue was removed from its base using a scalpel with a No. 15 blade and placed onto a filter paper to avoid curling or twisting artefacts.

For the incisional biopsy, the lip was stabilized with a forceps (OEPM No. 201200158) and the incision performed away from the mid-line using a No. 15 scalpel blade. This incision was directed horizontally for about 1.5 cm, just penetrating the epithelium and combined with a blunt dissection of the borders of the wound. Five minor salivary glands were harvested from each corpse.[6]

All specimens were immediately placed in a wide-mouthed container, coded, and fixed in a generous amount of 10% formalin buffered saline for 24 h.

A single pathologist cut all specimens longitudinally with a new disposable scalpel for every section to obtain three slices 200 μm apart from each specimen and orientated them before paraffin embedding. Samples were prepared in 4-μm sections, stained with haematoxylin and eosin, and processed by the same technician. All specimens were examined using an Optiphot-2 microscope (Nikon, Tokyo, Japan) equipped with a millimetre-calibrated eyepiece graticule (Graticules Ltd, Tonbridge, Kent, UK) in order to measure the length of the core of tissue obtained by punch procedure. Pathological analysis also assessed the presence of severed nerve fibres within the tissue samples and the number of glands obtained by each technique.

The scores obtained for each variable were recorded and the confidence intervals for the differences between techniques calculated using Epidat 3.1 statistical package (Xunta de Galicia, Santiago de Compostela, Spain).

## Results

The punch technique for minor salivary gland biopsy produced specimens of 7.2 ± 1.1 mm length. The procedure harvested one minor salivary gland per sample in three cases; another case showed two glands located at the same depth in the tissue sample and the last case showed no glandular tissue in the specimen.

All punch biopsy samples (100%) displayed severed nerve fibres, located deeper in the tissue than the minor salivary glands. Only one sample showed nerve fibres close to the glandular tissue, at a more superficial level (Fig. 1). No nerve fibres (0%) could be identified in the samples obtained by means of the linear incision technique (Fig. 2). While the linear incision approach permitted retrieval of at least five glands, punch biopsies did not provide enough material for the diagnosis of Sjögren's syndrome. The results are summarized in Table 1.

Pathological analysis revealed no handling-related artefacts (pseudo-cysts, crushing, fragmentation, haemorrhage, or fissures), although fixing alterations could be recognized (more appearing at the lip mucosa epithelium and the minor salivary glands), probably due to the use of frozen cadaveric material.

## Discussion

The selection of a particular technique for MSGB is limited by the absence of clinical trials and standardization of surgical complications, which seriously compromise the validity of the information available in the literature on this topic. Existing case series display a wide range of sample

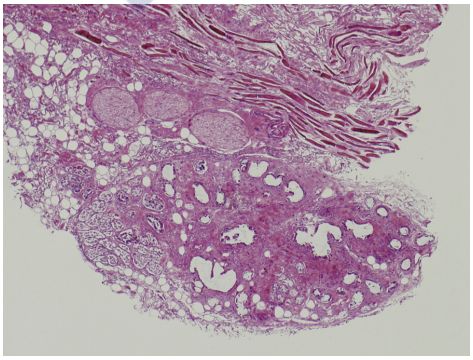

*Fig. 1.* Deep portion of a lower lip sample obtained by punch biopsy: sectioned nerve fibres can be seen close to a minor salivary gland (haematoxylin and eosin, 4×).

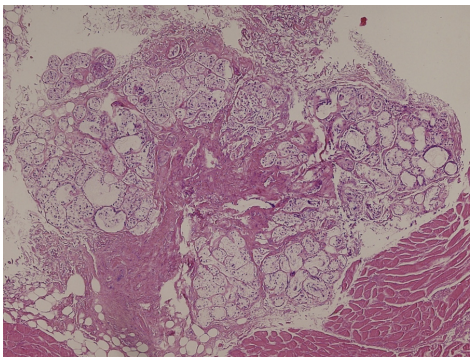

*Fig. 2.* Minor salivary gland obtained by linear incisional technique (haematoxylin and eosin, 4×).

sizes (6–502 patients), with different frequencies of complications associated with the variety of techniques employed.[1,4,5] Studies performing incisions shorter than 2 cm with a scalpel have reported complications ranging from none to 9.3%,[4] whereas those using larger incisions (2–3 cm) have described complications in the range of 3.7–31%.[7] Transient disorders of lip sensitivity were found to occur in up to 11.7% of the procedures,[4] and those studies with follow-up periods beyond 1 year have reported persistent lower lip hypoaesthesia in about 3.4–4% of the cases.[4] Only minimally invasive techniques[5,6] (excluding punch), based upon multiple 2–3-mm scalpel incisions on the inner side of the lower lip combined with a cup forceps to retrieve the tissue samples, have proved the absence of neurological morbidity after a follow-up period of 1.5 years.[4]

However, minor salivary gland punch biopsy has been suggested as an alternative to incisional biopsy techniques precisely because of the absence of risk to the patient, its simplicity (can be performed by a single operator), and also because it is less expensive.[2,3] This technique consists of obtaining the biopsy from the buccal side of the lower lip – which is stabilized by the patient him/herself – using a 4–5-mm punch, which permits the retrieval of a cylinder of tissue up to 8 mm in length[2,3]; no complications have been reported using this protocol in a series of 14 patients[3] and only 4% of transient numbness in a series of 50 cases with an unspecified follow-up period.[2]

Although this approach may seem interesting, the percentages of undesired events reported in these investigations[2,3] should be considered with caution, as they are unexpectedly well under those described for incisional techniques, where sensory nerves can clearly be seen and subsequently avoided, and also because they strongly disagree with our results obtained under controlled experimental conditions.

In general, sample size calculations may not be required for pilot studies, as they are focused mainly on feasibility rather than on statistical significance. However, it has to be kept in mind that studies with small sample sizes may not disclose differences between groups. Despite our study being performed on a small series of cadaveric material, the massive differences in terms of neural damage between the two techniques disclosed by this study seem to suggest that the chances of error are remote, irrespective of sample size issues. Moreover, post-mortem studies have frequently been employed when validating glandular biopsy for the diagnosis of SS.[8] Fresh, cryopreserved (frozen) corpses are the best model in terms of similarity of tissue quality and surgical handling. The age of the patients in the series (>50 years) may also be an advantage, because as persons grow older, fibrosis and reductions of the acinar volume occurs, which is a situation similar to that found in SS patients.[8]

Previous reports have described the collection of an average of six minor salivary glands using incisional techniques of 1.5 and 2 cm.[10,4,5,7] In addition, our study on MSGB using a 1.5-cm linear incision permitted retrieval of at least five glands, whereas punch biopsy did not provide enough gland material for the diagnosis of SS in any situation. Incisional techniques (including minimally invasive ones) have enabled the retrieval of an average of more than five glands accompanied by only 1.6% of undesired material, mostly fatty or fibromuscular in nature.[5] In contrast, sampling error is an inherent handicap of punch techniques, which sometimes require an additional biopsy on the other side of the lip,[2] thus increasing morbidity. The reduction of the surgical time to a ''few minutes''[2] does not seem to be an advantage of the punch biopsy either, as incisional biopsies using linear incisions take from 5 to 9 min.[7] Minimally invasive techniques can also be performed within a minimal operative time.

Alternative procedures to MSGB for the diagnosis of SS, such as sublingual salivary gland biopsy[9] and parotid biopsy,[10] have been found to cause no neural morbidity. These comparative studies seem to suggest that both procedures – sublingual and parotid biopsy – retain a diagnostic potential comparable to that of lip biopsy and may be associated with lower postoperative morbidity.[9,10]

Within the limitations of this type of study, our results discourage the use of punch techniques for biopsies of the minor salivary glands in the lip and provide information on the superiority of linear incisional biopsies in terms of neural damage. The results of this pilot study may promote the undertaking of clinical trials on patients in whom Sjögren's syndrome is suspected, comparing the morbidity associated with MSGB (linear incisional technique vs. minimally invasive) and major salivary gland biopsy.

*Table 1.* Number of glands and presence of nerve tissue in the specimens.

| Case | Age, years | Gender | Punch specimen length (mm) | No. of glands in each sample | | Presence of nerve tissue | |
|---|---|---|---|---|---|---|---|
| | | | | Punch | Linear incision | Punch | Linear incision |
| 1 | 76 | F | 5.5 | – | 5 | + | – |
| 2 | 72 | M | 7.0 | 1 | 5 | + | – |
| 3 | 79 | M | 7.0 | 1 | 5 | + | – |
| 4 | 70 | F | 8.5 | 1 | 5 | + | – |
| 5 | 73 | M | 8.2 | 2 | 5 | + | – |

F, female; M, male.

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## Competing interests

None declared.

## Ethical approval

Not required.

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# Minor salivary gland biopsy in Sjögren's syndrome: A review and introduction of a new tool to ease the procedure

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## Abstract

Objectives: To review the existing techniques for minor salivary gland biopsy (MSGB) in the lip and to suggest a new approach to ease the procedure and reduce post-operative complications.

Study Design: A comprehensive literature review and a descriptive study of a new surgical technique.

Results: Diverse incisions have been suggested for MSGB with different designs (ellipse, circular, linear), different directions (parallel, oblique, vertical) and a wide range of lengths (from 1 mm up to 3 cm), but no comparative studies supporting the advantages of a particular type of incision over the others could be retrieved. A variety of features of the existing techniques for MSGB are linked to undesired events and surgical complications which could be minimized by modifying certain aspects of these procedures. The technique described, together with the use of the S forceps, represents a significant improvement over the already described chalazion forceps because it allows for a better access and positioning of the lower lip, improves the ergonomic conditions of the assistant, and facilitates the identification of lip areas with more superficial gland lobules.

Conclusions: The suggested approach for lip MSGB includes a specifically designed instrument whose performance during lip biopsy may contribute to minimize post-operative complications.

***Key words:*** *Sjögren's syndrome, diagnosis, minor salivary gland biopsy, surgical technique, lower lip.*

## Introduction

The Sjögren's syndrome (SS) is an autoimmune exocrine disorder with signs and symptoms of dry mouth and keratoconjunctivitis sicca, which may sometimes display a wide range of systemic, non-glandular alterations (1,2). The prevalence of this syndrome has been estimated to range between 0.5% and 1% (3), with a female:male ratio of about 9:1 (1-3).

Histopathology in minor salivary gland (presence of focal lymphocytic sialadenitis with a focus score ≥1) is one out of the six diagnostic criteria set in the revised international classification for Sjögren's Syndrome

(2) for diagnosis of SS. It has recently become more important because of the consensus in considering only objective criteria to define a SS case, which has to meet at least 2 of the following 3 findings: 1. Positivity serum anti-SSA and/or SSB; 2. Ocular staining score >3; and 3. Presence of focal lymphocytic sialadenitis with a focus score >1 per 4 mm2 of glandular tissue (2).

A systematic review on minor salivary gland biopsy (MSGB) has proved diagnostic value for SS with high specificity (X±SD= 88.1±11.7) and sensitivity (X±SD= 78.8±11.2), as well as diagnostic confidence in terms of positive (X±SD= 87.6±9.5) and negative (X±SD= 79.0±16.9) predictive values (3). These results make this technique particularly useful for patients suspicious for SS with inconclusive clinical findings (4). MSGB may also contribute to diagnosis of amyloidosis, sarcoidosis, and confirmation of neonatal hemochromatosis (3,5,6).

Despite the different surgical approaches suggested for MSGB (use of chalazion forceps for tissue stabilization, usage of scalpel vs. punch, different incision sizes, and need or not for suturing), both immediate and mediate complications are continuously described in the literature, being the most relevant a long-lasting lower lip numbness occurring in up to 6% of MSGB procedures (7). These events support the need for a review of the technique to reduce morbidity. In this sense, we suggest the use of a specifically designed forceps for lip biopsy in SS patients that improves tissue stabilization, eases the procedure, and reduces complications.

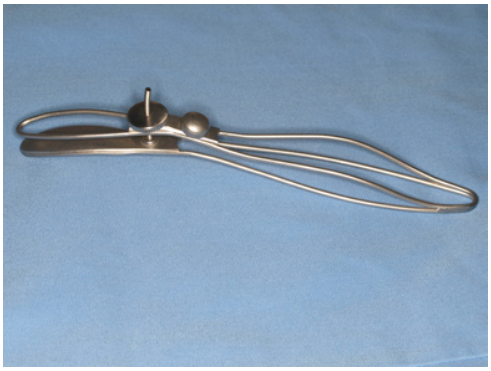

**Fig. 1.** "S" forceps.

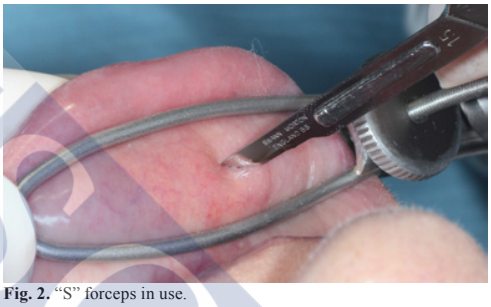

**Fig. 2.** "S" forceps in use.

## Material and Methods

The materials required for this technique include a syringe for intraoral local anaesthesia, scalpel with a No. 15 blade, non-toothed Adson forceps, 4/0 braided silk suture, and the "S" forceps for biopsy (OEPM nº 201200158) (Fig.1). This is a 18.5 cm long forceps with a fenestrated active end (5 $cm^2$). Both the fenestrated area (longitudinal to the axis of the forceps) and its wide size are conceived to provide an ample surgical field. The non-fenestrated blade of the forceps is slightly convex in shape to facilitate herniation of minor salivary gland lobules. There is a screw in the shank for adjustment of the space between the blades, thus permitting a variable and controlled pressure over the soft tissues during the surgical procedure. The handles of the forceps are at an angle with the blades to help traction and visibility of the surgical field. This angle also permits the forceps to work as a surgical separator improving accessibility by means of a traction-separation movement.

Technique

The biopsy site should be selected from the inner side of the lower lip, rich in minor salivary glands, avoiding the midline area due to its lesser content of glandular component (Fig. 2).

Local anesthesia is performed by perilesional infiltration or blockage of the mental nerve. Once anesthesia is achieved, the whole lower lip is stabilized using the S forceps, and the biopsy site selected taking advantage of the forceps design which forces the gland lobules to protrude through the fenestrated blade.

A horizontal linear incision of about 1 cm to 1.5 cm is performed away from the midline, combined with a blunt dissection of the borders of the wound. At this stage, the lobules are herniated towards the surface of the wound pushed by the non-fenestrared, convex, blade of the forceps (Fig. 3). Five to seven lobules can now be gently removed using the Adson tweezers and introduced into an abundant fixing solution (at least ten fold the volume of the tissue sampled). The wound is then sutured with interrupted single sutures. Use of magnification is recommended when performing the technique in order to identify superficial nerves and vessels and to diminish surgical morbidity.

Observations about the technique

MSGB of the lip is a key diagnostic tool for the diagnosis of systemic disorders and particularly of SS.

The technique described above, together with the use of the S forceps, represents a significant improvement over the already described chalazion forceps because

it allows for a better access and positioning of the lower lip, improves the ergonomic conditions of the assistant, and facilitates the identification of lip areas with more superficial gland lobules. It also permits a better bleeding control during surgery, an enhanced visualization of vessel and nerve endings, reduces the surgical time, and provides non-artefacted lobules for pathological analysis (Fig. 4).

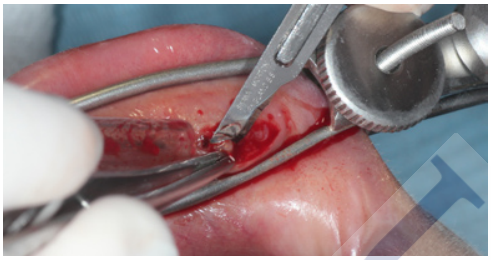

**Fig. 3.** Excision of a minor salivary gland.

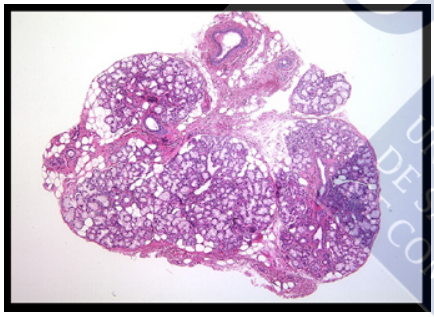

**Fig. 4.** Minor salivary gland (H&E x10).

## Discussion

Techniques and complications in MSGB

Despite there is a wide agreement on avoiding the glandular-free zone in the centre of the lower lip, it seems to exist a remarkable lack of standardisation of the MSGB technique when aimed at obtaining at least five glandular lobules for the diagnosis of SS (8).

Different incisions have been suggested with different designs (ellipse, circular, linear), different directions (parallel, oblique, vertical) and a wide range of lengths (from 1 mm up to 3 cm), but no comparative studies supporting the advantages of a particular type of incision over the others could be retrieved (9-19).

Most frequent immediate surgical complications include intra- and post-operative bleeding (9,11). Pain, inflammation, wound infection, suture dehiscence, and cheloid scars are described as mediate complications of glandular biopsy (7,9-15), but the so-called "disorders of lip sensitivity" are the most frequently reported complication (18,19), occurring in up to 11% of cases in large series (12). This finding has discouraged the use of a punch for MSGB because it removes lip mucosa together with the attached gland, and favoured techniques that permit identification and avoidance of sensory nerve endings (16). These complications may well justify that only patients in a community setting with negative results for anti-RO/la antibodies would be referred for MSGB (20).

Lip stabilization devices

In this sense, some authors have suggested the use of chalazion forceps, employed by ophtalmologists during chalazion exeresis, to ease biopsy of minor salivary gland from mobile lip tissue, as it permits tissue stabilization and to work under ischemic conditions (6,7). However, this instrument was originally designed for ophthalmology and has a number of shortcomings for oral use: the handles of the chalazion forceps are small-sized to allow finger control and are placed perpendicular to the main axis of the blades; this forces the assistant's hand to work on an uncomfortable position, too near to the surgical field. The size of the fenestration also limits the incision design, particularly when undertaking minimally invasive techniques with multiple 2 mm incisions along the inner face of the lower lip (13,18,19). An improved chalazion forceps was introduced by López-Jornet et al. (21): this forceps was larger than the original (20 cm.) and its active end provided a constant pressure of 1Kg/cm$^2$ on the tissues exerted by means of two flat plates (one of them with a round opening, sized 1.7 cm diameter). This design eases lip stabilization by the assistant, but it is impossible to graduate the pressure on the lip tissue and the fenestrated blade provides a reduced surgical field.

On the other hand, the forceps we suggest for MSGB, besides permitting a controlled pressure adapted to the surgical time and to the features of the lip of the patient (macrochelia, etc.), allows a more ergonomic hand grasp in such a way that keeps the assistant's hand away from the working area without disturbing the surgeon. Moreover, the width of the fenestrated blade in this forceps conditions neither the design nor the size of the incisions as well as permits minimally invasive techniques, where a wide surgical field is required to harvest glandular tissue all over th inner side of the lower lip (18).

## Conclusion

This forceps stabilizes lip tissues, avoids excessive intra-operative bleeding, permits better visibility of the surgical field, allows improved selection of tissue samples for pathological analysis and has a potential to minimize the morbidity related to iatrogenic nerve lesions.

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**Conflict of interest**

Dr Juan Seoane and Dr. Juan M. Seoane-Romero have designed the instrument described in this manuscript.

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