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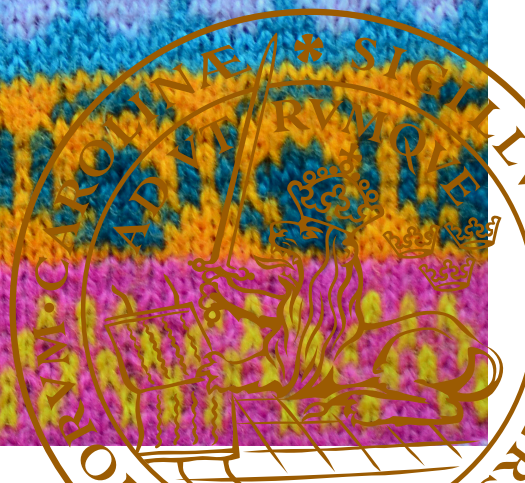
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# Pulmonary involvement in primary Sjögren's syndrome

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# Pulmonary involvement in primary Sjögren's syndrome

Anna Nilsson



**LUND**  
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DOCTORAL DISSERTATION

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To be defended in Agardhsalen at the Clinical Research Centre (CRC), Skåne  
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*Faculty opponent*

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<p><b>Abstract</b></p> <p>The aim of this thesis was to investigate pulmonary involvement in patients with primary Sjögren's syndrome (pSS), in terms of pulmonary function and chronic obstructive pulmonary disease (COPD) prevalence and its development over time in association with radiographic findings, pSS disease features and respiratory symptoms, taking cigarette smoking into account, as well as to study inflammatory markers in the airways of never-smoking patients with pSS compared to never-smoking controls.</p> <p>In study I, 51 consecutive SS patients were assessed by pulmonary function tests (PFTs) and the results were compared to population-based controls, taking cigarette smoking into account. pSS patients were also assessed by computed tomography (CT) of the chest and for pSS disease activity, respiratory symptoms, cigarette consumption as well as laboratory inflammatory and serological pSS features. COPD was a common finding in pSS patients, even among never-smoking patients. An obstructive pattern was the predominant PFT finding in pSS patients, although a superimposed restrictive lung disease could not be excluded.</p> <p>In study II, 37 pSS patients and 74 population-based matched controls were assessed using the forced oscillation technique (FOT). pSS patients showed FOT signs of obstruction affecting both the peripheral and central airways. Also, pSS patients with no spirometrical signs of obstructive airway disease and never-smoking pSS patients showed clear FOT signs of airway obstruction. Thus, FOT appears to be a sensitive method of detecting airway obstruction in pSS patients.</p> <p>In study III, induced sputum cytokines and leukocytes were assessed in 20 never-smoking patients with pSS and in 19 never-smoking population-based matched controls. Pulmonary function, disease activity, respiratory symptoms, as well as inflammatory and serological pSS features were assessed in the pSS patients. B-cell activating factor (BAFF), interleukin (IL)-6 and IL-8 were significantly increased in the induced sputum of pSS patients compared to population-based controls, suggesting a specific ongoing inflammatory disease process in the airways of pSS patients. Cytokine levels in induced sputum were not associated with PFTs, disease activity, respiratory symptoms or serological features of pSS.</p> <p>In study IV, 40 patients with pSS, who previously participated in study I, were re-assessed by PFTs and for pSS disease activity, respiratory symptoms, cigarette consumption, as well as laboratory inflammatory and serological pSS features and high-resolution CT after a mean follow-up time of six years. Signs of both airway and pulmonary parenchymal involvement were commonly found with a co-existence of both an obstructive and a restrictive pulmonary function pattern, in which the latter tended to deteriorate over time. COPD remained a common finding, even in never-smoking pSS patients. Radiographic signs of bronchial involvement and interstitial lung disease were common.</p> <p>This thesis demonstrated that signs of airway and pulmonary parenchymal involvement are common in patients with pSS and may therefore be underdiagnosed. COPD being common, even in never-smoking pSS patients, suggests that the disease <i>per se</i> is involved in COPD development. Special attention and liberal assessment of pulmonary involvement in pSS patients is mandated.</p>		
<b>Key words</b> primary Sjögren's syndrome, pulmonary disease, airways, COPD, pulmonary function, radiography, immunology, disease activity, respiratory symptoms, follow-up		
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# Pulmonary involvement in primary Sjögren's syndrome

Anna Nilsson



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Tage Danielsson





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## List of papers

This thesis is based on the following original papers and manuscripts:

- I. Nilsson AM, Diaz S, Theander E, Hesselstrand R, Piitulainen E, Ekberg O, Wollmer P, Mandl T. Chronic obstructive pulmonary disease is common in never-smoking patients with primary Sjögren's syndrome. *The Journal of Rheumatology* 2015;42:464-71.
- II. Nilsson AM, Theander E, Hesselstrand R, Piitulainen E, Wollmer P, Mandl T. The forced oscillation technique is a sensitive method for detecting obstructive airway disease in patients with primary Sjögren's syndrome. *Scandinavian Journal of Rheumatology* 2014;43(4):324-8.
- III. Nilsson AM, Tufvesson E, Hesselstrand R, Olsson P, Wollmer P, Mandl T. Increased B-cell activating factor, interleukin-6, and interleukin-8 in induced sputum from primary Sjögren's syndrome patients. *Scandinavian Journal of Rheumatology* 2019;48(2):149-56.
- IV. Nilsson AM, Aaltonen LH, Olsson P, Persson HL, Hesselstrand R, Theander E, Wollmer P, Mandl T. Mixed airway and pulmonary parenchymal disease in patients with primary Sjögren's Syndrome - a six year follow-up. Manuscript.

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## Abbreviations

ACA	Anti-centromere antibody
ACR	American College of Rheumatology
AECG	American-European Consensus Group
AHR	Airway hyper responsiveness
AIDS	Acquired immunodeficiency syndrome
ALBIA	Addressable laser bead immunoassay
AMA	Anti-mitochondrial antibody
ANA	Anti-nuclear antibody
AS	Ankylosing spondylitis
BAFF	B-cell activating factor
BAL	Bronchoalveolar lavage
BALT	Bronchial-associated lymphoid tissue
C	Complement component
CAT	COPD assessment test
CD	Cluster of differentiation
CK	Creatine kinase
COP	Cryptogenic organizing pneumonitis
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
csDMARD	Conventional synthetic DMARD
CT	Computed tomography
CTD	Connective tissue disease
D <sub>L,CO</sub>	Diffusing capacity of the lungs for carbon monoxide
DMARD	Disease-modifying antirheumatic drug
dsDNA	double-stranded Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
ESSDAI	EULAR Sjögren's Syndrome Disease Activity Index
ESSPRI	EULAR Sjögren's Syndrome Patient Reported Index
EULAR	European League Against Rheumatism
GC	Germinal centre
GFR	Glomerular filtration rate
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
HRCT	High-resolution computed tomography
HRQoL	Health-related quality of life
HUVS	Hypocomplementaemic urticarial vasculitis syndrome
FEV	Forced expiratory volume
FEV <sub>1</sub>	FEV in 1 s
FEV <sub>1rev</sub>	FEV <sub>1</sub> after reversibility test

FOT	Forced oscillation technique
F <sub>res</sub>	Resonant frequency
ICS	Inhaled corticosteroids
IFN $\alpha$	Interferon alpha
Ig	Immunoglobulin
IL	Interleukin
ILD	Interstitial lung disease
IOS	Impulse Oscillometry System
LABA	Long-Acting beta-2 Agonists
LAMA	Long-Acting Muscarinic Antagonists
LIP	Lymphocytic interstitial pneumonitis
M3R	Muscarine 3 receptor
MALT	Mucosa-associated lymphoid tissue
mMRC	modified Medical Research Council
MSSR	Malmö Sjögren's Syndrome Registry
MZBCL	Marginal zone B-cell lymphoma
NF- $\kappa\beta$	Nuclear factor- $\kappa\beta$
NK cell	Natural killer cell
NSAID	Non-steroid anti-inflammatory drugs
NSIP	Non-specific interstitial pneumonitis
OAD	Obstructive airway disease
OSS	Ocular staining score
PCR	Polymerase chain reaction
pDC	plasmacytoid Dendritic cell
PFT	Pulmonary function test
PsA	Psoriasis arthritis
pSS	primary Sjögren's syndrome
R	Resistance
RA	Rheumatoid arthritis
RCT	Randomized controlled trial
RF	Rheumatoid factor
RNA	Ribonucleic acid
RV	Residual volume
SABA	Short-Acting beta-2 Agonists
SGRQ	St George's Respiratory Questionnaire
SLE	Systemic erythematosus
SMA	Smooth muscle antibody
SSA/Ro	Sjögren's syndrome antigen A/Ro
SSB/La	Sjögren's syndrome antigen B/La
SSc	Systemic sclerosis
sSS	secondary Sjögren's Syndrome
Th1	Type 1 T-helper
TLC	Total lung capacity

TNF- $\alpha$	Tumour necrosis factor-alpha
VC	Vital capacity
VCrev	VC after reversibility test
UIP	Usual interstitial pneumonitis
UWS	Unstimulated whole saliva
X	Reactance



# Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune rheumatic disease that classically affects females. Various exocrine glands are affected and lymphocytic infiltration, B-cell hyperactivation and type I interferon (IFN) signature are immunological hallmarks of pSS. The disease is associated with an exocrine gland dysfunction, resulting in characteristic sicca symptoms, including dry mouth, dry eyes and dry symptoms in several other mucosal areas. pSS may also present with systemic manifestations and non-exocrine organ systems, including the lungs, may be affected in a widely heterogeneous manner and the disease can sometimes be severe. The Swedish ophthalmologist, Henrik Sjögren, described the systemic disease in his thesis from 1933. Secondary Sjögren's syndrome (sSS) is referred to in another clinical context; when sicca symptoms occur in association with other connective tissue diseases (CTDs), e.g. rheumatoid arthritis (RA) systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). This thesis concerns primary Sjögren's syndrome [1-4].

Respiratory symptoms are common in pSS and pleomorphic pulmonary involvement has been described [5]. Some studies indicate that pulmonary involvement in pSS affects the health-related quality of life (HRQoL) negatively and has even been shown to be associated with an increased mortality [6-8]. Knowledge about the development of pulmonary involvement in pSS over time is sparse and studies show conflicting results, possibly due to the use of different methodologies for evaluation of pulmonary involvement, different study designs and different classification criteria for pSS [9-14].

# Primary Sjögren's syndrome

## Epidemiology

pSS has been reported as the second most common rheumatic disease with an estimated prevalence of between 0.05% and 0.6% in different studies [15, 16]. There is variation in the reported prevalence due to both different study designs and classification criteria for pSS between studies. A recent Swedish study at the Karolinska Institute reported an annual incidence of 3.1/100,000 [17].

There is a significant female predominance in pSS, with over 90% of pSS patients being females. The reported female to male ratio has been reported as being between 9:1 and 14:1 in different studies. The peak incidence is at approximately 50 years and 55 years in a recent Swedish study [1, 17, 18].

## Etiopathogenesis and immunological disturbances

pSS is a chronic inflammatory autoimmune disease with a proposed multifactorial etiopathogenesis in which immunological disturbances with a focal lymphocytic infiltration of the exocrine glands, B-cell hyperactivation and autoantibodies are hallmarks. The autoimmune disease results in an exocrine dysfunction, inflammation and atrophy of glandular structures and, in some patients, even in a more systemic disease that affects various extraglandular organs. There has been progress in the understanding of the immunopathogenesis in pSS in recent years. An interaction of environmental factors, genetic factors and gender are hypothesised to contribute to the development of the disease. There has been speculation of preceding viral infections triggering the autoimmune response in pSS, e.g. Epstein-Barr virus (EBV), hepatitis C, retroviruses and enteroviruses [2, 3, 19].

### *The model of a vicious immunopathological cycle*

Briefly, the current immunopathological model in pSS is referred to in terms of a vicious cycle of immune activation involving both the innate and the adaptive immune system. A triggering factor (possibly viral infection) results in the activation of mucosal epithelial cells which, in turn, leads to the activation of the innate and adaptive immune system (natural killer cells (NK cells), plasmacytic dendritic cells (pDC), T and B lymphocytes). Activated T cells induce tissue damage through interferon  $\gamma$  (IFN $\gamma$ ) and support B-cell activation. B-cell hyperactivation leads to the secretion of autoantibodies (e.g. anti-Sjögren's syndrome antigen A (SSA)/Ro and anti-Sjögren's syndrome antigen B (SSB)/La) and to the formation of immune complexes. Further, the production of interferon- $\alpha$  (IFN- $\alpha$ ) leads to additional activation of the immune system. The IFN- $\alpha$ , in turn,

induces the production of B-cell activation factor (BAFF), through dendritic cells and epithelial cells. BAFF is another cytokine that has recently been highlighted as being of importance to pSS immunopathology by promoting B-cell maturation, proliferation and differentiation into plasma cells and B-cell survival. Other cytokines, including IL-6, are also involved in B-cell activation. It is suggested that both IFN $\gamma$  and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), produced by type 1 T-helper (Th1) cells, directly contribute to tissue damage [2, 19, 20].

As mentioned, the epithelial cells are likely to be involved in the immune activation and recruitment of T and B cells, mediated through cytokines such as BAFF, IL-6, IL-17 and IL-22, as well as chemokines such as CXCL10, CXCL12 and CXCL13. The epithelial cells in the salivary glands may also provide autoantigens and in this way contribute to the maintenance of autoimmunity. The term “autoimmune epithelitis” is commonly used, highlighting this hallmark of the immunopathogenesis of the disease [2, 19, 21].

Besides the identified activation of the adaptive immune system the innate immune system is also believed to be important in pSS immunopathogenesis, in which NK cells have recently been identified as important contributors, interacting with dendritic cells and producing IFN $\gamma$  [2].

The type I IFN system has also recently been identified as being highly important in the autoimmune pathways in pSS immunopathogenesis, supported by recent findings in genetic research, in which the mapping of gene expression shows a so-called interferon signature [2, 22].

Although it is suspected that microbiological triggering is involved in the development of autoimmunity in genetically susceptible individuals in pSS, no specific microbe has been established as causative, thus far [2]. Mofors et al. recently presented previous infections as risk factors for subsequent pSS development and stratified analysis demonstrated respiratory infections to be more prominent in pSS patients compared to controls before being diagnosed with pSS [23].

The formation of ectopic lymphoid structures, so-called germinal centres (GCs), has been highlighted as an important factor in the establishment of chronic autoimmune responses in target organs in pSS. Furthermore, the grade of lymphocytic infiltration and particularly the formation of GC, observed in the salivary glands in pSS, have been found to be prognostic factors of the disease and have been associated with extraglandular involvement and subsequent risk of development of lymphoma [24].

#### *Other possible factors involved in pSS etiopathology*

A genetic predisposition to pSS development is believed to exist and an increased incidence of pSS has been observed in family members of patients with pSS.

Some human leukocyte antigen (HLA) types have been shown to be associated with pSS (HLAB8, HLADw3 and HLADR3) [3, 19].

Considering the previously mentioned female predominance in pSS, factors associated with sex hormones have also been suggested to be involved in the development of pSS. As the disease commonly starts after the menopause, an oestrogen deficiency has been suggested to predispose pSS.

In addition, neuroendocrine disturbances have also been suggested to contribute in pSS pathology [3, 19, 25].

### *Autoantibodies in pSS*

Several types of autoantibodies, directed against specific nuclear or cytoplasmic antigens, are seen in pSS. Frequently, several different autoantibodies are shown in the same patient. The most common autoantibodies in pSS patients are: antinuclear antibodies (ANA), rheumatoid factor (RF), anti-SSA/Ro, anti-SSB/La and cryoglobulins. Anti-SSA/Ro and/or anti-SSB/La antibodies are shown in the majority of the patients with pSS and the presence of anti-SSA/Ro is included in both the American-European Consensus Group (AECG) and the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for pSS. The SSA/Ro and SSB/La antigens are small ribonucleoproteins, which are constituents of cellular ribonucleic acid (RNA). Different molecular forms of the antigen SSA/Ro have been described namely, two lymphocyte peptides of 52 kDa and 60kD, respectively [3, 21, 26-28].

Anti-SSA/Ro and anti-SSB/La as well as the other frequent autoantibodies seen in pSS (i.e. ANA, RF and cryoglobulins) have been associated with different phenotypes of the disease, mainly extraglandular features, and represent negative prognostic factors of the disease [21, 28]. Further details regarding the associations with specific pSS clinical features are presented in the section on clinical presentation.

Several other autoantibodies against non-nuclear antigens have also been reported in pSS, e.g. anti-muscarine 3 receptor (anti-M3R) antibodies, anti-smooth muscle antibodies (anti-SMA), anti-parietal cell antibodies, anti-mitochondrial antibodies (AMA), anti-double-stranded deoxyribonucleic acid (anti-dsDNA), and anti-centromere antibodies (ACA), with various frequencies and various clinical significances [1, 3, 21].

### *Hypocomplementaemia*

In approximately 10-25 % of patients with pSS, hypocomplementaemia, in terms of low levels of complement component 3 (C3) and/or complement component 4 (C4), has been shown. Complements are lowered as a result of complement consumption in inflammatory processes through enzyme cascades.

Hypocomplementaemia is also a negative prognostic factor that has been shown to be associated with lymphoma development, for example [3, 28, 29].

## **Clinical presentation**

### *Sicca symptoms, pain and fatigue*

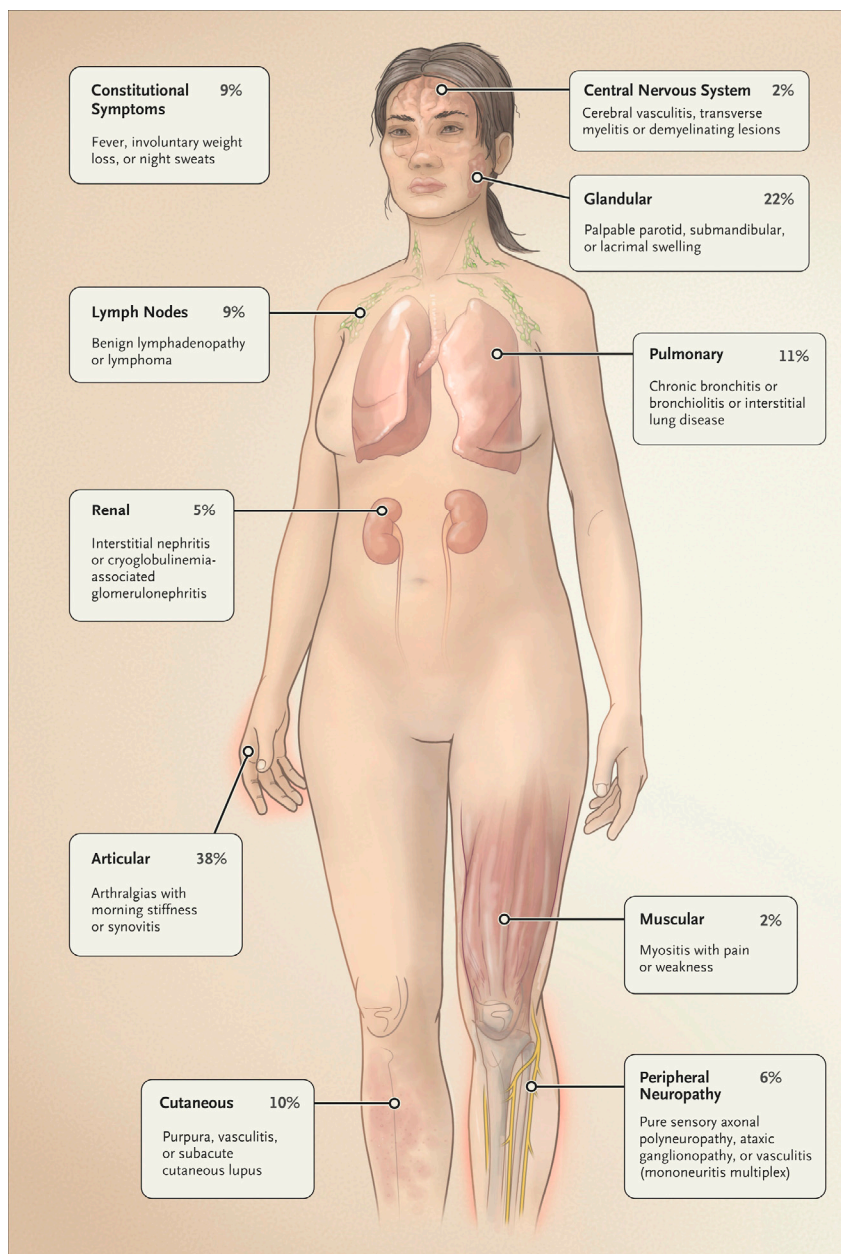
Dryness of the mouth, eyes and mucosal areas (sicca symptoms), fatigue and joint pain are characteristic symptoms of pSS and are present in the majority of patients [1, 18, 30].

Autoimmune inflammation of the exocrine glands in pSS is associated with exocrinopathy, a dysfunction of the exocrine glands. It has been proposed that both a reduction in secretion and impaired quality of secretions gives rise to sicca symptoms in pSS. Dryness of the mouth (xerostomia) may present as a need to drink and is associated with impaired oral health, such as increased risk of caries and oral candidiasis. Dryness of the eyes may present as a sensation of irritation and tiredness in the eyes with a frequent need of tear substitutes. Other mucosal areas are also commonly affected, such as the ear, nose and throat tract and the airways, presenting with a dry cough and hyperreactive airways, as well as vaginal mucosa, with painful intercourse and risk of vaginal candidiasis. Reduced HRQoL has been associated to the sicca symptoms in patients with pSS. Besides the sicca symptoms, pain and fatigue is commonly reported to affect patients with pSS and also contributes to a reduced HRQoL [1, 3, 8, 31].

### *Systemic manifestations*

In approximately one third of pSS patients, the disease presents with systemic manifestations and involvement of non-exocrine organ systems (Figure 1). The manifestations include arthralgia and arthritis, glandular involvement with enlargement of the parotid, submandibular and lacrimal glands, lymphadenopathy or even lymphoma, pulmonary involvement, including both airway and parenchymal involvement, cutaneous involvement, with purpura, vasculitis or subacute cutaneous lupus, involvement of the peripheral and central nervous system, renal involvement, with both renal tubular acidosis, interstitial nephritis or cryoglobulinemia-associated glomerulonephritis, myositis and constitutional symptoms (i.e. fever, night sweats and involuntary weight loss). In addition, both haematological and serological abnormalities are often found in pSS systemic disease and include anaemia, thrombocytopenia, neutro- or lymphopenia, hypergammaglobulinaemia, hypocomplementaemia and cryoglobulinaemia. The risk of lymphoma in pSS is elevated, with an approximately 15-20 times increased risk of B-cell lymphoma compared to the general population. Systemic manifestation of pSS may sometimes present as the first symptom of the disease [1, 18, 29, 30, 32, 33].





**Figure 1.** Systemic manifestations of primary Sjögren's syndrome. The percentages of the various manifestations are derived from reports in the Sjögren BigData Project, including more than 10,000 patients with pSS from 22 countries. Reproduced with the permission from of Professor Xavier Mariette [1].

### *Autoantibodies and hypocomplementaemia in relation to systemic pSS features*

Several of the most common autoantibodies shown in patients with pSS, i.e. anti-SSA/Ro, anti-SSB/La, ANA, RF and cryoglobulins, as well as hypocomplementaemia, have been associated with different phenotypes of the disease, mainly extraglandular features, and represent negative prognostic factors of the disease [3, 28].

Seropositivity for anti-SSA/Ro and anti-SSB/La autoantibodies is reported to be associated with several clinical and laboratory pSS features in pSS patients, particularly when the two autoantibodies are both present. The linked pSS features include parotid enlargement, lymphadenopathy, lymphoma, cutaneous vasculitis, neurologic involvement, pulmonary parenchymal disease, hypergammaglobulinaemia, hypocomplementaemia, RF seropositivity and cryoglobulins. In contrast, pSS patients without anti-SSA/Ro and anti-SSB/La autoantibodies might present other phenotypes of the disease, e.g. more infrequent extraglandular pSS features and more frequent male and elderly patients [1, 3, 28, 34].

ANA positivity is also closely associated with extraglandular and laboratory pSS features, e.g. hypergammaglobulinaemia, hypocomplementaemia and elevated erythrocyte sedimentation rate (ESR) and RF seropositivity in pSS, have been associated with risk of lymphoma, articular involvement and cutaneous vasculitis. Cryoglobulinaemia in pSS has been associated with a higher prevalence of extraglandular manifestations and requires special attention because of its association with a higher risk of B-cell lymphoma and life-threatening vasculitis [1, 3, 28].

Hypocomplementaemia in pSS has also been associated with several specific systemic features in pSS, including fever, articular involvement, polyneuropathy, renal involvement, cutaneous vasculitis and even lymphoma development, as well as an association with increased mortality in pSS. There is also an association between hypocomplementaemia, RF and cryoglobulinaemia, as all these are usually present in patients with concomitant cryoglobulinaemic vasculitis [3, 28, 29].

**Table 1.**

The 2002 American-European Consensus Group (AECG) classification criteria for pSS [27].

ITEM	
I. Ocular symptoms of dryness	<p>A positive response to at least one of the following questions:</p> <ul style="list-style-type: none"> <li>Have you had daily, persistent, troublesome dry eyes for more than 3 months?</li> <li>Do you have a recurrent sensation of sand or gravel in the eyes?</li> <li>Do you use tear substitutes more than 3 times a day?</li> </ul>
II. Oral symptoms	<p>Oral symptoms: a positive response to at least one of the following questions:</p> <ul style="list-style-type: none"> <li>Have you had a daily feeling of dry mouth for more than 3 months?</li> <li>Have you had recurrently or persistently swollen salivary glands as an adult?</li> <li>Do you frequently drink liquids to aid in swallowing dry food?</li> </ul>
III. Ocular signs	<p>Objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:</p> <ul style="list-style-type: none"> <li>Schirmer's I test, performed without anaesthesia (<math>\leq 5</math> mm in 5 minutes)</li> <li>Rose bengal score or other ocular dye score (<math>\geq 4</math> according to van Bijsterveld's scoring system)</li> </ul>
IV. Histopathology	<p>In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialadenitis, evaluated by an expert histopathologist, with a focus score <math>\geq 1</math>, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm<sup>2</sup> of glandular tissue.</p>
V. Salivary gland involvement	<p>Objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:</p> <ul style="list-style-type: none"> <li>Unstimulated whole salivary flow (<math>\leq 1.5</math> ml in 15 minutes).</li> <li>Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitory or destructive pattern), without evidence of obstruction in the major ducts.</li> <li>Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer.</li> </ul>
VI. Autoantibodies	<p>Presence in the serum of the following autoantibodies: 1. Antibodies to Ro(SSA) or La(SSB) antigens, or both.</p>
CLASSIFICATION RULES	
Primary SS	<p>In patients without any potentially associated disease, primary SS may be defined as follows:</p> <ol style="list-style-type: none"> <li>The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (Histopathology) or VI (Serology) is positive.</li> <li>The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V, VI)</li> </ol>
Secondary SS	<p>In patients with a potentially associated disease (for instance, another well defined connective tissue disease), the presence of item I or item II plus any 2 from among items III, IV, and V may be considered as indicative of secondary SS</p>
Exclusion criteria	<ul style="list-style-type: none"> <li>Past head and neck radiation treatment</li> <li>Hepatitis C infection</li> <li>Acquired immunodeficiency disease (AIDS)</li> <li>Pre-existing lymphoma</li> <li>Sarcoidosis</li> <li>Graft versus host disease</li> <li>Use of anticholinergic drugs (since a time shorter than 4-fold the half life of the drug)</li> </ul>

**Table 2.**

The 2016 American Collage of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for pSS.

The classification of primary Sjögren's syndrome (SS) applies to any individual who meets the inclusion criteria, does not meet the conditions listed as exclusion criteria and has a score of  $\geq 4$  points when the weights from the criteria items below are summed [26].

ITEM	WEIGHT/SCORE
Labial salivary gland with focal lymphocytic sialadenitis and focus score of $\geq 1$ foci/4mm <sup>2</sup> ‡	3
Anti-SSA/Ro-positive	3
Ocular Straining Score $\geq 5$ (or van Bijsterveld score $\geq 4$ ) in at least one eye §¶	1
Schirmer's test $\leq 5$ mm/5 min in at least one eye §	1
Unstimulated whole saliva flow rate $\leq 0.1$ ml/min§**	1
<b>INCLUSION CRITERIA</b>	
These inclusion criteria are applicable to any patient with at least one symptom of ocular or oral dryness, defined as a positive response to at least one of the following questions	
1 Have you had daily, persistent, troublesome dry eyes for more than 3 months?	
2 Do you have a recurrent sensation of sand or gravel in the eyes?	
3 Do you use tear substitutes more than three times a day?	
4 Have you had a daily feeling of dry mouth for more than 3 months?	
5 Do you frequently drink liquids to aid in swallowing dry food?	
or in whom there is suspicion of Sjögren's syndrome (SS) from the European League Against Rheumatism SS Disease Activity Index questionnaire (at least one domain with a positive item).	
<b>EXCLUSION CRITERIA</b>	
1 History of head and neck radiation treatment	
2 Active hepatitis C infection (with confirmation by PCR)	
3 AIDS	
4 Sarcoidosis	
5 Amyloidosis	
6 Graft-versus-host disease	
7 IgG4-related disease.	

‡The histopathologic examination should be performed by a pathologist with expertise in the diagnosis of focal lymphocytic sialadenitis and focus score count, using the protocol described by Daniels et al [43].

§Patients who are normally taking anticholinergic drugs should be evaluated for objective signs of salivary hypofunction and ocular dryness after a sufficient interval without these medications in order for these components to be a valid measure of oral and ocular dryness.

¶Ocular Staining Score described by Whitcher et al, van Bijsterveld score described by van Bijsterveld [44].

\*\*Unstimulated whole saliva flow rate measurement described by Navazesh and Kumar [45].

**Table 3.**

The EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI): domain and item definitions and scores [37, 40].

DOMAIN	ACTIVITY LEVEL	DESCRIPTION
<b>Constitutional</b> Exclusion of fever of infectious origin and voluntary weight loss	No=0	Absence of the following symptoms
	Low=3	Mild or intermittent fever (37.5°–38.5°C)/night sweats and/or involuntary weight loss of 5–10% of body weight
	Moderate=6	Severe fever (>38.5°C) / night sweats and/or involuntary weight loss of >10% of body weight
<b>Lymphadenopathy</b> Exclusion of infection	No=0	Absence of the following features
	Low=4	Lymphadenopathy ≥1 cm in any nodal region or ≥2 cm in inguinal region
	Moderate=8	Lymphadenopathy ≥2 cm in any nodal region or ≥3 cm in inguinal region, and/or splenomegaly (clinically palpable or assessed by imaging)
	High=12	Current malignant B-cell proliferative disorder*
<b>Glandular</b> Exclusion of stone or infection	No=0	Absence of glandular swelling
	Low=2	Small glandular swelling with enlarged parotid (≤3 cm), or limited submandibular or lachrymal swelling
	Moderate=4	Major glandular swelling with enlarged parotid (>3 cm), or important submandibular or lachrymal swelling
<b>Articular</b> Exclusion of osteoarthritis	No=0	Absence of currently active articular involvement
	Low=2	Arthralgias in hands, wrists, ankles and feet accompanied by morning stiffness (>30 min)
	Moderate=4	1–5 (of 28 total count) synovitis
	High=6	≥6 (of 28 total count) synovitis
<b>Cutaneous</b> Rate as 'No activity' stable long-lasting features related to damage	No=0	Absence of currently active cutaneous involvement
	Low=3	Erythema multiforma
	Moderate=6	Limited cutaneous vasculitis, including urticarial vasculitis, or purpura limited to feet and ankle, or subacute cutaneous lupus
	High=9	Diffuse cutaneous vasculitis, including urticarial vasculitis, or diffuse purpura, or ulcers related to vasculitis
<b>Pulmonary</b> Rate as 'No activity' stable long-lasting features related to damage, or respiratory involvement not related to the disease (tobacco use, etc.)	No=0	Absence of currently active pulmonary involvement
	Low=5	Persistent cough or bronchial involvement with no radiographic abnormalities on radiography Or radiological or HRCT evidence of interstitial lung disease with: No breathlessness and normal lung function test.
	Moderate=10	Moderately active pulmonary involvement, such as interstitial lung disease shown by HRCT with shortness of breath on exercise (NHYA II) or abnormal lung function tests restricted to: 70%>DL <sub>CO</sub> ≥40% or 80%>FVC≥60%
	High=15	Highly active pulmonary involvement, such as interstitial lung disease shown by HRCT with shortness of breath at rest (NHYA III, IV) or with abnormal lung function tests: DL <sub>CO</sub> <40% or FVC<60%
<b>Renal</b> Rate as 'No activity' stable long-lasting features related to damage, and renal involvement not related to the disease. If biopsy has been performed, please rate activity based on histological features first	No=0	Absence of currently active renal involvement with proteinuria <0.5 g/day, no haematuria, no leucocyturia, no acidosis, or long-lasting stable proteinuria due to damage
	Low=5	Evidence of mild active renal involvement, limited to tubular acidosis without renal failure or glomerular involvement with proteinuria (between 0.5 and 1 g/day) and without haematuria or renal failure (GFR ≥60 mL/min)
	Moderate=10	Moderately active renal involvement, such as tubular acidosis with renal failure (GFR <60 mL/min) or glomerular involvement with proteinuria between 1 and 1.5 g/day and without haematuria or renal failure (GFR ≥60 mL/min) or histological evidence of extra-membranous glomerulonephritis or important interstitial lymphoid infiltrate
	High=15	Highly active renal involvement, such as glomerular involvement with proteinuria >1.5 g/day or haematuria or renal failure (GFR <60 mL/min), or histological evidence of proliferative glomerulonephritis or cryoglobulinemia related renal involvement



<b>Muscular</b> Exclusion of weakness due to corticosteroids	No=0	Absence of currently active muscular involvement
	Low=6	Mild active myositis shown by abnormal EMG or biopsy with no weakness and creatine kinase ( $N < CK \leq 2N$ )
	Moderate=12	Moderately active myositis proven by abnormal EMG or biopsy with weakness (maximal deficit of 4/5), or elevated creatine kinase ( $2N < CK \leq 4N$ ),
	High=18	Highly active myositis shown by abnormal EMG or biopsy with weakness (deficit $\leq 3/5$ ) or elevated creatine kinase ( $>4N$ )
<b>PNS</b> Rate as 'No activity' stable long-lasting features related to damage or PNS involvement not related to the disease	No=0	Absence of currently active PNS involvement
	Low=5	Mild active peripheral nervous system involvement, such as pure sensory axonal polyneuropathy shown by NCS or trigeminal (V) neuralgia
	Moderate=10	Moderately active peripheral nervous system involvement shown by NCS, such as axonal sensory-motor neuropathy with maximal motor deficit of 4/5, pure sensory neuropathy with presence of cryoglobulinemic vasculitis, ganglionopathy with symptoms restricted to mild/moderate ataxia, inflammatory demyelinating polyneuropathy (CIDP) with mild functional impairment (maximal motor deficit of 4/5 or mild ataxia), Or cranial nerve involvement of peripheral origin (except trigeminal (V) neuralgia)
	High=15	Highly active PNS involvement shown by NCS, such as axonal sensory-motor neuropathy with motor deficit $\leq 3/5$ , peripheral nerve involvement due to vasculitis (mononeuritis multiplex, etc), severe ataxia due to ganglionopathy, inflammatory demyelinating polyneuropathy (CIDP) with severe functional impairment: motor deficit $\leq 3/5$ or severe ataxia
<b>CNS</b> Rate as 'No activity' stable long-lasting features related to damage or CNS involvement not related to the disease	No=0	Absence of currently active CNS involvement
	Moderate=10	Moderately active CNS features, such as cranial nerve involvement of central origin, optic neuritis or multiple sclerosis-like syndrome with symptoms restricted to pure sensory impairment or proven cognitive impairment
	High=15	Highly active CNS features, such as cerebral vasculitis with cerebrovascular accident or transient ischaemic attack, seizures, transverse myelitis, lymphocytic meningitis, multiple sclerosis-like syndrome with motor deficit.
<b>Haematological</b> For anaemia, neutropenia, and thrombopenia, only autoimmune cytopenia must be considered Exclusion of vitamin or iron deficiency, drug-induced cytopenia	No=0	Absence of autoimmune cytopenia
	Low=2	Cytopenia of autoimmune origin with neutropenia ( $1000 < \text{neutrophils} < 1500/\text{mm}^3$ ), and/or anaemia ( $10 < \text{haemoglobin} < 12 \text{ g/dL}$ ), and/or thrombocytopenia ( $100\,000 < \text{platelets} < 150\,000/\text{mm}^3$ ) Or lymphopenia ( $500 < \text{lymphocytes} < 1000/\text{mm}^3$ )
	Moderate=4	Cytopenia of autoimmune origin with neutropenia ( $500 \leq \text{neutrophils} \leq 1000/\text{mm}^3$ ), and/or anaemia ( $8 \leq \text{haemoglobin} \leq 10 \text{ g/dL}$ ), and/or thrombocytopenia ( $50\,000 \leq \text{platelets} \leq 100\,000/\text{mm}^3$ ) Or lymphopenia ( $\leq 500/\text{mm}^3$ )
	High=6	Cytopenia of autoimmune origin with neutropenia ( $\text{neutrophils} < 500/\text{mm}^3$ ), and/or anaemia ( $\text{haemoglobin} < 8 \text{ g/dL}$ ) and/or thrombocytopenia ( $\text{platelets} < 50\,000/\text{mm}^3$ )
<b>Biological</b>	No=0	Absence of any of the following biological features
	Low=1	Clonal component and/or hypocomplementemia (low C4 or C3 or CH50) and/or hypergammaglobulinemia or high IgG level between 16 and 20 g/L
	Moderate=2	Presence of cryoglobulinemia and/or hypergammaglobulinemia or high IgG level $>20 \text{ g/L}$ , and/or recent onset hypogammaglobulinemia or recent decrease of IgG level ( $<5 \text{ g/L}$ )

\*Defined as indolent not treated lymphoma or currently treated lymphoma or myeloma (or treatment ended from less than 6 months). Do not rate past treated lymphoma or myeloma in complete remission.

CIDP, chronic inflammatory demyelinating polyneuropathy; CK, creatine kinase; CNS, central nervous system; DLCO, diffusing CO capacity; EMG, electromyogram; FVC, forced vital capacity; GFR, glomerular filtration rate; Hb, haemoglobin; HRCT, high-resolution CT; IgG, immunoglobulin G; NCS, nerve conduction studies; NYHA, New York Heart Association Classification; Plt, platelet; PNS, peripheral nervous system.

## Classification criteria for pSS

No widely used diagnostic criteria for pSS has been developed for clinical practice, although different classification criteria for pSS have developed over time as tools for identification of patients for research purposes [26, 27, 35, 36]. These classification criteria for pSS may as well be of help in clinical practice. The most recently developed classification criteria are the 2002 AECG criteria, which have been in wide use and the 2016 ACR/EULAR criteria (Table 1 and 2) [26, 27]. The various criteria have developed over time and, following progress in pSS research, both specificity and sensitivity have been improving. In the most recent, ACR/EULAR criteria, both specificity and sensitivity are considered high and as these are easily utilised they are also suitable for use in clinical practice. Patients who fulfil the previously widely used global AECG criteria also often also fulfil the most recent ACR/EULAR criteria [26, 27].

### *The ACR/EULAR classification criteria*

The ACR/EULAR classification criteria are used in patients with either sicca symptoms or signs of systemic pSS manifestations assessed by the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (Table 3) [26, 37]. The pSS classification is then based on a scoring of weighted data from objective tests demonstrating reduced exocrine function of either the salivary or the lacrimal glands (1 point each) and signs of autoimmunity related to pSS, either anti-SSA/Ro positivity or a focal lymphocytic sialadenitis in a biopsy of the minor salivary gland of the lower lip (3 points each). Patients scoring  $\geq 4$  points are classified as pSS if no other rheumatic disease or any of the exclusion criteria exist (Table 2) [26].

### *Assessment of sicca symptoms*

Well-defined questions for assessing persistent patient-reported sicca symptoms of the mouth and eyes have been developed by the American-European study group on classification criteria for SS and the International Sjögren's Syndrome Criteria Working Group (Table 1 and 2). A positive response to any of these questions meets the inclusion criteria for pSS classification, according to the ACR/EULAR criteria [26, 27].

### *Assessment of systemic manifestations as a sign of pSS*

pSS systemic disease activity is evaluated by the ESSDAI and evaluates disease activity in pSS in 12 domains representing different organ systems (Table 3). Disease activity in at least one ESSDAI domain is considered pathological, when used as inclusion criteria for pSS classification, according to the ACR/EULAR criteria. The 12 ESSDAI domains are: 1: constitutional, 2: lymphadenopathy, 3: glandular 4: articular, 5: cutaneous, 6: respiratory, 7: renal, 8: muscular, 9: peripheral nervous system (PNS), 10: central nervous system (CNS),

11: haematological and 12: biological domain. The disease activity in each domain is assessed (none, low, moderate and high) and weighted according to its relative clinical importance and a total score is then calculated by adding the individual weighted domain scores [37, 38]. The disease activity in each domain is based on anamnestic information, clinical examination and laboratory analyses (C3, C4, Immunoglobulin G (IgG) levels, blood cell count, leukocyte differential count, glomerular filtration rate (GFR), urine analysis, cryoglobulins and creatine kinase (CK). High-resolution computed radiography (HRCT) of the chest and pulmonary function test (PFT) are recommended for assessing pulmonary involvement at least at diagnosis in addition to when suspected. Other complementary assessment methods are sometimes indicated. Guidelines for the use and interpretation of ESSDAI scores have been developed [37, 39].

The ESSDAI has been developed as a tool for estimation and grading of systemic disease activity in pSS and has subsequently been validated as a tool of assessment of systemic disease activity in pSS in clinical trials as well as in clinical practice [30, 40, 41].

#### *Functional measurements of the salivary and lacrimal glands*

- Salivary flow is assessed by a 15-minute unstimulated whole saliva (UWS) collection, in which saliva production of  $\leq 1.5$  ml ( $\leq 0.1$  ml/min) is defined as pathologically low. The assessment is easily performed by letting the patient's saliva drip into a container during 15 minutes. The test is most commonly performed in either a dentist's office or by a nurse in a rheumatology department.
- Tear flow is assessed by Schirmer's test, in which a flow of  $\leq 5$  mm after 5 minutes is considered pathological. During the test a strip of filter paper is placed in the conjunctival sac of the lower eyelid and the wetting of the paper in mm is measured after 5 minutes.
- Conjunctival and corneal dryness and damage are assessed by an ocular staining test, in which the stain marks dry and damaged conjunctival and corneal areas in which the epithelial cells are damaged and stained areas can be evaluated. Different stains, e.g. lissamine green and Rose-Bengal staining, as well as various scoring systems, have been used. Scores of  $\geq 5$  points are defined as pathological when assessed using the Ocular Staining Score (OSS), and  $\geq 4$  points using the van Bijsterveld score in at least one eye. The ocular staining tests are usually performed by an ophthalmologist [1, 3, 26, 27].

#### *Assessment of autoimmunity in pSS*

Signs of autoimmunity in pSS include signs of focal sialadenitis in the minor salivary gland and/or the presence of anti-SSA/Ro autoantibodies in a blood sample.

A minor salivary gland biopsy from the lower lip showing focal lymphocytic sialadenitis, defined as  $\geq 1$  lymphocytic foci of  $\geq 50$  lymphocytes /  $4 \text{ mm}^2$ , is used when classifying pSS, although a histopathologic analysis also provides important prognostic information. Histopathologically, lymphocytic infiltration particularly around the salivary ducts is seen, in which the majority are T-cells. The presence of germinal centres has been shown to be predictive of subsequent lymphoma. A minor salivary gland biopsy from the lower lip is a simple procedure and in Malmö the biopsy is performed by experienced professionals at the Department of Oral Medicine and the histopathological assessment at the Department of Oral Pathology.

Analysis of anti-SSA/Ro autoantibodies is performed at a department of immunology and commonly used methods include Immunoblot and addressable laser bead immunoassay (ALBIA) [1, 3, 24, 26, 27].

### *Special considerations in pSS classification and diagnosis*

Sicca symptoms are common in the population and common causes include exocrine gland atrophy with age, functional side effects of several common medications with anti-cholinergic effects such as anti-depressants and anti-histamines, as well as other modes of action such as diuretics and beta blockers. Stress also impairs exocrine secretion [3, 42].

sSS is referred to when autoimmune sicca symptoms are associated with other CTDs, i.e. SLE, RA, SSc and myositis. Although both pSS and sSS patients experience sicca symptoms, sSS has not been associated with classical systemic pSS manifestations [3].

Apart from sSS, other diseases that mimic pSS must be kept in mind, e.g. IgG4-related disease, sarcoidosis, amyloidosis, active hepatitis C infection, acquired immunodeficiency syndrome (AIDS), graft-versus-host disease (GVHD) and previous head and neck radiation treatment, as described in the exclusion criteria for pSS in the ACR/EULAR classification criteria [3, 26].

## **Clinical management**

### *Assessment*

Patients with pSS with a mild phenotype of the disease (sicca symptoms, fatigue and pain) without systemic manifestations are usually followed-up during infrequent visits, every to every second year, at outpatient clinics in either departments of rheumatology or even in primary care units and in collaboration with dentists and ophthalmologists. It is recommended that patients with systemic manifestations, high disease activity with high ESSDAI scores ( $\geq 14$  points) and/or negative predictive immunological markers for systemic manifestations, e.g.

hypocomplementaemia, are monitored more closely (every 3-6 months) and investigated and treated in collaboration with specific organ specialists, where necessary [1, 30, 47].

The assessment of systemic disease activity using the ESSDAI is suggested to be a useful tool in pSS diagnosis, to aid identification of patients who require closer follow-up as well as for monitoring systemic activity and treatment response at follow-up (Table 3). Complementary assessment methods, in addition to clinical examination and blood samples at visits might be needed. An HRCT of the chest and PFT for evaluation of pulmonary manifestations and ultrasonography of the major salivary glands may, for example, be useful as prognostic tools at both pSS diagnosis and at follow-up. The ESSDAI total score ranges from 0–123 points, where 0 represents no pSS disease activity and total scores <5 points represent low disease activity, scores between 5–13 points represent moderate activity and scores  $\geq 14$  points represent high activity [1, 30, 33, 38, 39, 48, 49].

Besides the ESSDAI, the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), a validated tool for assessment of patient-reported sicca symptoms, pain and fatigue, could also be used (Figure 2) [40, 46, 50].

Usually, pSS patients are managed and followed-up in outpatient clinics. However, a recent study has shown higher hospitalisation rates in pSS patients compared to the general population [47, 51].

1) How severe has your dryness been during the last 2 weeks ?

No dryness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Maximal imaginable dryness
	0	1	2	3	4	5	6	7	8	9	10	

2) How severe has your fatigue been during the last 2 weeks ?

No fatigue	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Maximal imaginable fatigue
	0	1	2	3	4	5	6	7	8	9	10	

3) How severe has your pain (joint or muscular pains in your arms or legs) been during the last 2 weeks ?

No pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Maximal imaginable pain
	0	1	2	3	4	5	6	7	8	9	10	

**Figure 2.**  
The EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) [40, 46].



## *Treatment*

Generally, treatment of pSS must be individualised and conditioned according to the manifestations of the disease. No widely used guidelines for pSS treatment have yet been published, although EULAR treatment guidelines are under development. Thus far, treatment has often been empirically based, as there have been few studies on pSS therapies, in particular, randomized controlled trials (RCTs). Clinical management and treatment of specific systemic features has largely been performed in accordance with the treatment guidelines of other CTDs, such as SLE. A multidisciplinary approach is recommended [1, 47, 52-54].

Principles regarding the management of sicca symptoms have focused on patient education, oral health, environmental modifications, avoidance of xerogenic drugs, various topical treatments, tear substitutes, and in selected cases, cholinergic drugs, increasing exocrine secretion, as well as treatment for complications such as protection of caries and treatment of fungal mucositis [1, 47].

In milder xerostomia, the stimulation of salivary flow using sugar-free chewing gum and lozenges and topical fluoride for caries protection is recommended. In moderate to severe sicca symptoms, cholinergic drugs (muscarinic agonists), such as pilocarpine, may improve symptoms in some patients, although a large proportion of patients discontinue treatment because of the side effects.

For mild symptoms of dry eyes, artificial tears are the first-line treatment in combination with environmental modifications, whilst in more severe diseases, topical anti-inflammatory drugs, e.g. topical cyclosporine, may be used for shorter periods. In severe cases, tear plugs, blocking tear drainage, could be used [1, 47].

For mild systemic symptoms such as fatigue, exercise has been recommended. It is suggested that pain and arthralgia in pSS are treated with analgesics, such as non-steroidal anti-inflammatory drugs (NSAID), whilst in more pronounced arthralgia, hydroxychloroquine and low-dose corticosteroids may be used. Mild arthritis in pSS could be similarly treated, although other conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) used for the treatment of arthritis in other rheumatic diseases, e.g. methotrexate and leflunomide, may also occasionally be used [1, 47].

Systemic manifestations of pSS could require systemic treatment, including both corticosteroids and immunomodulatory therapies. As mentioned above, specific and severe organ manifestations are commonly treated in accordance with guidelines for other CTDs and commonly used csDMARDs include hydroxychloroquine, methotrexate, mycophenolate mofetil, azathioprine, cyclosporine and cyclophosphamide. Hydroxychloroquine is commonly used in pSS, particularly in patients with skin manifestations and articular symptoms [1, 47]. In selected cases, B-cell depletion therapy (Rituximab) could be considered for treatment of particular systemic manifestations, such as cryoglobulaemic

vasculitis, with reported effects in a substantial number of patients in a small study of patients with primarily systemic pSS manifestations [55]. Another B-cell targeted therapy is anti-BAFF (Belimumab), recently approved for treatment of SLE and with suggested partial efficacy in pSS [56]. For very severe and even life-threatening manifestations such as vasculitis and CNS manifestations, which are very rare in pSS, high-dose corticosteroids together with cyclophosphamide pulses have been proposed, in combination with intravenous immunoglobulins or plasma exchange, based on case reports, data from similar diseases (vasculitis and SLE) and expert opinion [47]. Some special concerns regarding the management of pulmonary manifestations of pSS are presented below, in the section on pulmonary manifestations.

Several studies of targeted therapies in pSS have been conducted in the recent years, although it should be kept in mind that the heterogeneity of the disease, with its different phenotypes, makes it difficult to study. Novel targeted therapies for the treatment of pSS systemic disease activity, that are under evaluation include CTLA4 ligand (Abatacept), anti-IL-6 (Tocilizumab), co-administration of Rituximab and Belimumab, anti-CD40 as well as anti-BAFF receptor [1, 47, 53, 57].

# Pulmonary manifestations of pSS

## **Introduction, respiratory symptoms and clinical relevance**

Pulmonary involvement in pSS has been described as one of the major systemic manifestations of pSS and respiratory symptoms are common in pSS [5, 18, 33, 58, 59]. Recent studies have highlighted its association with mortality, morbidity and impaired HRQoL, which emphasises the importance of addressing this systemic feature [6-8, 30]. The described pleomorphic nature of pulmonary involvement in pSS may cause difficulties in identifying and assessing this systemic manifestation and could therefore be underdiagnosed in pSS. In general, there is a need for early detection and better assessment of pulmonary involvement in pSS, as well as the development of effective therapies that target and even prevent pulmonary manifestations in pSS patients [48, 60, 61].

*Respiratory symptoms* are commonly described in pSS and include dry cough, dyspnoea, wheezing, hyperreactive airways, and more rarely, chest pain [5, 31, 34, 48, 60]. Dry cough in pSS, first described by Henrik Sjögren in his original report, is common and around 50% of patients are affected [4, 5, 48, 58, 59]. Sicca symptoms of the airways, with lymphocytic infiltration and dysfunction of the laryngeal, tracheal and bronchial exocrine glands, may lead to a decrease in mucociliary clearance and is believed to be associated with a persistent and irritating dry cough [3, 5].

The respiratory symptoms may sometimes be correlated to objective findings in the respiratory tract, such as pulmonary function reduction and radiographic abnormalities, though there is often no such correlation. In contrast, objective signs of pulmonary involvement in pSS without respiratory symptoms are seen in more than 50% of the patients, approximately [5, 31, 59, 62-64].

The reported prevalence of pulmonary involvement varies greatly, ranging from 9-75%, depending on different definitions of pulmonary manifestations, assessment methods, study designs and the use of different classification criteria for pSS [5]. Clinically significant pulmonary disease is estimated to affect approximately 10% of the pSS patients, although one study reported 22% [5, 6, 28, 34, 61]. Pulmonary involvement in pSS, assessed by activity in the ESSDAI respiratory domain in systematic reviews, has been reported to affect 12-16% of patients [18, 48].

### *A pleomorphic pulmonary disorder*

The pulmonary manifestations of pSS have been described as “pleomorphic” with a large variation in anatomical localization, engaging several structures in a widely heterogeneous manner, as well as in clinical presentation and severity. Pulmonary manifestations described in pSS include xerotrachea and bronchial sicca

manifestations, signs of bronchial and bronchiolar involvement, several signs and types of interstitial lung disease (ILD), pulmonary cysts, emphysema and lymphoproliferative lung disease [5, 31, 48, 58, 65-68]. Pulmonary amyloidosis associated with pSS has also been described, but as a rare manifestation [5, 69]. Compared to other CTDs, pleural effusion and pulmonary fibrosis, in terms of honeycombing, are rarely seen in pSS [18, 48, 70]. Pulmonary vascular components can be affected but pulmonary hypertension is uncommon in pSS [5, 18, 71]. The pulmonary manifestations in pSS often overlap, although a predominance of small airway involvement has been described [12, 13, 48, 58, 72-74]. An overlap of lymphocytic infiltration and stroma changes, affecting structures surrounding the mucosa of the airways and the pulmonary parenchyma could exist [5, 66, 72].

### *Assessment*

HRCTs and PFTs have been described as sensitive techniques for assessing pulmonary involvement in pSS and have also been established as validated methods in the assessment of disease activity in the ESSDAI respiratory domain (Table 3) [40, 48]. Bronchoalveolar lavage (BAL) and histopathological analyses of tissues obtained in transbronchial and surgical lung biopsies are other invasive methods, used complementarily in some cases, for the better assessment of the type of pulmonary involvement in pSS [5, 58, 64, 68, 72, 75, 76]. In order to establish a definite diagnosis of ILD or airway disease, invasive methods such as a surgical lung biopsy may be necessary. However, this is only performed in a few patients when indicated [65, 68, 72]. Special considerations are required, as difficulties could be experienced in the interpretation of different findings. For example, a radiographic sign resembling an interstitial pattern could also derive from bronchial thickening and therefore be a sign of airway involvement [58]. HRCT findings and PFT findings are sometimes in congruence but are also reported to be poorly correlated [7, 12, 64, 73].

### *Clinical relevance*

Pulmonary involvement may sometimes be the first manifestation of pSS, but could also be detected during pSS diagnosis or later on after long standing disease [62, 77-79]. In most pSS patients pulmonary involvement is mild, but can sometimes be severe and even life threatening [7, 9, 62, 65, 77]. Recent studies have demonstrated the highly negative effects of pulmonary involvement in pSS with significantly impaired HRQoL and even increased morbidity and mortality [6-8, 30, 62].

The increased mortality reported in pSS patients with pulmonary involvement, defined by HRCT abnormalities and a reduced pulmonary function, have been described in several studies [6, 7, 30]. Early pulmonary manifestations, as evaluated by baseline disease activity in the ESSDAI respiratory domain, have

been found to be associated with subsequent overall mortality in pSS [30]. A four-fold increase in mortality risk after 10 years of disease amongst pSS patients with pulmonary involvement compared to patients without pulmonary involvement was reported in a retrospective study from Norway. The same study also reported reduced 5- and 10-year survival rates in pSS patients older than 50 years of age with pulmonary involvement compared to patients without pulmonary involvement. In that study, the pulmonary involvement was defined as a presence of HRCT findings and/or abnormal PFT results, together with respiratory symptoms [6]. Similarly, another retrospective study demonstrated a shorter overall median survival in patients with reduced pulmonary function and a high degree of HRCT findings compared to patients without such signs [7].

A reduction of HRQoL has been shown in pSS patients in comparison with the general population and Belenguer et al. identified pulmonary involvement as the extraglandular manifestation that contributed the most to a poor HRQoL in pSS [8].

## **Pathology and risk factors**

Based on clinical features and histopathology, two major phenotypes of pulmonary involvement have been identified namely, airway and parenchymal involvement. Similarly, a division into two major immunopathologic patterns for both the pSS disease in general and pulmonary involvement in particular, namely, “epithelial” vs. “extra-epithelial” disease has been described. The expressions of glandular and extra-glandular disease in pSS are other terms that refer to the division of the phenotypes of the disease. The phenotype, risk factors and the prognosis of the two forms have been described as differing [5, 61]. The histopathological pattern in pSS pulmonary involvement includes both airway and interstitial involvement [48, 58, 68, 72]. Concomitant small airway and parenchymal involvement have been demonstrated in around one half of the cases of pSS, in which lung biopsies have been performed on selected patients with ILD, indicating that small airway involvement could be underestimated [72]. Follicular bronchiolitis is one of the most commonly described histopathological findings in pSS and is described as a hallmark of the disease [5]. Lymphocytic infiltration in and around the bronchiolar mucosa may extend and also affect the lung parenchyma – and vice versa [5, 58, 72]. The different histological presentations are described in more detail below (in the sections on pSS airway disease and pSS parenchymal disease below).

Assessment by BAL in pSS has only been performed in a few studies and has shown both lymphocytosis, and elevated neutrophils [5, 11, 68]. Induced sputum in one pSS cohort has also shown lymphocytosis [31].

The current understanding of the exact immunopathological mechanisms of pSS pulmonary involvement is suggested to be similar to the immunological process in the salivary glands, with epithelial cells playing a critical role in the initiation, maintenance and symptomatology of the disease, in addition to the roles of the lymphocytes [2, 61, 66, 75, 76]. Also, a lower degree of epithelial integrity and an increased number of both neutrophils and mast cells, in addition to the increased T cells in bronchial biopsies have been shown in a small cohort of pSS patients, compared to healthy controls. The structural findings, although not the inflammatory cells, were described as resembling atopic asthma [75]. Finally, the increased lymphocytic infiltration found in pSS is also believed to precede bronchial-associated lymphoid tissue (BALT) lymphoma, in some cases [5, 66].

#### *Factors associated with pSS pulmonary involvement*

Identified risk factors of pulmonary involvement in pSS are mainly associated with pulmonary parenchymal disease and very few associated factors of airway disease have been identified. Generally, pulmonary parenchymal manifestations, as in other extra-epithelial manifestations of pSS, have been associated with hypergammaglobulinaemia, low complement levels and higher frequencies of anti-SSA/Ro and anti-SSB/La autoantibodies, whilst the epithelial manifestations of the airways are less associated with laboratory signs of inflammation or serological features of the disease [5, 9, 11, 34, 65].

Seropositivity for anti-SSA/Ro, anti-SSB/La, RF as well as hypergammaglobulinaemia, lymphopenia and impaired FVC and/or FEV<sub>1</sub> were all suggested as predictive of pulmonary parenchymal involvement in one retrospective study [34]. In addition, male sex, smoking history and higher age were more common in patients with pulmonary involvement [34]. Increased age, elevated neutrophils and hypoalbuminaemia were described as associated factors with pulmonary involvement in pSS in another recent retrospective study [59]. Anti-SSB/La positivity has also been reported to be associated with radiographic cystic lesions in pSS [70]. Finally, older age and oesophageal involvement were both identified as predictive parameters for both ILD onset and deterioration and Raynaud phenomenon was described as a predictive factor in another retrospective cohort [62].

In one study, pSS patients with bronchiectasis were found to be older at diagnosis, have a lower frequency of anti-SSA/Ro antibodies and a higher rate of hiatus hernia in comparison to pSS patients without bronchiectasis. The pSS patients with bronchiectasis in that study also had a higher frequency of respiratory infections and pneumonia in comparison with those without [80].

## **pSS airway disease**

Airway involvement has been described as the most frequent pulmonary manifestation of pSS, with a reported frequency ranging from 9-75%, depending on differences in assessment methods, study designs and patient selection [5, 58, 74]. The trachea, bronchi and bronchioles may be affected, in which small airway disease has been described as the main pulmonary manifestation in pSS [5, 18, 58, 74]. The main symptoms associated with bronchiolitis in pSS are dry cough, recurrent bronchitis and dyspnoea [5, 58, 74]. pSS bronchiolar disease may be isolated or associated with ILD, such as non-specific interstitial pneumonitis (NSIP), lymphocytic interstitial pneumonia (LIP) and pulmonary lymphoma [5, 72]. Thus, both obstructive and restrictive pulmonary function findings have been found in pSS. Distal airway disease in pSS has been difficult to evaluate, since spirometry mainly reflects airway obstruction of more proximal airways [5, 81].

### *Histopathology*

The principal histopathological finding of the airways in pSS is a bronchitis and bronchiolitis, with a peribronchial and/or peribronchiolar infiltration of mainly cluster of differentiation 4 (CD4+) T-lymphocytes and a peribronchial and peribronchiolar fibrosis, leading to small airway obstruction [3, 58, 66, 76]. Follicular bronchiolitis is the most frequently reported histopathological lesion in pSS, in which hyperplastic lymphocytic follicles are seen in bronchiolar and peribronchiolar areas. Lymphocytic bronchiolitis (without follicles) can also be seen. Follicular bronchiolitis can occur alone or coexist alongside lymphocytic bronchitis/bronchiolitis or LIP in pSS, and can also be seen in other autoimmune diseases such as RA or in viral infections [3, 5, 66, 72]. Neutrophils and mast cells have also been shown in the bronchial mucosa of pSS patients [75]. Respiratory symptoms have not been associated with airway cell infiltration in pSS [76]. As previously mentioned, transbronchial biopsies, as opposed to surgical lung biopsies, may underestimate abnormalities in the small airways. Due to the more invasive nature of biopsies, these are rarely performed in the diagnostic work-up of airway involvement in pSS [72].

### *Radiographic features*

Airway abnormalities have been described as the predominant radiographic finding in non-smoking pSS patients, found in 54% of patients [73]. Bronchial wall thickening, bronchiectasis, centrilobular nodules and air trapping have been frequently reported HRCT findings in pSS [5, 48, 66, 73, 74]. However, certain radiographic findings may be difficult to interpret. For example, radiographic abnormalities in conventional chest x-rays, reminiscent of ILD, have been suggested to represent thickened bronchioles, based on HRCT findings [58]. Furthermore, mucus impaction may impair bronchial

obstruction, leading to atelectasis, proximal airway obliteration and bronchiectasis [5]. Finally, obliteration of the airway lumen may evolve and a “check-valve” phenomenon may result in the development of cystic lesions and bullae formation [66, 70].

### **pSS parenchymal disease**

ILD is found in approximately 5% of pSS patients and different types of ILD patterns have been described [3, 48, 72]. Dyspnoea and cough are typical symptoms of pSS-associated ILD, though subclinical involvement may be present [62, 65]. Inspiratory crackles were observed in around 70% of a small retrospective cohort of pSS patients with ILD [65]. HRCT together with PFTs are considered to be sensitive methods of detecting and evaluating pSS-associated ILD [39, 48]. The most commonly reported interstitial HRCT findings in pSS include ground glass attenuation, subpleural small nodules, interlobular septal thickening, reticular pattern, cysts/bullae, consolidation, non-septal linear opacities, emphysema and honeycombing in subpleural areas [5, 7, 48, 65, 66, 73, 82]. For histopathological classification, surgical lung biopsy is the preferred method and multiple lung tissue samples may be necessary (RC, Shi). Non-specific interstitial pneumonitis (NSIP) is the most frequently described pattern in pSS, followed by usual interstitial pneumonia (UIP), lymphocytic interstitial pneumonia (LIP) and cryptogenic organizing pneumonia (COP) [62, 65, 72].

About one half of the cases of ILD in pSS are detected after pSS diagnosis and in around one quarter of cases ILD precedes pSS diagnosis [62, 79]. In a retrospective study, an acute/subacute symptomatic ILD onset was found in around one half of pSS patients, a progressive onset in approximately one quarter, whilst the remaining quarter were asymptomatic patients [62]. During follow-up, stabilization was seen in 47% of patients, deterioration in 37%, whilst an improvement was seen in 16% of patients [62]. In a small cohort, a poorer prognosis was reported in pSS patients with UIP compared to the other ILD patterns in one study [65].

Parenchymal bullae and cysts are often reported in pSS patients, seen in 20-39% [66, 70, 82, 83]. pSS airway disease, in which peribronchiolar lymphocytic cell infiltrations and sometimes amyloid depositions are seen, are believed to cause “check-valve” mechanisms that lead to bullous and cystic formations. Destruction of the alveolar walls is also suggested to contribute to the cystic lesions [66]. In addition, pulmonary cysts in pSS have been shown to be associated with anti-SSB/La and pulmonary lymphoproliferative disease and could be a predictor of systemic involvement [70].



### *Non-specific interstitial pneumonitis (NSIP)*

NSIP has been reported as being the most common histological ILD pattern in patients with pSS, seen in 60% of patients with pSS and ILD and in 45% of pSS patients with available histopathology in a large systematic review of systemic manifestations of pSS, including pulmonary histopathological findings [48, 68]. NSIP may also be idiopathic or seen in other settings such as hypersensitivity pneumonitis, drug-induced lung disease, infection or immunodeficiency [3].

In NSIP, the typical HRCT findings are ground glass attenuation with reticulation, linear interstitial pattern, traction bronchiectasis and little or no honeycombing [72]. Typical histopathological findings include a uniform interstitial involvement of varying degrees of chronic inflammation or fibrosis [3, 66].

### *Usual interstitial pneumonia (UIP)*

UIP in pSS is quite uncommon in pSS and is mainly associated with sSS, seen in other CTDs, but has been described in 16% of pSS patients in a large systematic review of systemic manifestations of pSS, including pulmonary histopathological findings [3, 5, 48]. A tendency of progressive lung disease has been seen in pSS-associated UIP in a small study [65].

In UIP, typical HRCT findings are variable distribution of patchy interstitial fibrosis, inflammation, honeycombing and normal parenchyma. The typical histopathological findings include fibroblast foci, with small aggregates of fibroblasts and myofibroblasts being mandatory [3].

### *Lymphocytic interstitial pneumonia (LIP)*

LIP was shown in 15% of pSS patients in a large systematic review that included of pulmonary histopathological findings in pSS [48]. Both a benign course, with stabilization or improvement to the evolution of BALT lymphoma has been described. Although not the most prevalent ILD pattern in pSS, LIP is classically associated with CTDs including SLE and pSS [3, 5].

Typical HRCT findings include a bilateral symmetric diffuse micronodular pattern with a predominantly centrilobular distribution and histopathological findings include a diffuse polyclonal lymphoid cell infiltrate surrounding the airways and expanding into the lung interstitium [3, 5].

### *Cryptogenic organizing pneumonia (COP)*

COP has been infrequently described in pSS, but has been observed in association with several CTDs, of which RA is the most common. The clinical presentation has been described as being reminiscent of community-acquired pneumonia and diagnosis is often delayed.

Findings on HRCT include bilateral patchy alveolar opacities with air bronchograms and normal lung volumes. The histopathology classically shows intra-alveolar buds of connective tissue and concentric stenosis of bronchiolar lumen [3, 5].

### **Other pulmonary manifestation in pSS**

Lymphoproliferative pulmonary disorders have been reported in pSS, mainly mucosa-associated lymphoid tissue (MALT) and marginal zone B-cell lymphomas (MZBCL), though high-grade B-cell lymphomas have also been reported (<10%). 20% of the lymphomas in pSS affect the lungs and the lungs are also the second most frequent location of extra-nodal lymphomas in pSS. Symptoms are often mild and slowly progressing. HRCT often shows a rather unspecific diffuse interstitial process and multiple nodular or reticulonodular opacities and biopsy is required for the diagnosis [5].

Pulmonary amyloidosis has previously been described in pSS patients but is a rare finding [5, 66, 69]. The same applies to pleuritis, which suggests other CTDs including SLE and RA [5]. Pulmonary hypertension has been reported to be rare in pSS and suggests systemic sclerosis or SLE. However, a very small proportion of patients may develop pulmonary amyloidosis, pleuritis and pulmonary hypertension as part of pSS [5, 18, 71].

### **Pulmonary function in pSS**

Different and conflicting pulmonary function findings, both obstructive and restrictive, as well as mixed patterns, with both deterioration and improvement over time, have been previously described in pSS [9-14, 48, 74, 84]. The correlations between PFT results and HRCT findings have varied and have even been poor in some studies [7, 12, 73]. In addition, both respiratory symptoms and laboratory signs of inflammation in pSS have been shown to be poorly associated with PFT results [7, 12, 14, 58]. Nevertheless, signs of airway obstruction have been commonly described in pSS patients [9, 10, 12, 14, 58, 74]. Restrictive pulmonary function findings have also been commonly reported [11, 48, 73]. In addition to the different pulmonary function patterns reported in pSS patients, the interpretation of the PFT results and divisions into obstructive and restrictive patterns may sometimes be difficult, since some of the PFT variables could be affected in similar ways by the two different pulmonary function patterns [81].

A reduction in forced vital capacity (FVC), diffusing capacity of the lungs for carbon monoxide ( $D_{L,CO}$ ) and total lung capacity (TLC) have been commonly reported in pSS patients, and a reduction of forced expiratory volume in 1 second ( $FEV_1$ ) and  $FEV_1/VC$  ratio in some studies [6, 7, 9-12, 14, 48, 73, 74].  $D_{L,CO}$  has

been found to correlate negatively with HRCT scores, reflecting interstitial involvement, in a retrospective study that showed increased mortality in pSS patients with pulmonary involvement [7]. However, in another study,  $D_{L,CO}$  reduction was shown to correlate with signs of small airway obstruction in pSS patients [13].

Finally, reduced maximal expiratory flows at 75%, 50% and 25% of VC, as a sign of small airway obstruction, have been shown in some studies [13, 14, 58, 74].

### *Pulmonary function development*

Only a few previous longitudinal studies have assessed pulmonary function in pSS. The majority included pSS patients classified according to previously used criteria for pSS. There have also been differences in patient selection, assessment methods and study designs, making the results difficult to compare. Data on cigarette consumption also differ. The reported conclusions regarding development over time are therefore conflicting in the different studies [9-12, 14].

Signs of small airway obstruction at baseline, with an improvement over time, have been shown in a seven-year follow-up study [14]. In contrast, another study showed an increase in airway obstruction after a four-year follow-up, but after ten years, patients from the same cohort showed an improvement of  $D_{L,CO}$  and no further deterioration of pulmonary function [9, 10]. Subtle restrictive deterioration was seen over time in another ten-year follow-up study [11]. Signs of increased airway obstruction over time, with a concomitant restrictive deterioration, have been recently demonstrated [12, 13]. The majority of pSS patients with a  $D_{L,CO}$  reduction over time were found to have signs of small airway obstruction [13].

Two recent studies have described frequent chronic obstructive pulmonary disease (COPD) development over time among pSS patients [12, 84]. In a ten-year follow-up study, performed by our group, of pulmonary involvement in pSS, 37% of patients were diagnosed with COPD in comparison with 7% at baseline, even though the vast majority of patients had not been smoking during follow-up, indicating that the disease *per se* is involved in COPD development [12]. A Taiwanese retrospective population-based cohort study showed a 1.4-fold increased cumulative risk of COPD development in female adults with pSS after a median follow-up period of eight years compared to female adult controls without pSS (adjusted for age and comorbidity) [84].

### *Airway hyper responsiveness*

Airway hyper responsiveness (AHR), in which increased cough after exposure to airway irritants (e.g. smoke, sprays, cold air) and reactivity in methacholine tests is seen, is also common in pSS, observed in 60% of the patients in one study [85]. AHR in pSS could also be associated with both small airway obstruction and ILD and may progress over time, as presented in an eight-year follow-up study [13].

## Management and treatment of pSS pulmonary disease

Systematic screening for pSS pulmonary disease at diagnosis of pSS is recommended, in view of its frequent presentation, the poor correlation with respiratory symptoms and its recently reported association with increased morbidity, mortality and impaired HRQoL [6, 8, 30, 48, 60]. A combination of both HRCT and PFTs is recommended, since the use of only one modality may result in underdiagnosis of pulmonary involvement in pSS [86]. Disease activity in the ESSDAI pulmonary domain is evaluated by HRCT and pulmonary function tests in addition to patient-reported symptoms from the respiratory tract (Table 3) [39, 40]. Baseline activity in the ESSDAI pulmonary domain is an important prognostic factor in pSS and has been associated with an increased risk of overall mortality [30]. Collaboration with lung specialists, radiologists, pathologist and thoracic surgeons is recommended [1, 60, 78]. Bronchoscopy with BAL and histological sampling from transbronchial and surgical biopsies could be used as complementary diagnostic tools, when indicated [60, 86]. Correspondingly, when ILD is diagnosed by a lung specialist, screening for symptoms and manifestations of CTDs, including pSS, is recommended [60, 78]. An assessment of pulmonary vascular components could be considered, though pulmonary hypertension has only been rarely described in pSS thus far [5, 18]. Considerations of comorbidities and differential diagnoses should also include other CTDs, pulmonary infections, congestive heart failure, ischemic heart disease and pulmonary arterial embolism [39, 87].

No specific guidelines for either the management or treatment of bronchial and pulmonary involvement in pSS have not yet been published, although EULAR treatment guidelines for topical and systemic treatment of pSS were reported at the EULAR meeting in Madrid in 2019 [52, 60, 61]. As in other systemic pSS manifestations, clinical management has been based on and performed in accordance with treatment guidelines for other CTDs [1, 47]. No RCTs that specifically address pulmonary involvement in pSS have been performed to date [53, 61]. As pulmonary manifestations tend to show a pleomorphic pattern, individualised concerns, taking symptoms, radiographic findings and pulmonary function into account, must be taken. Merely selected patients may be suitable for treatment [1, 60]. In prominent and progressive disease e.g. ILD, systemic treatment is indicated, i.e. corticosteroids, csDMARDs (cyclosporine, azathioprine, mycophenolate mofetil and cyclophosphamide) [1, 5, 60-62, 72]. Biological therapies targeting B or T cells have been suggested in the systemic treatment of pSS, of which only off-label use of Rituximab has been more widespread in clinical practice thus far [47, 60]. However several ongoing studies are assessing other biological therapies in pSS, although, none of them are specifically assessing pSS-associated pulmonary disease [53]. With regard to bronchial involvement, this is usually treated with inhalation bronchodilators and/or corticosteroids, although data on their role in pSS associated airway-disease is currently missing [47, 60, 61].

# COPD

COPD is a widespread respiratory disease that is one of the leading causes of morbidity and mortality globally [88, 89]. The disease is mainly associated with cigarette smoking, though other mechanisms are seen in approximately 20-25% [90-92]. COPD is an irreversible and usually progressive obstructive airway disease (OAD), characterized by inflammation of both airways and lung parenchyma, in which thickening of the small airways and emphysema are common HRCT findings [90]. COPD is a heterogeneous disease process with different phenotypes with respect to differences in risk factors, etiology, pathogenesis and symptomatic profile [88, 89, 93, 94]. Risk factors include genetic factors, environmental factors, e.g. exposure to cigarette smoking, indoor air pollution and autoimmune processes [88, 89]. Increasing knowledge of the inflammatory mechanisms behind COPD has emerged through current research, and several CTDs have recently been shown to also be associated with COPD development [95].

## Clinical presentation

The most common respiratory symptoms in COPD include dyspnoea, cough and/or increased sputum production. Another feature and prognostic factor of the disease are periods of acute deterioration of respiratory symptoms, exacerbations, and the exacerbations are sometimes linked to respiratory infections [88].

COPD has a negative impact on physical and psychological health with impaired HRQoL. There is a significant increase in mortality and hospitalisation rates among patients with COPD and COPD is the fourth leading cause of death in the world. Comorbidities in COPD are common, above all cardiovascular comorbidities [88].

## COPD definition and assessment

COPD should be suspected in patients with dyspnoea, chronic cough or increased sputum production, a history of recurrent lower respiratory tract infections and/or a history of exposure to risk factors for the disease [88].

Various degrees of pulmonary function reduction with an obstructive pattern are seen in COPD. The diagnosis is spirometrically confirmed and defined as a post-bronchodilator  $FEV_1/FVC$  ratio  $< 0.70$ , according to the Global Initiative for Chronic Obstructive Lung Disease (GLOD) criteria for COPD [88].

Grading of COPD is recommended and is useful in prognostic assessments and in therapeutic considerations regarding the management of COPD patients. The classification of severity of airflow limitation is based on post-bronchodilator  $FEV_1$  values and includes four COPD grades: GOLD 1 (mild) with a  $FEV_1 \geq 80\%$

of predicted, GOLD 2 (moderate) with a  $FEV_1 \geq 50\%$  and  $< 80\%$  of predicted, GOLD 3 (severe), with a  $FEV_1 \geq 30\%$  and  $< 50\%$  of predicted and GOLD 4 (very severe) with a  $FEV_1 < 30\%$  of predicted. Besides PFT, other assessment methods are recommended for grading and monitoring patients with COPD and include the modified Medical Research Council (mMRC), assessing respiratory symptoms as well as the COPD assessment test (CAT), assessing the impact of daily life and assessing the frequency of COPD exacerbation. In addition, particular attention to the presence of comorbidities, e.g. cardiovascular diseases, is recommended [88].

## **Epidemiology and pathology**

The current COPD prevalence in the general population is estimated to be approximately 10% in subjects older than 45 years of age [89, 94]. Correspondingly, the Swedish prevalence of COPD is 7-11% among adults  $> 40$  years of age and increases by age [96]. The main risk factor for COPD development is tobacco smoking, although 20-25% of patients with COPD have never smoked [88, 91, 92, 97, 98]. COPD development is believed to be caused by a complex interplay between long-term exposure to noxious gases and particles, as well as intrinsic factors [88, 89]. Environmental factors such as air pollution and occupational exposure, genetic factors, aging, female sex and exposure to infections are other factors presented as being associated with an increased risk of COPD development [88, 89, 91, 92]. Recent research in COPD pathology has also suggested that autoimmune mechanisms with bronchial epithelial damage are involved in COPD development [94, 99].

Both bronchiolitis and emphysema are described as pathological hallmarks of COPD [90, 94]. Typical histopathological features include inflammation and fibrosis of the terminal and respiratory bronchioles, as well as reduction and destruction of the terminal bronchioles through the terminal alveoli. Goblet and squamous cell metaplasia has been shown. In addition, alterations of the vasculature with intimal thickening and smooth muscle proliferation can be observed [90].

Several local immunological disturbances in both the airways and the lung parenchyma have been shown in COPD and both the innate and the adaptive immune system are involved in the inflammatory process. Oxidative stress has been highlighted as playing a key role in driving the inflammation seen in COPD and could result in activation of the proinflammatory transcription factor nuclear factor- $\kappa\beta$  (NF- $\kappa\beta$ ) [94]. It is suspected that an imbalance between the inflammatory-induced proteolytic enzymes and the anti-proteolytic activity, as well as an imbalance between apoptosis and cell proliferation in the lung, exists [90]. Dendritic cell activation resulting in activation of T cells targeted at the lung endothelium and epithelium have also been proposed to be involved in the process. Multiple proinflammatory mediators are involved in the inflammatory process in COPD,

including lipid mediators, free radicals, several chemokines and cytokines, e.g. IL-1 $\beta$ , IL-8, and TNF- $\alpha$ , and growth factors. [90, 94]. A neutrophilic inflammation has been predominantly shown in COPD, although an increased number of macrophages, T and B lymphocytes may also be seen in the airway lumen. In addition, increased numbers of eosinophils may be observed in some patients [94].

The increased airflow resistance seen in COPD is believed to be caused by a combination of small airway disease, parenchymal destruction (emphysema) and increased airway responsiveness in many cases [89, 90].

## **Associations between COPD and CTDs**

COPD has recently been reported to be more common in patients with CTDs: RA, ankylosing spondylitis (AS), psoriasis arthritis (PsA), SLE, hypocomplementaemic urticarial vasculitis syndrome (HUVS) and SSc, in addition to pSS, as previously mentioned in the section on pulmonary manifestations of pSS [12, 84, 95, 100]. Furthermore, patients with inflammatory arthritis and COPD showed increased mortality, hospitalisation rates and emergency visits, though the associations were mainly studied in RA patients [95].

## **Management**

In patients with COPD associated with tobacco smoke exposure, smoking cessation is the only treatment that has unequivocally been shown to reduce the rate of FEV<sub>1</sub> decline [101].

Several other treatments that effect the rate of decline in FEV<sub>1</sub> exist. The treatment goals are described as reducing symptoms and reducing risks of the disease. It is recommended that treatment of COPD is individualised and based on the COPD grade. The pharmacological treatment includes inhalation treatment with bronchodilators [long-acting beta-2 agonists (LABA) and muscarinic antagonists (LAMA), sometimes supplemented with short-acting bronchodilators] and inhaled corticosteroids (ICS), often in combination. Systemic treatment with corticosteroids and antibiotics might be necessary in the treatment of exacerbations. In severe COPD, systemic treatment with phosphodiesterase-4 inhibitors might be of use. Hypoxic patients may need oxygen therapy. In addition to pharmacological COPD treatment, physiological interventions, rehabilitation and patient education are other important aspects of the management of patients with COPD. COPD is clinically managed at primary care centres or at departments of respiratory medicine for more severe cases. For all patients, a multi-professional care regimen is recommended [88].

International and Swedish national guidelines for COPD assessment and treatment has been developed [88, 102].

# Aims

The main objective of this thesis was to investigate pulmonary involvement in primary Sjögren's syndrome.

The specific aims of the thesis were:

- I. To assess the prevalence of COPD in consecutive patients with pSS and to study its relationship to cigarette smoking, radiographic features, respiratory symptoms, pSS disease activity, laboratory inflammatory and serological features of pSS (Paper I).
- II. To assess signs of OAD in patients with pSS without prior physician-diagnosed OAD using the forced oscillation technique (FOT) (Paper II).
- III. To study cytokine and leukocyte levels in induced sputum from never-smoking patients with pSS compared to never-smoking healthy controls (Paper III).
- IV. To assess changes in lung volumes, lung function and COPD development over time in consecutive patients with pSS, as well as the associations between pulmonary function, radiographic findings, respiratory symptoms and clinical features of pSS, taking cigarette consumption into account (Paper IV).





# Materials and methods

## Study designs

Paper I: Cross-sectional cohort study

Paper II: Cohort study, methodological pilot study

Paper III: Small cohort study, methodological pilot study

Paper V: Cross-sectional follow-up study

## Setting

Patients with pSS from the city of Malmö and surroundings are regularly monitored at the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö. In 2018, the population of the county of Skåne (Scania) in Southern Sweden was 1.4 million [103]. The county of Skåne has five rheumatology outpatient clinics, namely, the Department of Rheumatology at Skåne University Hospital in Lund and Malmö and four rheumatology outpatient clinics at secondary care hospitals in Helsingborg, Kristianstad, Simrishamn and Trelleborg. The municipalities of Malmö and Lund also have two smaller private care rheumatology outpatient clinics, which mainly care for patients with different arthritides. Malmö is the largest city in the region with a population of 339,313 in the municipality (2018) and Lund is the second largest city with a population of 122,948 in the municipality (2018) [104]. Since 2010 the University Hospitals of Lund and Malmö have been merged into one unit – the Skåne University Hospital. Outpatient clinics as part of the Department of Rheumatology at Skåne University Hospital are located in both Lund and Malmö. Since 1984, when the Malmö Sjögren's Syndrome Registry (MSSR) was founded as a research registry by Associate Professor Rolf Manthorpe, the vast majority of patients diagnosed with pSS in the catchment area of Skåne University Hospital in both Lund and Malmö have been monitored at the Department of Rheumatology at Skåne University Hospital in Malmö. Consecutive pSS patients have continuously been included in the MSSR. A small proportion of pSS patients living in other parts of the county of Skåne are also followed at the Department of Rheumatology at Skåne

University Hospital in Malmö and are included in the MSSR. By the end of 2018, MSSR involved a total of 489 patients, of which 361 were still alive. pSS patients in the MSSR are classified according to both the AECG 2002 and ACR/EULAR 2016 criteria for pSS. Initially, pSS patients enrolled in the registry were classified according to the previously used Copenhagen and European criteria for pSS and patients now also fulfil the more recent criteria above [26, 27, 105, 106]. Data registered in the MSSR include disease specific diagnostic and clinical data for pSS, as well as data regarding certain comorbidities. pSS patients included in the registry are believed to be representative of the pSS population in general when compared to studies [18]. Based on data from the MSSR available from the end of 2018, the mean disease activity at diagnosis as evaluated by the ESSDAI was 4.7 points and the mean ESSPRI score at diagnosis was 5.7 points. 13% of pSS patients showed activity in the ESSDAI respiratory domain, 8% with mild and 5% with moderate activity, respectively.

## Participants

### Patients

All pSS patients in the four studies (studies I-IV) fulfilled both the AECG and the ACR/EULAR classification criteria for pSS [26, 27].

#### *Study I*

51 consecutive pSS patients (mean age 60 years, range 29–82 years, 49 females), seen as outpatients from May to December 2012 at the Department of Rheumatology at Skåne University Hospital in Malmö were included in this cross-sectional study. Of the 56 patients who were invited, five declined to participate. The prevalence of previously diagnosed OAD, i.e. COPD or asthma, was not known. 10% of the patients had previously been diagnosed with ILD, based on radiographic signs of ILD. 27 of the pSS patients were never-smokers, 20 were former smokers and four were current smokers. Nine patients were treated with inhalation of combined LABA and ICS, two patients with ICS only, two with Short-Acting beta-2 Agonists (SABA) and one patient with LAMA, all of whom had to refrain from the use of these 24 hours prior to the PFT examination. 16 of the pSS patients were treated with low-dose corticosteroids (2.5–10 mg) and 21 with DMARDs (16 with hydroxychloroquine, one with azathioprine, one with cyclosporine and three had previously been treated with Rituximab).

### *Study II*

37 pSS patients (median age 64, range 38–77 years, 37 females) from the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö were included in this cohort study. The patients were invited and assessed when participating in a previous follow-up study of pulmonary function in pSS in 2010 [12]. Exclusion criteria were prior physician-diagnosed OAD, i.e. COPD or asthma. Four of the 41 participants in the previous follow-up study reported that they had previously been diagnosed with OAD by a physician and were excluded. 14% had been previously diagnosed with ILD at some time, based on clinical respiratory symptoms together with radiographic signs of ILD. 24 of the pSS patients were never-smokers, ten were former smokers and three were current smokers. Three patients were treated with inhalation of SABA. Five of the pSS patients were treated with low-dose corticosteroids (2.5–10 mg) and ten with csDMARDs (six with hydroxychloroquine, three with methotrexate and one with cyclosporine).

### *Study III*

In this small cohort study, 20 never-smoking patients with pSS (mean age 66 years, range 33–84 years, 19 females) seen at the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö during 2014 were included. The participants were recruited among pSS patients who had participated in either of the two previous studies that evaluated pulmonary manifestations of pSS (17 from study I, the cross-sectional study, and three from the previous follow-up study by our group [12]). The exclusion criteria were ever-smoking and current use (last month) of inhalation steroids. Seven of the pSS participants were previously diagnosed with COPD according to the GOLD criteria for COPD. Two participants had previously been diagnosed with ILD at some time, based on clinical respiratory symptoms together with radiographic signs of ILD, none of whom had more severe fibrosis or honeycombing. Six of the pSS patients were treated with low-dose oral corticosteroids (2.5–10 mg) and seven with csDMARDs (six with hydroxychloroquine and one with methotrexate).

### *Study IV*

40 pSS patients (mean age 66 years, range 42–81 years, 39 females) were included in this six-year follow-up study. The patients were recruited from the previous cross-sectional study (study I) on pulmonary involvement among consecutive pSS patients at the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö from 2012–2013. In 2018, all 51 pSS patients were alive and were invited to the follow-up study, eleven of whom declined to participate. The mean follow-up time was six years (range 5–6 years) and the mean disease duration was 18 years (range 5–34 years). 20 of the pSS patients were never-smokers, 17 were former smokers and three were current smokers.

Nine patients were treated with a combination of LABA and ICS, one with LABA only, seven with SABA and two with LAMA, all of whom had to refrain from the use of these 24 hours prior to the PFTs. Nine of the pSS patients were treated with low-dose corticosteroids (2.5–10 mg) and eleven with csDMARDs (ten with hydroxychloroquine and one with cyclosporine).

## Controls

### *Studies I, II and IV*

The PFT controls comprised 186 population-based female subjects, 100 of whom were never-smokers and 86 current smokers (mean age 45, range 20–70 years) and 270 population-based male subjects, 124 of whom were never-smokers and 146 current smokers (mean age 45, range 20–70 years), all attending a general health survey in Uppsala [107, 108]. Based on the PFT results of the controls, expected PFT values could be calculated using a linear regression model into which age, height, weight and cigarette smoking were entered as covariates for females and males separately.

### *Study II*

The FOT controls were recruited from a population-based survey including 213 female subjects from Malmö, who denied having been previously diagnosed with OAD by a physician [109, 110]. From this group, two controls, matched for gender, age, height, weight and tobacco consumption were selected for each pSS patient. 74 controls (median age 64, range 47–77 years, 74 females) were therefore included.

### *Study III*

The 19 never-smoking sputum induction controls (mean age 70 years, range years, 46–84, 18 females) were randomly selected from the Swedish population registry and all lived in the city of Malmö and its surroundings. The controls were matched for gender and date of birth of the 20 pSS participants. Out of a total of 134 who were invited, 19 were considered appropriate with regard to the exclusion criteria and agreed to participate. The exclusion criteria were: ever-smoking, prior diagnosis of rheumatic disease, asthma or COPD and the use of systemic corticosteroids, as well as inhalation treatment with bronchodilators or ICS in the last 6 months.

# Methods

## Pulmonary function tests

The pSS patients in all studies (studies I–IV) were assessed by pulmonary function tests (PFTs), which are commonly used when assessing airway and lung physiology and allow estimation of both obstructive and restrictive pulmonary function patterns. The PFTs, including calibration, were performed according to current standards (MasterScreen PFT System, Intramedic, Sollentuna, Sweden) at the Department of Clinical Physiology at Skåne University Hospital in Malmö. The PFTs included static and dynamic spirometry, from which vital capacity (VC), total lung capacity (TLC), residual volume (RV), forced expiratory volume in 1 second (FEV<sub>1</sub>) and FEV<sub>1</sub>/VC ratio and RV/TLC ratio could be calculated. The diffusing capacity of the lungs for carbon monoxide (D<sub>L,CO</sub>) was measured using the single-breath technique. FEV<sub>1</sub> and VC were measured before and after inhalation of 1.0 mg terbutaline and from which FEV<sub>1</sub> reversibility could be calculated. Clinically significant reversibility was defined as an increase of 12% and 200 ml in FEV<sub>1</sub>. TLC and RV were measured by body plethysmography [81, 111-114].

In *obstructive pulmonary function abnormalities* a decreased FEV<sub>1</sub>/VC ratio (<5th percentile of predicted) is seen.

In *restrictive pulmonary function abnormalities* a reduced TLC (<5th percentile of predicted) is seen.

In *mixed pulmonary function abnormalities*, in which obstruction and restriction coexist, both a reduced FEV<sub>1</sub>/VC ratio and TLC is seen (<5th percentile of predicted) [81].

The spirometric definition of COPD is a post-bronchodilator FEV<sub>1</sub>/FVC ratio < 0.70, according to the GOLD criteria for COPD [81, 88].

## The forced oscillation technique

The forced oscillation technique (FOT) is a validated non-invasive method that is proposed to be sensitive in assessing airway obstruction, detecting even smaller changes in pulmonary function, compared to spirometry, and the method was used in patients and controls in study II. The technique can also be useful in distinguishing the anatomical localization of airway obstruction. The FOT is based on an assessment of physiological responses in the airways, during the application of pressure oscillations generated by a loudspeaker. The oscillations are composed of multiple frequencies and are superimposed in the airways for 30 seconds of normal breathing. FOT assessments were conducted at the Department of Clinical

Physiology at Skåne University Hospital in Malmö and the Jaeger Impulse Oscillometry System (IOS), (MasterScreen IOS Viasys GmbH, Hoechenberg, Germany) was used. During the test, the subjects sat upright, had a nose clip and firmly supported their cheeks with their hands. FOT measurements were made after inhalation of 1.0 mg terbutaline, since the FOT measurements were all conducted immediately after the spirometry and the reversibility test [109, 115-117].

The FOT allows assessment of resistance (R) and reactance (X) at multiple oscillation frequencies, ranging from 5 to 35 Hz, as well as the resonant frequency ( $F_{res}$ ).

*Resistance* comprises the pressure-flow relationship of the portion of the pressure oscillation that is in phase with airflow, whilst reactance is related to those portions of the pressure oscillation that are not in phase with airflow.

*Reactance* reflects the combined effect of elastic/capacitive and inertial properties in the pressure-flow relationship.

*Resonant frequency* is the point at which the opposing elastic and inertial forces are equal.

In OAD, measurements by FOT show increased resistance and  $F_{res}$  and a decreased reactance. Furthermore, FOT can also be useful in distinguishing the anatomical localization of obstruction since resistance at low frequencies is considered to reflect total airway resistance, whilst resistance at higher frequencies is considered to reflect central airway resistance [115, 116].

## **The sputum induction technique**

The sputum induction technique, performed on pSS patients and controls in study III, allows non-invasive sampling of the airways, providing the opportunity to study cells and markers of inflammation [118, 119]. The method is proposed to be useful in both early diagnosis of airway diseases and as a method of monitoring treatment [120-123]. The method has been used in COPD and asthma but has only been reported as being used in one study that assessed patients with pSS [31, 122, 123]. Induced sputum is achieved through inhalation of isotonic or hypertonic solutions, administered by nebulization, and induces a small amount of airway secretion that can be expectorated and analysed [118].

The airway surface liquid, often referred to as mucus, is a thin layer of fluid covering the luminal surface of the airways. The components of airway mucus include mucins, which mainly contribute to the viscosity of the mucus, proteoglycans, neutral lipids, anti-proteases, anti-oxidants, anti-microbial proteins, secretory IgA, cytokines, as well as various cells [124]. Induced sputum leukocyte

differential cell count is the most validated marker, in which eosinophilia has been shown to correlate to exacerbations and predict therapeutic response to corticosteroids, especially in asthma, but also in COPD [120, 121, 123]. In COPD, various inflammatory cells of both the innate (neutrophils, macrophages, eosinophil, mast cells and dendritic cells) and the adaptive (T and B lymphocytes) immune system have been shown in induced sputum, in which neutrophil and, to some extent, also eosinophil counts are increased [94, 121, 123]. Induced sputum leukocyte differential cell count has previously been evaluated in pSS patients in a study in which lymphocytosis was reported [31].

Inflammatory cytokines, previously described as being involved in COPD pathogenesis, include IL-1 $\beta$ , IL-8, and TNF- $\alpha$ , which have been described as being elevated in induced sputum in COPD patients. BAFF has been shown to be increased in lymphoid follicles in the lungs of patients with COPD [94]. Correspondingly, inflammatory cytokines previously described as being involved in pSS pathogenesis include TNF- $\alpha$ , BAFF, type I IFNs, IL-1 $\beta$  and IL-6 [2]. Based on this, the selection of induced sputum cytokine analyses in study III was made.

#### *Sputum induction performance*

Sputum induction was performed at the department of Clinical Physiology at Skåne University Hospital in Malmö. Sputum was induced through inhalation of nebulised 3% NaCl 3x5 min. Lung function was measured 1 min. after each induction time point and induction was interrupted if lung function (FEV<sub>1</sub>) decreased  $\geq 20\%$ . Subjects were asked to rinse their mouths, blow their noses and try to expectorate sputum between each dose of nebulised saline. Sputum induction continued until an adequate sample volume was obtained. Sputum samples were kept refrigerated and were processed within two hours. Sputum induction was carried out in accordance with the standardized technique described by Paggiaro et al. [118, 119, 125, 126].

#### *Sputum processing*

Sputum quality was evaluated on a scale of 1–6 (1=plugs easy to collect, 6=just saliva). Sputum plugs were sorted and treated with four volumes of 0.65 mM dithiothreitol (DTT) in phosphate-buffered saline (PBS) for 30 mins at 4°C. An additional four volumes of PBS were added, followed by filtration through a 60  $\mu$ m filter and a final centrifugation (200 g for 5 mins). The supernatant was frozen at -80 °C until subsequent analysis and the cells were subjected to Cytospin (30,000 cells/glass). Cells were stained with May-Grunewald/Giemsa and differential counts were performed.



### *Sputum analysis*

Sputum supernatants were analysed for BAFF, IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$  using the respective human DuoSet ELISAs (BAFF: DY124, IL-1 $\beta$ : DY201, IL-6: DY206, IL-8: DY208 and TNF- $\alpha$ : DY210) and IFN- $\alpha$  using the VeriKine human ELISA Human Interferon Alpha Multi-Subtype Serum ELISA Kit (all from R&D systems, Abingdon, UK). Samples were run in duplicate within the same ELISA plate with a maximum in-between variation of less than 5%. Cytokine levels  $\geq 2$ SD above the mean levels in the controls were considered abnormal. The detection level limit for the various cytokines were as follows: BAFF: 0.2 pg/ml, IL-1 $\beta$ : 1 pg/ml, IL-6: 0.25 pg/ml, IL-8: 0.5 pg/ml, TNF- $\alpha$ : 0.5 pg/ml, and IFN- $\alpha$ : 2.5 pg/ml. Subjects with cytokine levels below the detection level limit were considered to have 0 pg/mL.

### **Computed tomography of the chest**

In study I, radiographic features of the lungs were evaluated using conventional computed tomography (CT) of the chest and in the follow-up study, study IV, by HRCT at the Department of Radiology at Skåne University Hospital in Malmö. Signs of interstitial lung disease using HRCT had been rarely detected in a previous follow-up study at our centre that had evaluated pulmonary involvement in pSS patients, although signs of bronchial involvement and obstructive signs were frequently seen [12]. Signs of air trapping and other signs of obstructive pulmonary disease could be evaluated by conventional CT scans and, for radiation hygiene reasons, conventional CT, including both inspiratory and expiratory images, was chosen in study I. The CT images in study I were assessed by a chest radiologist who was blinded with regard to the clinical characteristics and PFT results of the patients. The presence of a reticular pattern, ground glass attenuation, honeycombing, central as well as traction bronchiectasis, emphysema, pulmonary cysts and air trapping was evaluated.

HRCT was performed in 39 of the pSS patients in study IV (one patient did not consent). The images were interpreted visually by a radiologist (HLA). The findings were defined by the Fleischner Society guidelines for imaging studies [127]. The presence of emphysema, cysts, nodules, signs of bronchial involvement (defined as central bronchiectasis or bronchial wall thickening) and ILD signs (defined as ground glass attenuation, a reticular pattern, traction bronchiectasis or honeycombing) was registered.

## Laboratory analyses

In all studies (studies I–III), blood samples were collected at the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö. Analyses included laboratory tests both as part of ESSDAI evaluation and clinical follow-up, in particular: ESR, C-reactive protein (CRP), blood cell counts, including leukocyte differential counts, GFR, IgG, C3, and C4, re-assessment of serologies performed in the diagnostic procedures of the pSS disease and brain natriuretic peptide (NT-ProBNP), in order to rule out concomitant congestive heart failure. The analysis methods used for the immunological tests were: EuroblotOne for anti-SSA/Ro and anti-SSB/La antibodies, indirect immunofluorescence using Hep 2010 cells as substrate for ANA, Phadia Immucap 250 for RF, nephelometry using an Immage 800 (Beckman Coulter Inc., Brea, CA, USA) for IgG, C3 and C4.

## The EULAR Sjögren's Syndrome Disease Activity Index

pSS disease activity was assessed by one of two physicians (Thomas Mandl or Anna Nilsson) using the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) in all patients (studies I–IV) during their visit to the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö.

The ESSDAI is a validated instrument that assesses current systemic activity in pSS, divided into 12 organ-specific domains, based on well-defined anamnestic and clinical data. The domains represent different organ systems and the activity in each is divided into 3–4 levels (level 0: no activity, 1: low activity, 2: moderate activity, 3: high activity). The scores of each domain are also weighted according to relative importance and the added domain score comprises the ESSDAI total score. The total score ranges from 0–123 points, where 0 represents no pSS disease activity, total scores <5 points represent low disease activity, scores between 5–13 points represent moderate activity and scores  $\geq 14$  points represent high activity (Table 3) [38–40]. The ESSDAI is further presented in the introduction.

## Questionnaires

All the questionnaires were written in Swedish and the participants completed their forms during the visit to the outpatient clinic at the Department of Rheumatology at Skåne University Hospital in Malmö.

### *The EULAR Sjögren's Syndrome Patient Reported Index*

Patient-reported pSS-associated symptoms, namely, sicca symptoms and pain and fatigue, were all evaluated using the EULAR Sjögren's Syndrome Patient

Reported Index (ESSPRI), which is a validated instrument for assessing pSS-associated symptoms in all studies (studies I–IV). Patients grade the extent of their symptoms over the last two weeks on a Likert scale for each of the three symptoms described above. The scores in each item range from 0–10 points, where 0 represents no pSS-associated symptoms. The mean of the symptom scores comprises the ESSPRI total score [40, 46].

#### *The Swedish version of the St George's Respiratory Questionnaire*

The validated Swedish version of the St George's Respiratory Questionnaire (SGRQ) was conducted for the evaluation of respiratory and COPD-associated symptoms and their impact in all studies (studies I–IV). The SGRQ is a 76-item questionnaire that has been developed for assessing health-related quality of life in patients with obstructive pulmonary disease. The SGRQ items are divided into three domains: respiratory symptoms – assessing the degree of problems caused by specific respiratory symptoms; activity – assessing the restriction of activity caused by dyspnoea and impact – assessing the impact of the disease on daily life. The score of each item is weighted and the added score comprises the SGRQ total score, which ranges from 0 to 100% of potential distress. The total scores for each domain above could also be calculated [128–130].

#### *The COPD Assessment Test*

The COPD Assessment Test (CAT) is a validated short and simple questionnaire for assessing and monitoring COPD in clinical practice. The CAT was conducted on the pSS patients in study IV. The test comprises eight items covering cough, phlegm, chest tightness, breathlessness, activity limitation at home, confidence leaving home, sleep and energy. Patients grade their symptoms of each item on a Likert scale, ranging from 0–5 points. The added score of the eight items comprises the CAT total score and ranges from 0 to 40 points. Lower scores indicate better health, CAT scores of < 10 points indicate less symptomatic COPD while CAT scores of  $\geq 10$  points indicate more symptomatic COPD [131, 132].

#### *The modified Medical Research Council dyspnoea scale*

The modified Medical Research Council (mMRC) dyspnoea scale evaluates dyspnoea-associated disability and is used in the clinical assessment of patients with COPD. The mMRC was used in all studies (studies I–IV). Through the mMRC, patients estimate their disability caused by dyspnoea in 0–4 grades, where 0 represents breathlessness only during strenuous exercise and 4 the most dyspnoea-associated disability [88, 133].

### *Structured questionnaire evaluating cigarette consumption*

In all studies (studies I–IV), cigarette consumption was evaluated using a structured questionnaire, assessing smoking status, start/stop year of smoking and mean cigarette consumption for current and former smokers, which enabled a pack-year assessment. A pack year is defined as twenty cigarettes smoked every day for one year (Figure 3).

Smoking habits			
A. Have you ever been smoking on a regular basis		<input type="checkbox"/> YES	<input type="checkbox"/> NO
If YES	<input type="checkbox"/> cigarettes?	If YES, when did you start?	Age: .....years
	<input type="checkbox"/> pipe?		
	<input type="checkbox"/> cigars?		
B. Have you quit smoking? <input type="checkbox"/> YES <input type="checkbox"/> NO			
If you have quit smoking, when did you quit?			
Age: .....years			
C. Your current smoking habits ..... cigarettes/day			
.....		gr pipe tobacco/week (1 package = 50 g)	
.....		cigars/week	
D. During the whole period you have been smoking: how much have you been smoking on average?			
..... cigarettes/day			
..... gr pipe tobacco/week (1 package = 50 g)			
..... cigars/week			

**Figure 3.**

Structured questionnaire evaluating cigarette consumption (translated into English).

## Statistics

**Study I.** The normally distributed data was and presented as mean  $\pm$  SD. A multiple linear regression model was used for calculating the predicted PFT values. The paired samples Student's t-test was used when comparing measured and expected PFT results and the Student's t-test was used when comparing normally distributed continuous variables between groups. Differences in categorical data were analysed using the Chi-square test or Fisher's exact test. Pearson's correlation coefficient was calculated for correlations.

**Study II.** Due to the small sample size and non-normally distributed variables, data were presented as median (interquartile range). The Mann-Whitney U test and Wilcoxon's matched pairs test were used for comparisons. The Spearman rank correlation test was used for correlations.

**Study III.** Due to the small size of the cohort and non-normally distributed variables, data were presented as median (interquartile range). The Mann-Whitney U test was used when comparing continuous variables between different pSS groups, as well as between pSS patients and controls. The Spearman's correlation coefficient was calculated for correlations.

**Study IV.** The normally distributed data were presented as mean  $\pm$  SD and the non-normally distributed data as median (interquartile range). The paired samples Student's t-test was used when comparing measured and predicted PFT results, as well as when comparing previous results with the actual PFT results for normally distributed variables and the Wilcoxon Signed-Rank Test for non-normally distributed variables. The Student's t-test was used for comparison of normally distributed continuous variables between independent groups and the Mann Whitney U test for non-normally distributed continuous variables. The Chi-square test and Fisher's exact test were used for comparisons of categorical data. The McNemar's test was used when comparing pairwise frequencies of categorical data. Pearson's correlation coefficient was calculated for correlations of normally distributed continuous variables whilst Spearman's correlation coefficient was calculated for non-normally distributed variables.

Data were analysed for normality by the Shapiro-Wilks test and visual inspection of histograms and Q-Q-plots.

P-values  $<0.05$  were considered statistically significant.

No formal power calculation was performed due to the pilot study nature of these studies.

## Ethics

The studies were all approved by the Regional Ethical Review Board for Southern Sweden (study I: LU 2012/98), (study II: LU 2009/8), (study III: LU 2013/6), (study IV: LU 2018/26). Studies I and IV were also approved by the Radiation Protection Committee at Skåne University Hospital. All patients gave their written informed consent in accordance with the Declaration of Helsinki.

# Results and discussion

## Paper I

### **Chronic obstructive pulmonary disease is common in never-smoking patients with primary Sjögren's syndrome**

In this study, pulmonary function, radiographic pulmonary findings, use of CT, pSS disease activity, pSS-associated symptoms, pSS, respiratory symptoms and cigarette consumption as well as laboratory inflammatory and serological pSS features were assessed in 51 consecutive pSS patients. PFT results were compared to previously studied population-based controls, taking gender, age, height, weight and cigarette consumption into account.

### **Results**

#### *Pulmonary function reduction*

pSS patients mainly showed an obstructive pulmonary function reduction compared to the predicted values and a high proportion of COPD among pSS patient was shown, even among never-smoking pSS patients

The PFTs revealed decreased VC, FEV<sub>1</sub>, FEV<sub>1</sub>/VC ratio and D<sub>L,CO</sub> as well as an increased RV and RV/TLC ratio in pSS patients in comparison with the predicted values (Table 4a). Similar PFT abnormalities were demonstrated in the never-smoking pSS patients (Table 4b).

According to the GOLD criteria for COPD, COPD was shown in 41% of pSS patients, but also in nearly one third (30%) of never-smoking pSS patients (Figure 4). In ever-smoking pSS patients, COPD was seen in 54% (Figure 4). According to the GOLD grading of COPD, the majority of pSS patients with COPD showed a mild degree of COPD disease (61%), whilst the remaining 39% had a moderate degree of COPD (Table 4a). pSS disease duration was similar in patients with or without COPD (17 years vs. 17 years,  $p = 0.939$ ). However, none of the four never-smoking patients with a short pSS disease duration, defined as <5 years from diagnosis, had COPD.

**Table 4a.**

Results of the PFTs in 51 patients with pSS compared to predicted values. Clinically significant reversibility was defined as FEV<sub>1</sub> improvement >12% and > 200 mL.

PFT results	pSS patients, n=51		Predicted values	p-values pSS vs predicted
	Absolute numbers	Percent of predicted	Absolute numbers	
VC, l	3.3 ± 0.8	93 ± 14	3.6 ± 0.9	0.001
TLC, l	5.6 ± 1.0	100 ± 13	5.6 ± 0.7	0.750
RV, l	2.3 ± 0.5	114 ± 23	2.0 ± 0.4	0.000
RV/TLC ratio	0.41 ± 0.1	115 ± 18	0.36 ± 0.1	0.000
FEV <sub>1</sub> , l	2.3 ± 0.6	86 ± 15	2.7 ± 0.7	0.000
FEV <sub>1</sub> /VC ratio	0.69 ± 0.08	93 ± 10	0.75 ± 0.03	0.000
D <sub>L,CO</sub> , mmol/min kPa	6.5 ± 1.9	92 ± 18	7.0 ± 1.6	0.002
D <sub>L,CO</sub> < 80% of predicted, n (%)	12 (24)	NA	NA	
COPD, n (%)	21 (41)	NA	NA	
Mild COPD	61	NA	NA	
Moderate COPD	39	NA	NA	
Severe COPD	0	NA	NA	
Clinically significant reversibility, n (%)	2 (4)	NA	NA	

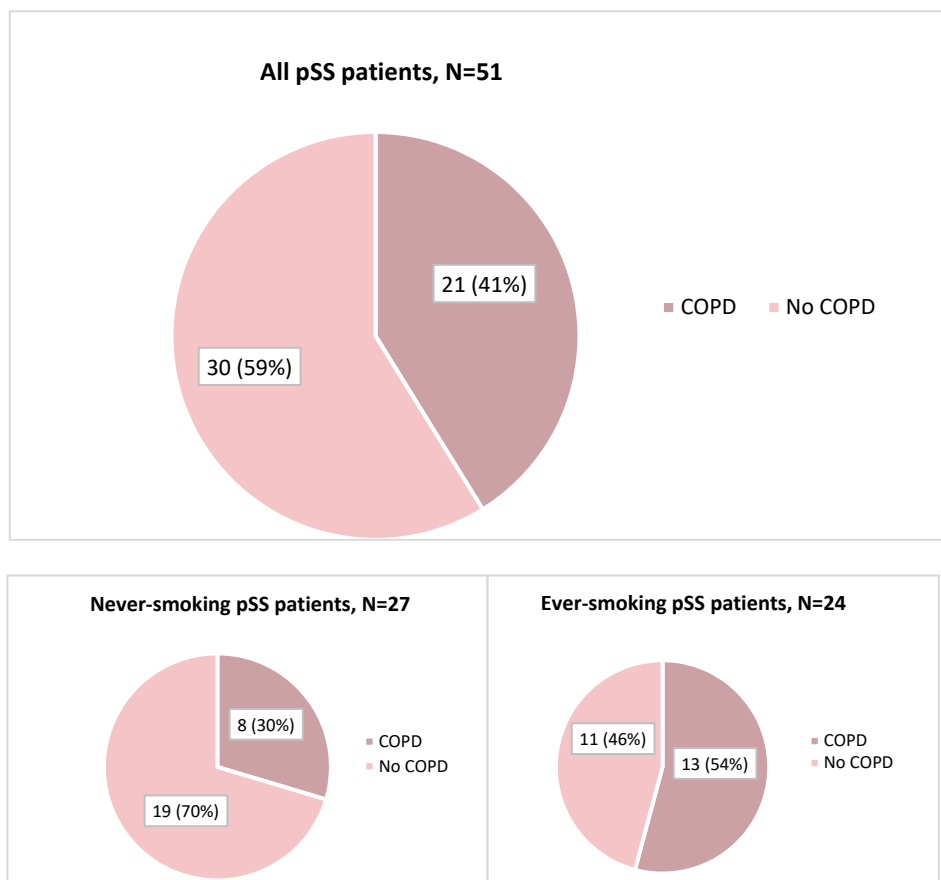
Results are presented as mean ± SD or percentages with abnormal findings.

**Table 4b.**

Results of the PFT in the 27 never-smoking patients with pSS compared to predicted values. Clinically significant reversibility was defined as FEV<sub>1</sub> improvement >12% and > 200 mL.

PFT results	Never-smoking pSS patients, n=27		Predicted values	p-values Never-smoking pSS vs predicted
	Absolute numbers	Percent of predicted	Absolute numbers	
VC, l	3.5 ± 0.9	96 ± 15	3.8 ± 1.0	0.060
TLC, l	5.8 ± 1.2	102 ± 11	5.6 ± 0.9	0.300
RV, l	2.2 ± 0.5	121 ± 19	1.9 ± 0.4	0.000
RV/TLC ratio	0.39 ± 0.1	119 ± 20	0.34 ± 0.1	0.000
FEV <sub>1</sub> , l	2.5 ± 0.6	88 ± 14	2.9 ± 0.8	0.000
FEV <sub>1</sub> /VC ratio	0.72 ± 0.08	94 ± 8	0.76 ± 0.03	0.001
D <sub>L,CO</sub> , mmol/min kPa	7.1 ± 1.9	102 ± 11	7.6 ± 1.9	0.065
D <sub>L,CO</sub> < 80% of predicted, n (%)	5 (18)	NA	NA	
COPD, n (%)	8 (30)	NA	NA	
Mild COPD	75	NA	NA	
Moderate COPD)	25	NA	NA	
Severe COPD	0	NA	NA	
Clinically significant reversibility, n (%)	0	NA	NA	

Results are presented as mean ± SD or percentages with abnormal findings



**Figure 4.** Proportion of COPD in all 51 patients with pSS as well as the 27 never-smoking and the 24 ever-smoking patients. Results are presented as absolute numbers and percentages.



## CT findings

Radiographic findings were common in pSS patients with 65% of patients presenting some kind of radiographic abnormality. The most common radiographic findings were air trapping (35%), a reticular pattern (33%), bronchiectasis (33%) (25% central and 8% peripheral traction bronchiectasis respectively) and cysts (29%). No patient demonstrated honeycombing. Radiographical signs of ILD (represented by ground glass attenuation or traction bronchiectasis) were demonstrated in 17% of pSS patients (Table 5).

Among the pSS patients with COPD, radiographic emphysema, central bronchiectasis and a reticular pattern were significantly more common in patients with COPD than those without (Table 5). Otherwise, radiographic findings and PFT results were generally poorly associated.

**Table 5.**

Results of the ESSDAI respiratory domain and symptoms, evaluated using the ESSPRI and the SGRQ and radiographic findings of CT of the chest in all 51 pSS patients as well as pSS patients with (+) and without (-) COPD. Radiographic signs of ILD are represented by traction bronchiectasis, ground glass attenuation or honeycombing.

Signs and symptoms	All pSS patients, n=51	pSS + COPD, n=21	pSS - COPD, n=30	p-values pSS + vs - COPD
ESSDAI respiratory domain score > 0 points	55	52	57	0.762
Low activity	39	38	40	0.891
Moderate activity	14	10	17	0.685
High activity	2	5	0	0.412
ESSPRI Total score	6 ± 2	6 ± 3	6 ± 2	0.281
SGRQ Total score	21 ± 16	24 ± 17	19 ± 16	0.232
SGRQ Symptom score	27 ± 22	31 ± 20	24 ± 22	0.185
SGRQ Activity score	33 ± 24	36 ± 27	31 ± 23	0.527
SGRQ Impact score	13 ± 14	15 ± 15	11 ± 13	0.403
<b>Radiographic findings</b>				
Radiographic abnormalities	65	81	53	0.073
Air trapping	35	38	33	0.726
Reticular pattern	33	52	20	0.016
Central bronchiectasis	26	43	13	0.024
Traction bronchiectasis	8	5	10	0.634
Cysts	29	24	33	0.463
Emphysema	14	29	3	0.015
Ground glass attenuation	12	5	17	0.381
Honeycombing	0	0	0	NA
ILD signs	17	10	23	0.277

Results are presented as mean ± SD or percentages with abnormal findings.

*pSS features and respiratory symptoms in relation to PFT and CT findings*

55% of pSS patients demonstrated disease activity in the respiratory domain of the ESSDAI (score > 0 points). However, the ESSDAI respiratory domain scores and respiratory symptoms, as evaluated by the SGRQ, did not significantly differ between pSS patients with and without COPD (Table 5). The SGRQ total score correlated inversely with the percentage of VC predicted ( $r = -0.34$ ,  $p = 0.013$ ),  $D_{L,CO}$  predicted ( $r = -0.32$ ,  $p = 0.022$ ) and TLC predicted ( $r = -0.29$ ,  $p = 0.039$ ), respectively. A significant correlation was found between the SGRQ and ESSPRI total scores ( $r = 0.40$ ,  $p = 0.004$ ).

The ESSDAI total score also correlated inversely with the percentage of VC predicted ( $r = -0.40$ ,  $p = 0.004$ ),  $D_{L,CO}$  predicted ( $r = -0.34$ ,  $p = 0.014$ ) respectively, as expected, but also inversely with the percentage of TLC predicted ( $r = -0.40$ ,  $p = 0.004$ ). However, if the respiratory domain score was excluded from the ESSDAI total score, no correlation with the PFT results was demonstrated. Laboratory inflammatory findings i.e. serum levels of CRP, ESR, IgG, C3, C4 and the presence of anti-SSA/Ro, anti-SSB/La, ANA and RF, were poorly associated with both PFT results and radiographical findings (data not shown).

In ever-smoking pSS patients, VC, FEV<sub>1</sub>, FEV<sub>1</sub>/VC and  $D_{L,CO}$  were significantly reduced and the proportion of anti-SSB/La seropositive patients was increased. Otherwise, there were no consistently significant differences in PFT results, radiographical findings or laboratory findings between never- and ever- smoking pSS patients (Table 6).

**Table 6.**

Disease characteristics and demographics, signs and symptoms, evaluated using the ESSPRI and the St George's SGRQ, results of the PFT and radiographic findings in the 27 never-smoking and the 24 ever-smoking patients with pSS. Clinically significant reversibility was defined as FEV<sub>1</sub> improvement >12% and > 200 mL. Radiographic signs of ILD are represented by traction bronchiectasis, ground glass attenuation or honeycombing.

Characteristics	Never-smoking pSS, n= 27	Ever smoking pSS, n=24	p- values Never- vs ever- smoking pSS
Age, yrs	58 ± 14	63 ±10	0.227
Disease duration, yrs	19 ±14	15 ± 10	0.321
Anti-SS-A antibody seropositives	74	83	0.508
Anti-SS-B antibody seropositives	33	63	0.037
IgG, g/l	13.5 ± 5.1	14.2 ± 5.2	0.624
C3, g/l	1.02 ± 0.26	1.04 ± 0.25	0.604
C4, g/l	0.17 ± 0.07	0.19 ± 0.07	0.395
Lower lip biopsy – focus score ≥ 1	85	58	0.058
ESSDAI Total score	7 ± 7	7 ±5	0.499
ESSPRI Total score	6 ± 2	6 ±2	0.681
SGRQ Total score	20 ± 16	24 ± 17	0.365
<b>PFT results</b>			
VC, l	3.5 ± 0.9	3.1 ± 0.7	0.034
TLC,l	5.8 ± 1.2	5.4 ± 0.8	0.643
RV, l	2.2 ± 0.5	2.3 ±0.4	0.389
FEV <sub>1</sub> , l	2.5 ± 0.6	2.1 ± 0.6	0.009
FEV <sub>1</sub> /VC ratio	0.72 ± 0.08	0.67 ± 0.08	0.026
D <sub>L,CO</sub> (mmol/min kPa)	7.1 ± 1.9	5.7 ± 1.6	0.013
COPD, n (%)	8 (30)	13 (54)	0.076
Clinically significant reversibility, n (%)	0	2 (8)	0.216
<b>Radiographic findings</b>			
Radiographic abnormalities (any of the below)	60	71	0.388
- Air trapping	37	33	0.782
- Reticular pattern	22	46	0.074
- Central bronchiectasis	19	33	0.226
- Traction bronchiectasis	15	0	0.113
- Cysts	26	33	0.562
- Emphysema	8	21	0.232
- Ground glass attenuation	15	8	0.671
- Honeycombing	0	0	NA
- ILD signs	26	8	0.147

Results are presented as mean ± SD or percentages with abnormal findings.

## Discussion

In this cross-sectional study, COPD was a common finding among pSS patients and was shown in 41% of patients and in almost one third (30%) of never-smoking pSS. A predominantly obstructive pulmonary function pattern was shown, compared to the predicted values, as assessed by the PFTs. The associations between PFT results and pSS disease activity and respiratory symptoms were only moderate, whereas the associations with radiographic findings were limited and, with laboratory inflammatory and serological features, even poor.

### *Strengths and limitations*

The strengths of this study were the use of consecutive patients with pSS, the use of the widely-accepted AECG criteria for pSS and the use of population-based PFT controls, taking cigarette smoking into account. The relatively small number of patients was one limitation of this study. Another limitation was the lack of population-based controls for the SGRQ and the chest CT scans. Moreover, CT of the chest is less sensitive in detecting radiographic pulmonary parenchymal abnormalities compared to HRCT, with a risk of underestimation of ILD. However, the frequency of pulmonary fibrosis among pSS patients, as evaluated by HRCT in the previous longitudinal study at our centre, was low [12].

### *COPD and airway obstruction – reflections and possible explanations*

As COPD was a frequent finding in consecutive pSS patients in this study, this suggests that COPD may be underdiagnosed in pSS patients. To date, this manifestation of pSS has only been sparsely studied. A frequent development of COPD among pSS patients has previously been reported in one recent longitudinal study by our group, in which COPD was shown in 37% of patients at follow-up, although baseline PFT assessments of pSS patients in the previous study were mainly performed on patients with respiratory symptoms [12]. Another recent retrospective register-based study reported an increased risk of COPD in pSS patients compared to population-based controls, although information regarding both classification and diagnostic criteria for pSS and COPD, as well as data on cigarette smoking, were lacking in that study [84].

Taken together, the PFTs of pSS patients mainly showed signs of obstructive pulmonary disease, with reduced VC, FEV<sub>1</sub>, FEV<sub>1</sub>/VC ratio and D<sub>L,CO</sub> and increased RV and RV/TLC ratio, compared to the predicted values. In both obstructive and restrictive pulmonary diseases, VC may be reduced, but as TLC was normal and the RV/TLC ratio increased in pSS patients, the findings suggested a predominantly obstructive pulmonary disease [81]. Also, reduced D<sub>L,CO</sub> might be observed in several pulmonary abnormalities, e.g. in ILD, but also in emphysema [81]. The suggested predominance of an obstructive pulmonary function pattern in pSS patients, however, does not exclude a minor superimposed restrictive pulmonary disease.

The high proportion of COPD in the pSS patients in this study, which was equally observed in the never-smoking pSS patients, as well as the observation of a mainly obstructive pulmonary function pattern compared to the predicted values – taking cigarette smoking into account – suggests that the disease *per se* could be involved in COPD development. The understanding of the exact pathogenetic mechanisms behind small airway disease in pSS is limited. This could be attributed to several possible contributory factors. The inflammatory lymphocytic cell infiltrates in the exocrine glands of the airways and follicular bronchiolitis, shown in pSS, may result in obliteration and obstructive pulmonary function changes [5, 31, 58, 72, 75]. Parenchymal pulmonary components, which affect the elasticity of the lungs and thereby the mechanical properties of the airways, may also contribute [81]. Other common physiological abnormalities seen in pSS patients, e.g. xerotrachea with impaired mucociliary clearance and airway hyper responsiveness, could also possibly be involved in local alterations predisposing to COPD [5, 13, 75, 85].

### *Radiographic findings*

The most common radiographic findings shown in this study, i.e. air trapping, cysts, a reticular pattern and bronchiectasis, have also been previously reported in pSS patients [5, 6, 11, 68, 70, 73]. Airway abnormalities have been described as the characteristic pulmonary manifestation in pSS and can also be observed in association with other pulmonary manifestations, although a retrospective study identified ILD as the most frequent type of pulmonary finding among pSS patients, followed by emphysema [66, 73, 79]. Difficulties are also sometimes experienced in radiographic interpretation. For example, it has been suggested that radiographic patterns that resemble interstitial changes are derived from wall thickening of the segmental bronchi in pSS patients, thus reflecting pSS airway disease [58]. In this study, the radiographic findings and PFT results were poorly associated, though some of the radiographical findings were significantly more common in patients with COPD in comparison to patients without COPD, e.g. central bronchiectasis and emphysema, but also a reticular pattern, as a reflection of the above interpretation [58].

### *Modest correlations between pulmonary function and respiratory symptoms*

In this study, the correlations between PFT results and respiratory symptoms, assessed by SGRQ, were only moderate. Notably, the SGRQ total score correlated inversely with the percentages of predicted VC, TLC and  $D_{L,CO}$  values, respectively, but not with the percentages of the predicted  $FEV_1$  or the  $FEV_1/VC$  ratio. The lack of correlation with the latter two could be explained by the multiple reasons for respiratory symptoms in pSS, e.g. airway sicca, presumably affecting the majority of pSS patients, symptoms possibly related to both airway obstruction and interstitial lung disease. Another possible explanation could be that respiratory symptoms reflect components of restrictive lung disease in pSS, as higher scores were associated with a lower TLC and  $D_{L,CO}$ .

### *Implications*

The results of this study, which suggest that COPD is common in pSS patients, even in never-smoking patients, as well as signs of a predominantly obstructive pulmonary function reduction, support and emphasise a liberal assessment of pulmonary function in pSS patients both at diagnosis and at follow-up. The results also suggest that pSS disease *per se* may be involved in the development of COPD and highlight the need for studies of larger populations with both short and long disease duration, as well as studies regarding its etiopathogenesis and development over time. If better treatment options for pSS were to become available in the future, then pulmonary manifestations and COPD development in pSS could possibly be prevented. In the meantime, pSS patients with COPD should be assessed and treated according to GOLD recommendations for COPD.

## Paper II

### **The forced oscillation technique is a sensitive method for detecting obstructive airway disease in patients with primary Sjögren's syndrome**

In this study, pulmonary function, using the FOT, was assessed in 37 female pSS patients without prior physician-diagnosed obstructive airway disease (OAD) and compared to population-based controls, matched for gender, age height, weight and tobacco consumption. In addition to the FOT results, PFT results were available and predicted PFT values were calculated based on population-based controls, taking gender, age height, weight and tobacco consumption into account. The associations between the FOT results of the pSS patients and the PFT results, pSS features, respiratory symptoms and tobacco consumption, were studied.

## Results

Both FOT and PFTs showed pulmonary function reductions in pSS patients compared to FOT controls and the predicted PFT values, respectively. Signs of airway obstruction were found using both methods.

The FOT results of pSS patients revealed a significantly increased resistance, decreased reactance and an increased resonant frequency compared to controls (Table 7). Similar differences in FOT results were observed when comparing never-smoking pSS patients to controls as well (data not shown).

The PFT results of the pSS patients revealed a significantly reduced VC, FEV<sub>1</sub> and D<sub>L,CO</sub> and a significantly increased RV compared to the predicted values (Table 7).

OAD was shown in 14 of the pSS patients (12 with COPD and 2 with an abnormal reversibility test), as evaluated by PFTs. 21 patients had no OAD and two could not be classified due to the lack of a reversibility test. Both pSS patients with and without OAD, as evaluated by spirometry, had significantly increased resistance and decreased reactance compared to the FOT controls (Figure 5), although a statistically significant increased resonant frequency was only shown in pSS patients who showed OAD compared to the FOT controls (15.39 (13.07, 18.07) vs. 9.89 (9.09, 13.48),  $p < 0.001$ ).

There were some correlations between the FOT results and the PFT results. Resistance at all frequencies correlated negatively to VC, FEV<sub>1</sub>, and D<sub>L,CO</sub>, whilst the reactance, particularly at higher frequencies, correlated positively to VC, FEV<sub>1</sub> and D<sub>L,CO</sub> (data not shown).

### *FOT results in relation to pSS features and respiratory symptoms*

Tobacco consumption, as evaluated by pack years, correlated significantly negatively to the FEV<sub>1</sub>/VC ratio ( $r_s = -0.39$ ,  $p=0.018$ ), whilst neither resistance, reactance nor  $F_{res}$  showed any correlation with tobacco consumption in pSS patients. Lastly, there were no correlations between FOT results and respiratory symptoms, as evaluated by the SGRQ, nor with pSS features, as evaluated by ESSDAI, ESSPRI or levels of IgG, C3, C4, ESR and CRP.

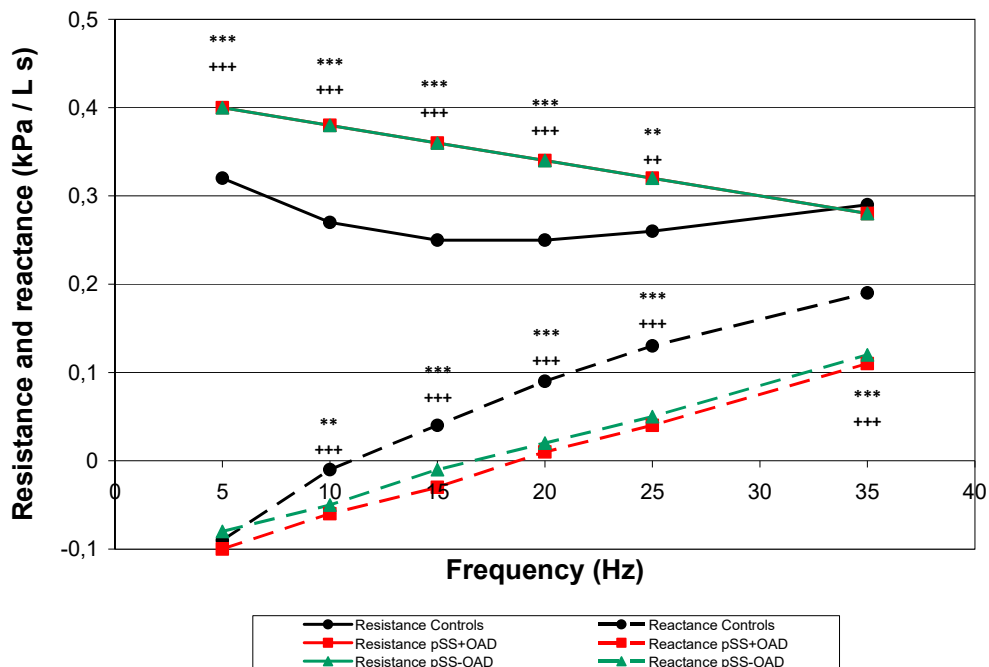
**Table 7.**

Resistance (R) and reactance (X) at different oscillation frequencies as well as resonance frequency ( $F_{res}$ ), assessed by the FOT in 37 patients with pSS and 74 population-based controls, matched with regard to age, gender, height, weight and tobacco consumption. In addition, spirometry parameters in the 37 pSS patients compared with expected values, based on 186 population-based females attending a general health survey. The expected values were corrected with regard to age, height, weight and tobacco consumption.

FOT	pSS patients (n=37)	Controls (n=74)	p-value
R 5 Hz (kPa/L s)	0.40 (0.39, 0.41)	0.32 (0.27, 0.38)	<0.001
R 10 Hz (kPa/L s)	0.38 (0.37, 0.39)	0.27 (0.22, 0.34)	<0.001
R 15 Hz (kPa/L s)	0.36 (0.35, 0.37)	0.25 (0.21, 0.31)	<0.001
R 20 Hz (kPa/L s)	0.34 (0.33, 0.35)	0.25 (0.21, 0.31)	<0.001
R 25 Hz (kPa/L s)	0.32 (0.31, 0.33)	0.26 (0.22, 0.32)	<0.001
R 35 Hz (kPa/L s)	0.28 (0.27, 0.29)	0.29 (0.25, 0.34)	0.343
X 5 Hz (kPa/L s)	-0.09 (-0.10, -0.08)	-0.09 (-0.12, -0.07)	0.251
X 10 Hz (kPa/L s)	-0.06 (-0.07, -0.04)	-0.01 (-0.03, 0.01)	<0.001
X 15 Hz (kPa/L s)	-0.02 (-0.03, -0.01)	0.04 (0.02, 0.06)	<0.001
X 20 Hz (kPa/L s)	0.01 (0.01, 0.03)	0.09 (0.07, 0.11)	<0.001
X 25 Hz (kPa/L s)	0.05 (0.04, 0.07)	0.13 (0.11, 0.16)	<0.001
X 35 Hz (kPa/L s)	0.12 (0.11, 0.14)	0.19 (0.17, 0.21)	<0.001
F <sub>res</sub> (Hz)	13.22 (11.40, 15.47)	9.89 (9.09, 13.48)	0.002
Spirometry	pSS patients (n=37)	Expected values	p-value
VC (L)	3.10 (2.75, 3.40)	3.35 (3.18, 3.80)	0.001
TLC (L)	5.30 (4.90, 5.85)	5.50 (5.24, 5.78)	0.172
RV(L)	2.20 (1.90, 2.55)	2.06 (1.83, 2.24)	0.010
FEV <sub>1</sub> (L)	2.20 (1.85, 2.55)	2.51 (2.36, 2.79)	<0.001
FEV/VC	0.74 (0.67, 0.77)	0.75 (0.73, 0.77)	0.081
D <sub>L,CO</sub> (mmol/min kPa)	6.40 (5.10, 7.30)	6.67 (6.08, 7.67)	0.004

Results are presented as median (IQR).





**Figure 5.**

Median values of resistance (continuous lines) and reactance (dashed lines) at different oscillation frequencies assessed using the FOT in patients with pSS, with (+OAD) and without (-OAD) spirometric signs of obstructive airway disease, as well as in population-based controls.

\*\*\*  $p < 0.001$  pSS+OAD vs controls, \*\*  $p < 0.01$  pSS+OAD vs. controls

+++  $p < 0.001$  pSS-OAD vs controls, ++  $p < 0.01$  pSS-OAD vs. controls

## Discussion

In this study, pSS patients with prior physician-diagnosed OAD showed signs of obstructive pulmonary disease, assessed by the FOT and PFTs, and in which the results were compared to population-based controls, taking tobacco consumption into account. The FOT revealed an increased resistance and a decreased reactance as well as an increased  $F_{res}$  in pSS patients. Also, never-smoking pSS patients showed similar FOT abnormalities. It is noteworthy that even patients with no spirometric signs of OAD showed FOT signs of airway obstruction, although the FOT results correlated with the PFT results.

### *Strengths and limitations*

The strengths of this study were the assessment of pulmonary function using both FOT and spirometry, the use of population-based controls, taking cigarette smoking into account, as well as the use of the widely-accepted AECG criteria for

pSS. The limitations were the small number of patients, the lack of a positive control group with established COPD and the poor ability of the technique to assess restrictive pulmonary disease.

### *Obstructive airway findings and reflections*

pSS patients with signs of airway obstruction are in accordance with previous studies which suggest that small airway disease is one of the most common pulmonary findings in pSS, although restrictive pulmonary disease has also been previously described in pSS patients [5, 9-12, 14, 58]. The presence of similar FOT signs of airway obstruction in this study, even among never-smoking pSS patients, suggests that the disease *per se* may be involved in the pathogenesis of obstructive airway disease in pSS patients. Studies that assess the pulmonary function in non-smoking pSS patients and pSS patients with low tobacco consumption have previously demonstrated signs of both an obstructive and a restrictive pulmonary disease [10, 12, 58]. In a recent study by our own group, pSS patients were even found to frequently develop COPD over time, despite the fact that the majority of patients did not smoke during follow-up [12]. It has been suggested that the lymphocytic infiltration of the small airway walls, shown in pSS patients and leading to airway lumen obstruction, explains the pulmonary physiological abnormalities observed in pSS, though other mechanisms, including mucosal dryness in the airways, leading to decreased mucociliary clearance, may also contribute [5, 58].

### *Pulmonary function results in relation to pSS features and respiratory symptoms*

Respiratory symptoms were poorly correlated with FOT results. The poor correlation with respiratory symptoms may be due to respiratory symptoms in pSS being very common and unspecific, with respiratory symptoms being associated with dry airways, OAD and with more severe pulmonary parenchymal disease. The poor association between pulmonary function, respiratory symptoms and laboratory signs of inflammation is also in accordance with several previous reports [12, 14, 58].

### *Implications*

In this study, signs of airway obstruction, both in small and more central airways, using the FOT, were demonstrated, even in pSS patients with no spirometric signs of OAD. This suggests that the FOT is a sensitive method of assessing airway obstruction in pSS patients, in accordance with previous evaluations of the method in patients with airflow obstruction [109, 115]. Furthermore, the possible clinical applications of the technique in pSS patients include early detection of airway obstruction in patients with subclinical airway involvement. Future follow-up studies of FOT in relation to PFTs would be interesting in order to possibly identify patients at risk of COPD development. Hypothetically, if better treatment

options that targeted the inflammatory mechanisms in pSS-associated airway involvement were available, FOT, as a sensitive method, could be of value in monitoring the effects of such treatments.

## Paper III

### **Increased B-cell activating factor, interleukin-6 and interleukin-8 in induced sputum from primary Sjögren's syndrome patients**

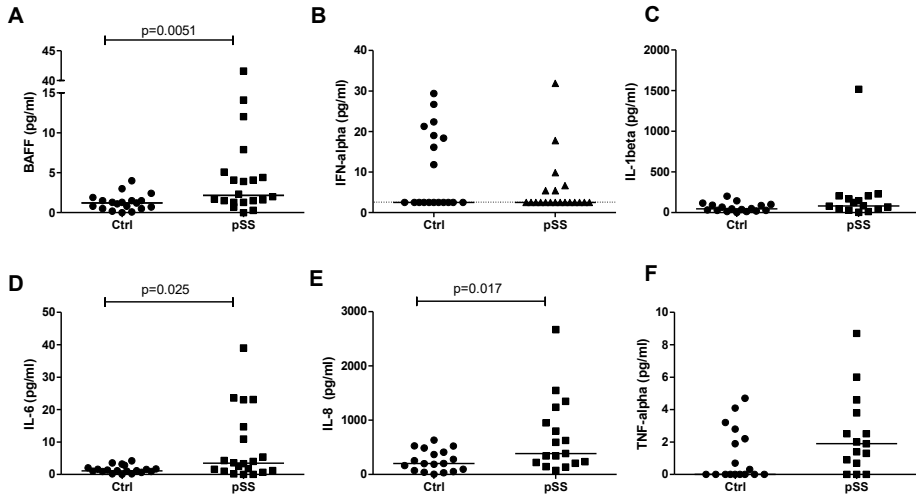
In this study, induced sputum cytokines and leukocytes were assessed in 20 never-smoking patients with pSS and the results were compared to never-smoking gender and age-matched population-based controls. In addition, PFTs, pSS features and respiratory symptoms were evaluated and assessed for correlations with the induced sputum results in pSS patients.

### **Results**

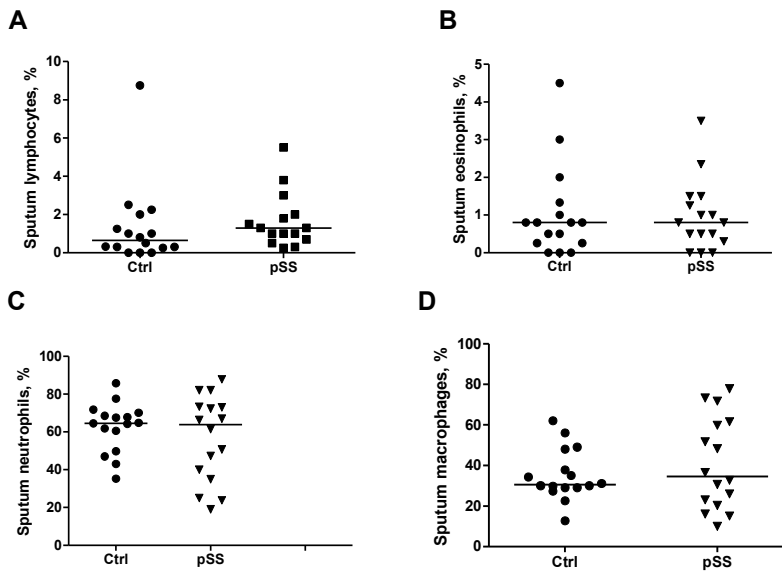
The quality of sputum supernatant samples was similar in pSS patients and controls [score 4.0 (3.0-5.0) and 5.0 (4.0-6.0),  $p=0.12$ ]. Induced sputum cytokines (i.e. BAFF, IL-1 $\beta$ , IL-6, IL-8, IFN- $\alpha$  and TNF- $\alpha$ ) were assessed in all patients and controls, whilst cell slides (i.e. lymphocytes, neutrophils, eosinophils and macrophages) were obtained in 16 pSS patients and 16 controls.

In pSS patients BAFF, IL-6 and IL-8 were significantly increased in sputum supernatant compared to controls [BAFF: 2.2 (1.4-4.9) pg/ml vs. 1.2 (0.5-1.5) pg/ml,  $p = 0.0051$ ; IL-6: 3.5 (1.1-13.8) pg/ml vs. 1.1 (0.9-2.0) pg/ml,  $p = 0.025$ ; IL-8: 384 (211-1094) pg/ml vs. 199 (65-426) pg/ml,  $p = 0.017$ ] (Figure 6). No significant differences were shown in levels of IL-1 $\beta$ , IFN- $\alpha$  and TNF- $\alpha$  between pSS patients and controls (Figure 6). Significant correlations were observed between BAFF and IL-6 ( $r_s = 0.65$ ,  $p = 0.002$ ), BAFF and IL-8 ( $r_s = 0.60$ ,  $p = 0.011$ ) and between IL-8 and IL-1 $\beta$  ( $r_s = 0.58$ ,  $p = 0.024$ ) in pSS patients.

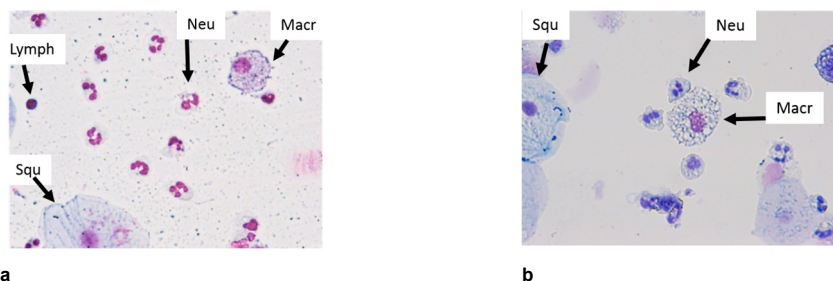
The proportions of cells in induced sputum did not significantly differ between pSS patients and controls (Figure 7, 8a and b). A numerically increased proportion of induced sputum lymphocytes was noted in pSS patients compared to controls, though the difference did not reach a statistically significant level [1.2 (0.6-2.0) % vs. 0.7 (0.3-1.8) %;  $p = 0.224$ ] (Figure 7, 8a and b). Finally, the induced sputum lymphocytes and BAFF levels in induced sputum in pSS patients were significantly positively correlated ( $r_s = 0.54$ ,  $p = 0.032$ ).



**Figure 6.** Results of sputum supernatant levels of BAFF, IL-1 $\beta$ , IL-6, IL-8, IFN- $\alpha$  and TNF- $\alpha$  in 20 pSS patients and 19 population-based controls (Ctrl).



**Figure 7.** Results of sputum differential counts of lymphocytes, eosinophils, neutrophils and macrophages in 16 pSS patients and 16 population-based controls (Ctrl). Evaluable sputum cell slides were obtained from 16 subjects, respectively.



**Figure 8.**

Microphotographies of induced sputum cells in a primary Sjögren's syndrome patient (a) and a population-based control (b).

Lymph = Lymphocyte

Neu = Neutrophil

Macr = Macrophage

Squ = Squamous cell

### *Induced sputum results in relation to PFT results, pSS features and respiratory symptoms*

Among the seven pSS patients with COPD, the proportion of neutrophils in induced sputum supernatant was increased in comparison with patients without COPD [72.8 (60.3–83.5) % vs. 49.1 (24.7–68.0) %,  $p = 0.042$ ], whilst other cell proportions and cytokine levels did not differ significantly. Cytokine levels in induced sputum in pSS patients generally correlated poorly with the pulmonary function test results.

Only a few correlations between induced sputum supernatant cytokine levels and pSS features, as well as respiratory symptoms, were observed among pSS patients. Serum levels of IgG correlated significantly with both IL-6 in induced sputum ( $r_s = 0.47$ ,  $p = 0.035$ ) and TNF- $\alpha$  ( $r_s = 0.55$ ,  $p = 0.034$ ). No significant difference in sputum supernatant cytokine levels was shown between anti-SSA/Ro and anti-SSB/La seropositive vs. seronegative pSS patients. Nor did the ESSDAI total score, ESSPRI total score, SGRQ total score, C3 and C4, respectively, show any significant correlation with sputum supernatant cytokine levels.

When comparing pSS patients with and without DMARDs, no significant differences were observed in induced sputum cytokine levels or in cell proportions. However, in pSS patients with concomitant corticosteroids, the proportion of lymphocytes in induced sputum was significantly increased [2.0 (1.4–4.3) % vs. 1.0 (0.3–1.3) %,  $p = 0.027$ ], whilst the other induced sputum cell proportions and cytokine levels were similar in pSS patients with and without corticosteroids.

## Discussion

In this study, BAFF, IL-6 and IL-8 were significantly increased in induced sputum from never-smoking pSS patients, in comparison with never-smoking controls. However, cytokine levels were not associated with pSS disease activity, pulmonary function tests, respiratory symptoms, or laboratory inflammatory or serological features of the disease.

### *Strength and limitations*

The strengths of this study were the use of never-smoking pSS patients who fulfilled the widely-accepted AECG criteria and the ACR/EULAR criteria for pSS, as well as the use of gender and age-matched never-smoking population-based controls. The principal limitation was the small number of patients and controls; the study only included a few patients with more severe bronchial and pulmonary disease, limiting the possibilities of subgroup analyses. Other limitations, relating to the limited amount of sputum supernatant obtained during one examination, were the limited number of analysed inflammatory mediators and cells, as well as the lack of more sensitive techniques for assessment of inflammation in this study. Furthermore, systemic immunosuppressants and corticosteroids affecting some of the inflammatory markers cannot be ruled out. Finally, the representativeness of the sputum findings for the local inflammatory process in airway mucosa in relation to lung parenchyma is not yet fully clear [120, 123].

### *Induced sputum cytokines and cells in relation to pSS and COPD*

In line with the findings of increased BAFF in induced sputum in pSS patients in this study, BAFF has been highlighted as an important mediator of B-cell hyperactivation and differentiation in pSS [2, 25, 134]. It is suggested that BAFF plays a key role in the immunopathology of pSS, in which lymphocytic infiltration and the formation of ectopic lymphoid structures, so-called germinal centres (GC), in exocrine glands and extraglandular organs are described as a histopathological hallmark of the disease [2]. IL-6, also shown to be significantly increased in the induced sputum of the pSS patients in this study, has been proposed to contribute to the lymphocytic infiltration in salivary glands and GC formation in pSS [2, 135]. The lymphocytic infiltration and GC formation in the small airways is proposed to lead to bronchiolitis and bronchial obstruction, as frequently shown in pSS patients [5, 12, 58, 75]. Consequently, IL-6 has also been shown to be associated with pulmonary involvement in pSS [136]. Small airway involvement with signs of airway inflammation has been addressed as an extraglandular feature that could be underdiagnosed in pSS – with some recent studies even proposing that pSS is associated with frequent COPD development [5, 12, 31, 58, 75, 77, 84]. Bronchiolitis is a hallmark of COPD and recent research has suggested that autoimmune mechanisms associated with bronchial epithelial damage are involved in COPD development [94]. Elevated neutrophils in induced sputum in COPD has

been associated with both COPD severity and with exacerbations, in accordance with the increased proportion of sputum neutrophils shown in pSS patients with COPD compared to patients without COPD [94]. IL-8, also known as a neutrophil chemotactic factor, was shown to be elevated in induced sputum in pSS patients, and is also frequently shown in high levels in the induced sputum of patients with COPD [94]. However, induced sputum IL-1 $\beta$  levels in pSS patients were not shown to be elevated, although IL-1 $\beta$  is also suggested to be a proinflammatory cytokine that contributes to neutrophilic inflammation in COPD [94].

In this study, the proportion of lymphocytes in induced sputum was only significantly increased in pSS patients with concomitant systemic corticosteroids compared to patients without, which could be due to pSS patients with bronchial pulmonary disease being more likely to be treated with corticosteroids. In contrast to a previous report, which demonstrated an increase in lymphocytes in induced sputum in pSS patients, no statistically significant increase in lymphocytes in pSS patients compared to controls was shown in this study [31]. Also, it has been established that the type 1 interferon system is of importance to pSS immunopathogenesis, although the level of induced sputum IFN- $\alpha$  was not significantly increased in pSS patients in this study [2, 25, 134]. Since there are different types of I IFN subtypes, an assessment of the IFN type I signature in induced sputum could be a more valid analysis than an assessment of protein levels using an ELISA, as was conducted in this study [137]. Finally, induced sputum levels of TNF- $\alpha$  were not significantly elevated in pSS patients, even though it is proposed that TNF- $\alpha$  contributes to both pSS and COPD immunopathology, and has been shown to be increased in the induced sputum of patients with COPD [2, 94].

#### *Induced sputum in association with pSS features and respiratory symptoms*

The lack of associations between induced sputum cytokine levels and disease activity, pSS-associated symptoms and respiratory symptoms, as well as the PFT results, may have several explanations. For example, the small size of the study, the discordance between the level of inflammation in different exocrine glands and extraglandular organs, as well as the respiratory symptoms in pSS, not only reflect the inflammatory process in the airways. Sicca symptoms, respiratory symptoms and changes in pulmonary function may not only be due to actual airway inflammation, but could also be due to end organ damage, particularly in patients with a long-standing disease. Furthermore, the pSS patients in this study could show better pulmonary function test values than pSS patients in general, as only never-smoking pSS patients were included.

### *Implications*

The increased levels of BAFF, IL-6 and IL-8 in the induced sputum of pSS patients in this study suggest that these cytokines may be involved in the immunopathogenesis of pSS-associated airway disease. The impact of the findings on pulmonary function over time in pSS patients is not clear and longitudinal studies of pulmonary function and pulmonary disease in pSS, taking induced sputum cytokine and cell levels into account, would be interesting. The sputum induction technique is a well-established and clinically used assessment method in respiratory medicine and is proposed to be a useful tool in achieving insights into the pathogenesis of different pulmonary and airway diseases [123]. Thus, the induced sputum cytokine and cellular analysis in pSS patients might be of value in research, to provide insights into the immunopathogenetic mechanisms of pSS-associated bronchial and pulmonary disease and in finding suitable targets for future therapies in pSS extraglandular disease, including pSS-associated airway and pulmonary disease. For example, B-cell targeted therapies, such as Rituximab, are already in use for treating severe extraglandular manifestations of pSS and Belimumab is currently being studied in pSS [47, 53, 55, 56]. In addition, IL-6 inhibitors such as Tocilizumab are also under evaluation for the treatment of systemic pSS disease [53]. Thus, induced sputum analysis could be a useful tool in monitoring and predicting the effects of novel treatments [123].

## Paper IV

### **Mixed airway and pulmonary parenchymal disease in patients with primary Sjögren's Syndrome - a six-year follow-up**

This study was a six-year follow-up study of the previous study on pulmonary involvement in consecutive pSS patients (Study I). 40 of the 51 baseline pSS patients chose to participate and pulmonary function, radiographic pulmonary findings use of HRCT, pSS features and respiratory symptoms and cigarette consumption were assessed. Predicted PFT results were achieved based on values from previously studied population-based controls, taking gender, age, height, weight and cigarette consumption into account.

### **Results**

#### *Pulmonary function test results - changes over time*

A pulmonary function reduction over time in comparison with the predicted values was demonstrated and COPD was still a common finding among pSS patients, with a rather unchanged frequency over time.



The PFTs demonstrated significantly decreased percentages of predicted TLC, RV, RV/TLC ratio and  $D_{L,CO}$  and an increased percentage of predicted  $FEV_1/VC$  ratio at follow-up compared to percentages of predicted values at baseline. The proportion of pSS patients with a  $D_{L,CO} < 80\%$  of predicted increased significantly over time, from only 3% at baseline to almost one third (30%) at follow-up (Table 8a). Similar PFT findings were seen in never-smoking and ever-smoking pSS patients at follow-up, without significant difference in percentages of predicted PFT results between the groups (data not shown).

COPD was demonstrated in 38% of pSS patients at baseline and in 40% of patients at follow-up, though no statistically significant difference in COPD prevalence was demonstrated over time (Table 8a, Figure 9a and b). The proportions of mild and moderate COPD did not change significantly over time, although there was a numerical shift of patients with mild COPD towards moderate COPD from baseline to follow-up (Table 8a, Figure 9a and b). The proportion of COPD in ever-smoking and never-smoking pSS did not significantly differ at follow-up, although COPD was numerically increased in ever-smoking patients (55%) compared to never-smoking patients (25%) ( $p=0.053$ ) (Figure 9c-f). In addition, cigarette consumption was significantly increased in pSS patients with COPD at follow-up compared to patients without COPD at follow-up, as evaluated by pack years (4 (0; 21) vs. 0 (0; 3);  $p=0.034$ ).

Among the pSS patients with COPD at follow-up, the percentages of predicted TLC, RV and RV/TLC ratio decreased significantly from baseline to follow-up (Table 8b). The remaining PFT results did not significantly differ between baseline and follow-up among patients with COPD (Table 8b).

**Table 8a.**

Results of the PFTs presented as percentage of predicted values in 40 pSS patients at baseline in 2012 compared to follow-up in 2018. Clinically significant reversibility was defined as FEV<sub>1</sub> improvement >12% and > 200 ml after inhalation of 1.0 mg terbutaline.

PFT results in pSS patients, N=40	Baseline	Follow-up	p-values
VC (% of predicted)	97 ± 14	96 ± 14	0.472
TLC (% of predicted)	101 ± 12	97 ± 10	0.000
RV (% of predicted)	113 ± 20	101 ± 18	0.000
RV/TLC ratio (% of predicted)	112 (100; 120)	103 (92; 112)	0.000
FEV <sub>1</sub> (% of predicted)	91 ± 15	91 ± 16	0.989
FEV <sub>1</sub> /VC ratio (% of predicted)	96 (89; 100)	98 (89; 104)	0.012
D <sub>L,CO</sub> (% of predicted)	95 ± 13	91 ± 15	0.016
D <sub>L,CO</sub> < 80 % of predicted, N (%)	1 (3)	12 (30)	0.001
COPD, N (%)	15 (38)	16 (40)	1.000
- Mild COPD, N (% of the above)	10 (67)	7 (44)	0.375
- Moderate COPD, N (% of the above)	5 (33)	9 (56)	0.125
- Severe and very severe COPD, N	0	0	NA
Clinically significant reversibility, N (%)	3 (8)	2 (5)	1.000

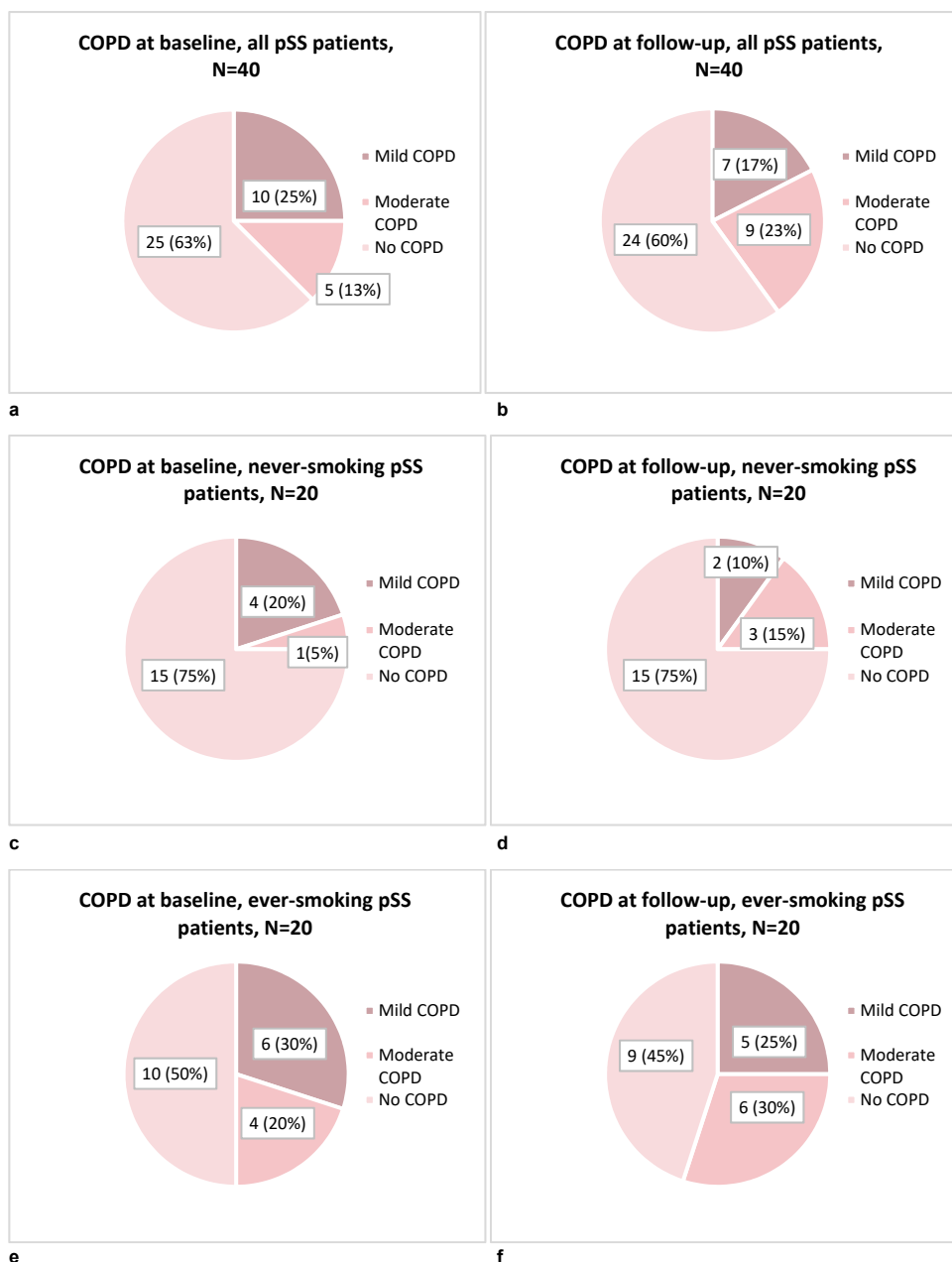
Results are presented as mean percentages of predicted (± S.D.), median percentages of predicted (IQR) and proportional findings as numbers and percentages.

**Table 8b.**

Results of the PFTs at baseline in 2012 compared to follow-up in 2018, presented as percentages of predicted values, amongst the 16 patients with pSS and COPD at follow-up.

PFT results in patients with pSS and COPD at follow-up, N=16	Baseline	Follow-up	p-values
VC (% of predicted)	96 (85; 106)	97 (81; 109)	0.804
TLC (% of predicted)	101 (87; 113)	98 (86; 108)	0.021
RV (% of predicted)	110 (98; 134)	101 (88; 114)	0.004
RV/TLC ratio (% of predicted)	116 (99; 129)	107 (92; 123)	0.006
FEV <sub>1</sub> (% of predicted)	85 (70; 88)	77 (67; 94)	0.804
FEV <sub>1</sub> /VC ratio (% of predicted)	86 (79; 98)	87 (79; 95)	0.454
D <sub>L,CO</sub> (% of predicted)	88 (78; 99)	92 (71; 98)	0.454
D <sub>L,CO</sub> < 80 % of predicted, N (%)	1 (6)	6 (38)	0.063
COPD, N	13	16	
(% of pSS with COPD at FU)	(81)	(100)	0.250
- Mild COPD, N	8	7	
(% of pSS with COPD at FU)	(50)	(44)	1.000
- Moderate COPD, N	5	9	
(% of pSS with COPD at FU)	(31)	(56)	0.125
- Severe and very severe COPD, N	0	0	NA

Results are presented as mean percentages of predicted ± SD, median percentages of predicted (IQR) and proportional findings as percentages.



**Figure 9.**

Fraction of mild and moderate COPD, according to the GOLD criteria for COPD, in all 40 patients with pSS at baseline (a) and follow-up (b) as well as the 20 never-smoking pSS patients at (baseline (c) and follow-up (d) and the 20 ever-smoking pSS patients at baseline (e) and follow-up (f). Results are presented as absolute numbers and percentages.

### *HRCT findings*

Radiographic abnormalities at follow-up were common among the 39 pSS patients, who underwent HRCT, demonstrated in 82% of the patients. Signs of bronchial involvement (represented by central bronchiectasis or bronchial wall thickening) and signs of ILD (represented by ground glass attenuation, a reticular pattern or traction bronchiectasis) were equally common, demonstrated in 39% each (Table 9). The most prevalent individual HRCT findings were cysts, central bronchiectasis and emphysema, observed in 36%, 28% and 21% of the patients, respectively (Table 9).

The correlations between radiographic findings and PFT results were generally poor. Comparing pSS patients with and without COPD at follow-up, of the individual HRCT findings, only emphysema was significantly more common in pSS patients with COPD at follow-up compared to patients without (38% vs. 9%) (Table 9). It is noteworthy that 50% of pSS patients with radiographic emphysema were never-smokers. Furthermore, among pSS patients with COPD at follow-up who had radiographic emphysema, the majority (67%) were never-smokers. Finally, never-smoking and ever-smoking pSS patients did not significantly differ in the proportions of patients with and without any of the individual or summarized HRCT findings.

Among pSS patients with radiographical signs of bronchial involvement, a decreased percentage of predicted FEV<sub>1</sub> was found compared to patients without such signs (87 (74; 95) vs. 97 (87; 101);  $p=0.030$ ), whilst other PFT results did not significantly differ between patients with and without bronchial involvement. Finally, no significant difference was observed when comparing PFT results in pSS patients with or without radiographic ILD signs.

### *pSS features and respiratory symptoms in relation to PFT and HRCT findings*

Generally, pSS disease features, i.e. disease duration, focal sialadenitis, anti SS-A and SS-B seropositivity and levels of C3, C4 and IgG were poorly associated with both PFT and radiographic findings and did not significantly differ between patients with and without COPD. However, in pSS patients with radiographic ILD signs, anti-SS-A seropositivity was shown in all patients, whilst in 71% of patients without ILD signs and the difference was significant ( $p=0.031$ ). Furthermore, C4 was found to be significantly decreased and IgG significantly increased in patients with ILD signs in comparison to patients without radiographic ILD signs (0.13 (0.08; 0.19) vs. 0.20 (0.17; 0.24);  $p=0.044$ ) and (15.7 (13.5; 19.9) vs. 11.0 (8.3; 14.8);  $p=0.010$ ), respectively. It is noteworthy that pSS patients with  $D_{L,CO} < 80\%$  of predicted at follow-up were significantly younger compared to patients with  $D_{L,CO} > 80\%$  (64 (59;66) vs. (71 (62;74);  $p=0.039$ ). In contrast, age did not differ between patients with and without COPD at follow-up. Finally, when comparing pSS patients with and without COPD, no significant differences were found, either in pSS disease activity or pSS-associated symptoms, as evaluated by ESSDAI and ESSPRI, nor in systemic treatment for pSS.

**Table 9.**

Results of the ESSDAI respiratory domain and respiratory symptoms evaluated by Swedish versions of the CAT, mMRC and SGRQ in all 40 patients with pSS, as well as the 16 pSS patients with (+) and the 24 pSS patients without (-) COPD at follow-up.

Results of HRCT of the chest in 39 of the pSS patients, as well as in the 16 pSS patients with (+) and the 23 pSS patients without (-) COPD. Bronchial involvement includes central bronchiectasis or bronchial thickening and ILD signs include ground glass attenuation, a reticular pattern, traction bronchiectasis or honeycombing.

Respiratory and radiographic findings	All pSS patients N=40	pSS patients + COPD N=16	pSS patients - COPD N=24	p-values pSS + vs - COPD
ESSDAI respiratory domain score > 0, N (%)	14 (35)	5 (31)	9 (38)	0.685
Low activity, N (%)	9 (23)	3 (19)	6 (25)	0.717
(% of above)	(64%)	(60%)	(67%)	1.000
Moderate activity, N (%)	5 (13)	2 (13)	3 (13)	1.000
(% of above)	(36%)	(40%)	(33%)	1.000
High activity, N (% of above)	0 (0)	0 (0)	0 (0)	NA
CAT total score	10 (6.00; 15.75)	10 (7.00; 18.25)	10 (4.00; 12.5)	0.174
CAT <10	19 (48)	8 (50)	11 (46)	0.796
CAT ≥ 10	21 (52)	8 (50)	13 (54)	0.796
mMRC	2 (1; 2)	2 (2; 3)	2 (0; 2)	0.126
SGRQ Total score	22.0 (11.4; 31.6)	27.8 (18.2; 34.0)	18.3 (8.1; 30.8)	0.134
- SGRQ Symptom score	27.0 (9.3; 42.4)	34.4 (14.6; 46.4)	20.6 (7.2; 39.5)	0.279
- SGRQ Activity score	41.5 (23.3; 55.0)	50.7 (37.6; 66.2)	35.6 (13.5; 50.7)	0.017
- SGRQ Impact score	8.0 (1.6; 18.5)	9.0 (3.6; 18.6)	7.2 (0.4; 18.5)	0.469
HRCT findings assessed in 39 patients	N=39	N=16	N=23	
HRCT abnormalities, any of the below, N (%)	32 (82)	13 (81)	19 (83)	1.000
Cysts, N (%)	14 (36)	6 (38)	8 (35)	0.862
Central bronchiectasis, N (%)	11 (28)	7 (44)	4 (17)	0.146
Emphysema, N (%)	8 (21)	6 (38)	2 (9)	0.045
Ground glass attenuation, N (%)	7 (18)	2 (13)	5 (22)	0.678
Reticular pattern, N (%)	7 (18)	5 (31)	2 (9)	0.101
Traction bronchiectasis, N (%)	5 (13)	2 (13)	3 (13)	1.000
Bronchial wall thickening, N (%)	4 (10)	1 (6)	3 (13)	0.631
Nodules, N (%)	3 (8)	3 (19)	0 (0)	0.061
Honeycombing, N (%)	0 (0)	0 (0)	0 (0)	NA
Bronchial involvement, N (%)	15 (39)	8 (50)	7 (30)	0.217
Central bronchiectasis (% of the above)	73	88	57	0.282
Bronchial wall thickening (% of the above)	27	13	43	0.282
ILD signs, N (%)	15 (39)	7(44)	8 (35)	0.447
Mixed bronchial and ILD signs, N (%)	5 (13)	4 (25)	1 (4)	0.139

Results are presented as medians (IQR) and proportional findings as percentages.

The ESSDAI respiratory domain score and respiratory symptoms evaluated by the CAT, mMRC and SGRQ scores did not significantly differ between pSS patients with and without COPD at follow-up, besides the SGRQ Activity score, which significantly increased in pSS patients with COPD at follow-up (Table 9).

Among pSS patients with HRCT findings, the CAT total score significantly increased (10 (7.3; 16) vs. 6 (2; 7);  $p=0.022$ ) compared to patients without HRCT findings. Finally, among pSS patients with radiographic signs of bronchial involvement, the CAT total score was significantly increased compared to patients without such findings (11 (9; 21) vs. 8.5 (5.3; 12.5);  $p=0.047$ ).

## Discussion

In this six-year follow-up study of consecutive patients with pSS, a mixed pattern of both airway and pulmonary parenchymal disease was shown. pSS patients significantly decreased percentages of predicted TLC, RV, RV/ TLC ratio and  $D_{L,CO}$  and increased the percentage of the predicted  $FEV_1/VC$  ratio at follow-up compared to baseline. Although COPD was still a common finding in pSS patients, the proportion of patients with COPD did not significantly change over time (38% at baseline vs. 40% at follow-up). In contrast, pulmonary function in pSS patients mainly showed a progression of restrictive variables from baseline to follow-up. In line with the latter findings, the proportion of pSS patients with  $D_{L,CO} < 80\%$  of predicted increased significantly from 3% at baseline to 30% at follow-up. Signs of bronchial involvement and ILD signs were common HRCT findings, affecting 39% of the patients, respectively. Limited associations between respiratory symptoms, pSS disease features, PFT variables and HRCT findings were observed, in accordance with previous reports [7, 12, 14, 58, 63].

The mixed and heterogeneous findings with regard to both pulmonary function and radiographic findings, representing both airway and pulmonary parenchymal findings, the diverse radiographic signs of bronchial involvement and ILD signs, emphysema, cysts and noduli, are in line with the previously described pleomorphic pSS pulmonary features [5, 11-13, 58, 61, 66, 72, 74]. Few previous studies have investigated pulmonary function changes over time and results are difficult to compare due to the use of different classification criteria for pSS, various modalities when assessing pulmonary function, as well as differences in study design [9-12, 14].

The pSS patients in this study showed mild and moderate COPD. Although the proportions of COPD did not increase over time, a statistically non-significant shift from mild towards moderate COPD at follow-up was observed. Only a few studies have previously reported COPD as a common finding in patients with pSS [12, 84, 138]. The previous longitudinal study of pulmonary disease in pSS by our group showed a significantly increased frequency of COPD during follow-up [12].

However, the pSS patients in that study were all investigated due to the presence of respiratory symptoms at baseline, whilst the current study included consecutive pSS outpatients, regardless of the presence of respiratory symptoms. The fact that COPD is still common even among never-smoking pSS patients suggests that the disease *per se* is involved in COPD development. The lymphocytic infiltration leading to bronchiolitis and bronchiolar wall thickening, resulting in lumen obliteration, as observed in pSS patients, may explain the obstructive pulmonary functional pattern [58, 72, 75]. Several previous studies have reported a predominance of small airway obstruction in pSS patients, with a suggested superimposed restrictive component, which may progress during follow-up, in line with pulmonary function findings and its development over time of this study [10-12, 31, 58, 75].  $D_{L,CO}$  reduction, which has been shown to progress over time in this study, may, for instance, be observed as a sign of pulmonary parenchymal disease, as a consequence of loss of the alveolar area in emphysema and may be associated with obliteration of the terminal bronchioles [81]. Other possible explanations of a reduced  $D_{L,CO}$  could be diastolic dysfunction or development of pulmonary arterial hypertension, often found in rheumatic diseases [71, 81]. However, in this study, echocardiographic assessment was only performed on patients with significantly increased NT-proBNP, for whom signs of at least a systolic dysfunction and pulmonary hypertension had been ruled out. In addition, pulmonary hypertension in pSS has been reported to be rare [5, 18]. In line with the decrease of  $D_{L,CO}$  over time among pSS patients in this study, a decrease in  $D_{L,CO}$  has also been previously described in longitudinal follow-up studies of pulmonary function in pSS, one of the studies even demonstrating an association with signs of small airway disease [9-14].

Only limited associations between PFT results and radiographic findings among pSS patients were found in this study, in line with previous reports [7, 12, 138]. Interestingly, the only radiographic abnormalities significantly associated with any of the pulmonary function variables in this study were emphysema with COPD and signs of bronchial involvement with a decreased percentage of predicted  $FEV_1$ . Radiographic emphysema was also commonly shown in pSS patients and, as mentioned, was more common in pSS patients with COPD. It is noteworthy that one half of pSS patients with emphysema were never-smokers, suggesting the disease *per se* is involved in both the airway and parenchymal pulmonary phenotypes of COPD [90, 94]. Radiographic cysts were also a common finding among the pSS patients in this study, in accordance with previous studies that reported cystic lesions to be a characteristic pulmonary radiographic pSS feature, possibly caused by airway obstruction, resulting in “check-valve” mechanisms, and alveolar wall destruction [5, 7, 66, 73, 82].

### *Implications*

Considering the generally limited associations between respiratory symptoms, pulmonary function and HRCT findings, with recent studies that address an increased morbidity and mortality in pSS patients with pulmonary disease in mind, the assessment of pulmonary involvement in pSS must be emphasised and should be liberally performed [6, 7, 30]. Special concerns regarding COPD as a pulmonary manifestation in pSS are mandated and might as well be considered to be a separate pulmonary manifestation in the ESSDAI respiratory domain.

### *Strength and limitations*

The strengths of this study were the use of consecutive pSS patients, all patients fulfilling the widely-established AECG and ACR/EULAR classification criteria for pSS, as well as the use of population-based PFT controls, taking both gender, age, height, weight and cigarette consumption into account. Another strength was the use of well-established modalities in the assessment of both airway and pulmonary parenchymal disease, as well as validated tools in assessing both pSS disease activity, pSS-associated symptoms and respiratory symptoms associated with COPD. The limitations were the relatively small number of pSS patients, HRCT images being obtained only at follow-up, the lack of PFT controls older than 70 years of age, as well as the lack of pulmonary function variables specifically reflecting small airways.

### *Conclusion*

In conclusion, both airway and pulmonary parenchymal disease were commonly found in pSS patients, with a coexistence of both obstructive and restrictive pulmonary function findings, in which the latter tended to progress over time. Also, the HRCTs showed a heterogeneous pulmonary pattern with a combination of both bronchial and pulmonary parenchymal findings. COPD was still a common finding at follow-up, even among never-smoking pSS patients, suggesting that the disease *per se* is involved in the development of COPD. Pulmonary involvement may be underdiagnosed in patients with pSS and special attention to the clinical assessment of patients with pSS is necessary, with careful assessment and monitoring of pulmonary involvement, even in patients with no respiratory symptoms.





# General discussion

Respiratory symptoms are common in pSS patients and pulmonary involvement in pSS has been described as one of the major systemic manifestations of pSS [5, 30, 33, 58, 59, 139]. Pulmonary involvement in pSS patients has recently been addressed as being associated with increased mortality and morbidity, as well as impaired HRQoL, which emphasises the importance of early detection of this systemic feature [6-8, 30]. The pleomorphic nature of pulmonary involvement in pSS patients makes identifying and monitoring pulmonary involvement in pSS difficult and might therefore be underdiagnosed [48, 60]. Only a few previous longitudinal studies that assess pulmonary function changes over time in pSS patients have been performed, with conflicting results, and the results are difficult to compare, due to differences in modalities when assessing pulmonary function, study designs and classification criteria for pSS [9-14]. A previous longitudinal follow-up study by our group demonstrated frequent COPD development over time in pSS patients, although the patients in that study were all assessed as a result of respiratory symptoms at baseline [12]. Thus, these studies aimed at assessing pulmonary involvement in consecutive pSS patients and also studying more sensitive methods of early detection of pulmonary involvement in pSS. If earlier detection of pulmonary involvement was possible and targeted therapies were available in the future, pulmonary manifestations of pSS could be more effectively treated, thereby preventing deterioration or even possibly development of these.

In these studies, pulmonary manifestations in pSS patients were found to be common features of the disease. A heterogeneous presentation, with signs of both airway and pulmonary parenchymal involvement, as well as a combination of these was found. However, respiratory symptoms and clinical and laboratory pSS disease features were generally poorly associated with pulmonary function findings, radiographic findings and inflammatory markers in induced sputum.

Airway obstruction was the main pulmonary function pattern, shown in consecutive pSS patients, in which results were compared with population-based controls, taking cigarette consumption into account. It was noteworthy that COPD was a common finding in approximately 40% of pSS patients and COPD frequency remained stable over time during the six-year follow-up. The fact that COPD is a common finding even among never-smoking pSS patients suggests that the disease *per se* is involved in COPD development in pSS. In line with the PFT

results, FOT showed signs of small airway obstruction in pSS patients and results were similar even in never-smoking pSS patients, again implying that the disease *per se* is involved in airway obstruction in pSS. In addition, even in pSS patients with no spirometric signs of OAD, FOT revealed signs of both central and small airway obstruction, suggesting that FOT is sensitive in detecting subclinical OAD in pSS patients. A co-existence of a superimposed restrictive pulmonary function component was suggested and which progressed over time. A progression of  $D_{L,CO}$  reduction was shown among pSS patients during the six- year follow-up, possibly influenced by several factors, including both airway, parenchymal and vascular components [81]. It has been suggested that the lymphocytic infiltration associated with bronchiolar wall thickening and follicular bronchiolitis, shown in pSS, leads to an obstructive pulmonary function pattern in pSS patients [58, 75]. In accordance with the findings that indicate OAD in pSS patients and a reduction of  $D_{L,CO}$  over time in these studies, signs of small airway obstruction in association with  $D_{L,CO}$  reduction over time in pSS patients have also been shown in a recent longitudinal study [13].

Studying inflammatory markers in the airways, the elevated markers shown in induced sputum from never-smoking pSS patients have been presented as crucial inflammatory mediators in both pSS and COPD immunopathology and include BAFF, IL-6, IL-8 in pSS patients and neutrophils in pSS patients with COPD [2, 75, 94, 99]. Corresponding to the predominantly lymphocytic inflammation in pSS immunopathology, an increase in lymphocytes was shown in induced sputum among pSS patients with systemic corticosteroids, which might be explained by pSS patients who have more prominent inflammation being more often treated with systemic corticosteroids [5, 58, 75].

Several similarities with regard to pathogenesis are described in pSS and COPD with an immunologic activation, believed to develop in genetically susceptible individuals as a response to an external trigger, involving both innate and humoral responses and in which epithelial cells appear to play an important role. The inflammation then gives rise to tissue damage with subsequent exposure of autoantigens, resulting in further autoimmune reactions in a vicious cycle [2, 94]. As a reflection of the above, a substantial proportion of individuals with COPD have never smoked and women are more frequently affected in this group [91, 98]. Besides tobacco exposure and environmental and occupational exposure to airway pollution, the role of autoimmune mechanisms has been highlighted in COPD etiopathology [89, 94, 99]. Correspondingly, increased rates of COPD development among patients with different CTDs have also recently been reported [95]. Although the exact mechanisms are not clear, the CTDs *per se* are hypothesised to be involved in COPD development, rather than as a result of confounding factors [95]. Parallel to the inflammatory process in the airways, parenchymal inflammation and parenchymal destruction, as described in COPD,

the inflammatory process in pSS also involves both the airways and the parenchyma [5, 58, 72, 90, 94].

The radiographic abnormalities in pSS patients entailed both airways and parenchyma and radiographic signs of bronchial involvement and ILD signs were equally common HRCT findings in study IV. Radiographic emphysema was among the most common individual radiographic abnormalities, together with cystic lesions and central bronchiectasis at HRCT during follow-up, whilst air trapping, cystic lesions and a reticular pattern were the most common CT findings at baseline. It is noteworthy that 50% of pSS patients with radiographic emphysema during follow-up were never-smokers, indicating that the disease *per se* is also involved in the parenchymal changes commonly demonstrated in COPD. Cystic lesions have previously been described as a characteristic radiographic pulmonary feature of pSS and possible pathophysiological explanations are airway obstruction, leading to “check-valve” mechanisms resulting in cysts, as well as alveolar wall destruction [5, 7, 66, 73, 82]. Cystic lesions are also described as a manifestation of LIP, though this feature has been reported as being quite uncommon in pSS and lung biopsy is necessary for an unequivocal diagnosis [5, 66, 72]. No further categorising of radiographic findings in terms of different interstitial pneumonias, described in pSS, was performed in these studies, due to interpretational difficulties in such categorisation, with many of the individual radiographical findings overlapping between categories, ILD being quite uncommon in pSS and, finally, as more accurate categorisation should be based on histopathology [5].

Generally, the associations between pulmonary function results, radiographic findings, symptoms and pSS disease features, as well as respiratory symptoms, were poor. In addition, the inflammatory signs in induced sputum were poorly associated with disease features and respiratory symptoms. The poor associations could have several explanations, including respiratory symptoms being associated with various pulmonary manifestations, e.g. airway sicca, functional abnormalities, such as airway hyper responsiveness, both bronchial and parenchymal manifestations, as well as combinations of these. Furthermore, respiratory symptoms and changes in pulmonary function might not only reflect current inflammation but could also be associated with damage resulting from long-standing inflammatory processes that affect the tissue in both airway and pulmonary parenchyma. Finally, inflammation in the exocrine glands and other extraglandular organs may not parallel the inflammatory process in the airways.

Considering the generally limited associations between pulmonary function, respiratory symptoms, pSS features, radiographic findings and inflammatory findings of the airways in these studies, as well as increased morbidity and mortality in pSS patients with pulmonary disease, assessment of pulmonary involvement in pSS patients must be emphasised and should be liberally performed [6, 7]. Furthermore, the studies indicating that pSS is associated with

COPD development imply that special attention should be paid to COPD in the clinical management of pSS patients, including interventions for smoking cessation in smoking pSS patients. Considering the current era of successfully targeted immunomodulatory therapies in rheumatology and also in respiratory diseases, treatments that target inflammatory processes in the airways and lung parenchyma in pSS could become available in the future [53, 99]. As a reflection of the increased levels of BAFF in induced sputum in never-smoking pSS patients, B-cell directed therapies have already shown promising results in pSS patients with systemic disease [53, 55, 56]. An assessment of induced sputum could be useful in identifying central cytokines and immune targets suitable for such future treatments and could also serve as a tool for monitoring airway inflammation [123]. Finally, FOT, as a sensitive method of detecting airway obstruction in pSS patients, could also be valuable in the clinical management of pSS patients, in both detecting and monitoring airway involvement, as well as the possible effects of future treatments.

# Conclusions

- Pulmonary involvement is common in pSS and has a heterogeneous pattern, signs of both airway and parenchymal involvement being common, as well as mixed patterns.
- COPD is a common finding, even among never-smoking pSS patients, suggesting that the disease *per se* is involved in the development of COPD.
- There is a predominance of an obstructive pulmonary function pattern in pSS, although a minor superimposed restrictive pulmonary component may exist.
- The restrictive pulmonary function component in pSS appears to progress during six years of follow-up, while COPD prevalence remains stable.
- The FOT is a sensitive method for detecting obstructive airway disease in pSS patients and could be considered as a complementary method for early diagnosis and assessment of airway obstruction in the clinical management of pSS patients.
- Induced sputum in never-smoking pSS shows signs of an inflammatory process in the airways with increased levels of BAFF, IL-6 and IL-8 compared to population-based controls.
- Analysis of induced sputum supernatant in pSS patients could be a useful method in both further immunopathological research and in the clinical management of pSS patients, to help assess airway inflammation.
- Radiographic signs of both airway and pulmonary parenchymal involvement are common in pSS patients, as well as mixed findings.
- Pulmonary function findings in pSS generally show poor associations with radiographic findings, pSS disease features and respiratory symptoms, as well as inflammatory findings in induced sputum.
- Pulmonary involvement may be underdiagnosed in patients with pSS and special attention in the clinical assessment of patients with pSS is mandated, with liberal assessment and monitoring of pulmonary involvement, even in patients with no respiratory symptoms.



# Future perspectives

The results of the studies in this thesis raise further research questions that must be addressed in future multidisciplinary studies. Some research questions and possible future perspectives are listed below.

*Can the results be confirmed?*

As the studies in this thesis were small, further studies regarding pulmonary involvement in pSS in larger and possibly multicentre settings, which also enable subgroup analyses of different pulmonary manifestations, would be interesting.

*Is it possible to predict COPD development using early and more sensitive assessment methods in pSS?*

One set-up could constitute a prospective longitudinal study design, including consecutive newly diagnosed pSS patients, assessing pulmonary involvement, using the sensitive method FOT as well as assessment of inflammatory markers in induced sputum, together with more conventional methods, PFTs and HRCT. This would make it possible to define the natural history and development of both pSS-associated airway and parenchymal involvement in pSS over time.

*How common are pSS and other CTDs among COPD patients?*

A cross-sectional assessment of never-smoking COPD patients from population-based cohorts, for example, the Swedish BRONCHO-SCAPIS study, regarding signs of pSS, would be interesting. Furthermore, prospective longitudinal studies using such a population-based cohort in assessing the subsequent development of pSS and other CTDs in association with pulmonary diseases would be another interesting approach.

*Which autoimmune pathways are involved in the immunopathology of pSS airway and pulmonary disease? Are there similarities in COPD and pSS immunopathology that could be targeted using similar therapies?*

Several other inflammatory markers and possible external triggers in the airways could be assessed in the induced sputum supernatant in patients with both pSS and COPD in order to broaden the understanding of the immunological processes at play in both disease entities. With the guidance of such insights, future studies in developing and assessing the effects of possible targeted therapies could be established.



Other assessment methods for immunopathological research, e.g. histopathological analysis and BAL, could add further insights, although these methods might only be used in selected cases, as they are more invasive.

*Other interesting research questions for further studies*

- What is the impact on morbidity and mortality of COPD and different pulmonary manifestations in pSS patients, regardless of respiratory symptoms?
- How do airway and parenchymal manifestations of pSS interact?
- Are there other possible common and inflammatory factors that drive COPD development in pSS and in other CTDs?
- How does non-Sjögren's sicca syndrome, with no concomitant autoimmune disorder, affect the airways and lung parenchyma?
- How does the microbiome interfere with the immune responses in pSS? Is there an alteration of the microbiome locally in the lower or upper airways? How does oral health and an altered microbiome in the mouth affect the environment and immune reactions in the peripheral airways in pSS and other CTDs?

# Populärvetenskaplig sammanfattning

## Lungengagemang vid primärt Sjögrens Syndrom

### Primärt Sjögrens syndrom

Primärt Sjögrens syndrom har fått sitt namn efter den svenske läkaren Henrik Sjögren, som beskrev sjukdomen i sin avhandling 1933. Primärt Sjögrens syndrom är en av de vanligaste autoimmuna reumatiska sjukdomarna och sjukdomen drabbar framförallt kvinnor. Vid autoimmuna sjukdomar angriper kroppens eget immunförsvar olika vävnader i kroppen och ger en inflammation. Sjögrens syndrom leder till inflammation och försämrad funktion av kroppens utsöndrande körtlar, såsom saliv- och tårkörtlar. Besvärande torrhet i munnen, ögonen och i slemhinnor överlag är vanliga symptom vid Sjögrens syndrom. Många patienter lider också av uttalad trötthet och värk i kroppen. Hos ungefär en tredjedel av patienterna drabbas även andra organ, såsom lungor, leder, hud, njurar och nerver av inflammationen.

Det är vanligt med torra och irriterade luftvägar vid Sjögrens syndrom. Utöver detta kan sjukdomen ge en inflammation både i luftvägarna och i själva lungvävnaden. I nuläget finns viss kunskap om förekomsten av lungengagemang vid Sjögrens syndrom, och begränsad kunskap om uppkomsten, utvecklingen över tid och om behandlingen. Sista åren har studier visat försämrad livskvalitet och en ökad dödlighet hos patienter med primärt Sjögrens syndrom och lungpåverkan.

Det finns idag ingen bot för Sjögrens syndrom, behandlingen av sjukdomen fokuserar i dagsläget i de flesta fall på att lindra symptom. När inre organ är drabbade ges ibland behandling med antiinflammatoriska läkemedel, till exempel kortison och olika typer av antireumatiska läkemedel. Under senare år har flera nya så kallade ”biologiska” antireumatiska behandlingar utvecklats. Behandlingarna har till viss del visat sig lovande när sjukdomen drabbar inre organ. Forskning pågår för att utveckla fler specifikt riktade läkemedel som skulle kunna hjälpa patienterna.

## **Kroniskt obstruktiv lungsjukdom (KOL)**

KOL är en kronisk (livslång) lungsjukdom som drabbar både luftvägarna och lungorna. Sjukdomen gör att de små luftrören blir inflammerade och trånga och att lungvävnaden bryts ned och bildar så kallat emfysem, stora luftinnehållande områden. Vanliga symptom är hosta, andfäddhet och perioder med akuta försämringar i luftvägarna. Sjukdomen leder till minskad lungfunktion och påverkar det dagliga livet och livskvalitet beroende på sjukdomens omfattning. Sjukdomen är dessutom förenad med en ökad risk för andra sjukdomar till exempel hjärtkärlsjukdom. KOL är en av de vanligaste dödsorsakerna i världen. Rökning är den största riskfaktorn för KOL, men kring en fjärdedel av patienterna med KOL har aldrig rökt. Andra riskfaktorer för KOL är till exempel åldrande, luftföroreningar i miljön och tidigare luftvägsinfektioner. Det har också visat sig att autoimmun inflammation verkar bidra i uppkomsten av KOL. Forskning i fältet pågår.

Diagnosen KOL ställs med hjälp av lungfunktionsundersökning (spirometri). Vid KOL visar undersökningen trånga luftvägar och minskad lungfunktion. Den viktigaste behandlingen för rökare med KOL är att sluta röka. Den medicinska behandlingen består i dagsläget av olika luftrörsvidgande inhalationsmediciner och ibland inflammationsdämpande inhalationsmediciner (kortison). Antibiotika och kortison som tabletter kan behövas vid infektioner. Fysisk träning är viktigt vid KOL. När sjukdomen blivit svår behövs ofta syrgasbehandling. Forskning kring behandlingen av KOL pågår och det är möjligt att nya "biologiska" inflammationsbromsande mediciner kan komma att få en plats även i KOL-behandling i framtiden.

## **Bakgrund och målsättning med studierna**

En tidigare studie vid reumatologiska kliniken i Malmö visade att de Sjögrenpatienter som någon gång under sin uppföljning hade genomgått lungfunktionsundersökning ofta utvecklade KOL över tid. Detta trots att de flesta av patienterna aldrig hade rökt. Sjögrens syndrom tycktes alltså kunna bidra till uppkomst av sjukdomen KOL. Detta sambandet visste man mycket litet om, så fler studier behövdes.

Målet med denna avhandling var att undersöka hur vanligt det är med lungfunktionsnedsättning och KOL är hos Sjögrenpatienter och hur lungfunktionen utvecklas över tid. Målet var också att undersöka om det fanns kopplingar mellan lungfunktion och olika sjukdomsytttringar av Sjögrens syndrom, luftvägssymptom, inflammationstecken i luftvägarna eller fynd på skiktröntgen. Sådana kopplingar skulle man i så fall kunna ha nytta av för att värdera risk för lungengagemang och tidigt upptäcka lungengagemang hos Sjögrenpatienter och på sikt hjälpa dessa patienter bättre.

## Avhandlingsdelarnas resultat och möjlig användning

Avhandlingen har baserats på fyra delarbeten, där hittills tre av arbetena har publicerats i internationella reumatologiska tidskrifter. Studierna ägde rum på Reumatologiska kliniken på Skånes Universitetssjukhus (SUS) i Malmö mellan 2012-2018, där patienterna undersöktes avseende sjukdomsyttningar av Sjögrens syndrom vid läkarbesök. Patienterna fick också bland annat fylla i frågeformulär om hosta, andfåddhet och rökvanor samt fick genomgå lungfunktionstester och vanlig och högupplöst skiktröntgen (CT och HRCT) av lungorna.

Första delarbetet var en tvärsnittundersökning, som innefattade 51 Sjögrenpatienter. Lungfunktionstesterna visade att KOL var vanligt och sågs hos 40 % av patienterna. Även bland patienterna som aldrig hade rökt var KOL vanligt och sågs hos nära en tredjedel av dessa patienter. Detta gör att man kan misstänka att sjukdomen primärt Sjögrens syndrom i sig verkar kunna bidra till uppkomst av sjukdomen KOL. Skiktröntgen (CT) visade att både luftrörsförändringar och olika typer av förändringar i själva lungvävnaden var vanligt hos patienterna. Man behöver alltså vara frikostig med att undersöka patienter med Sjögrens syndrom för att upptäcka lungengagemang.

Det sista delarbetet var en sexårsuppföljning av lungfunktionen hos Sjögrenpatienter från det första delarbetet. I detta arbete användes högupplöst skiktröntgen av lungorna (HRCT), som är en känsligare undersökning än vanlig skiktröntgen (CT). KOL var fortsatt vanligt och sågs fortsatt hos kring 40 % av patienterna. Oftast rör det sig om en mild-måttlig svårhetsgrad av KOL. Gasutbytet, som är ett sätt att mäta lungornas funktion, försämrades däremot efter sex år. Orsaken till detta är inte klar, men flera faktorer kan bidra. Skiktröntgen visade fortsatt att både inflammation i luftvägarna och olika förändringar i lungvävnaden var vanligt hos Sjögrenpatienterna.

I andra delarbetet undersöktes 37 Sjögrenpatienter med så kallad forcerad oscillationsteknik (FOT), som är en känslig metod för att påvisa tecken på trånga luftvägar. Undersökningen är enkel och går ut på att ljudvågor, av olika frekvenser, skickas från ett munstycke vidare ner i luftvägarna. Mätningar av hur ljudvågorna studsar tillbaka kan sedan visa tecken på trånga luftvägar på olika nivåer i luftvägsträdet. FOT visade tecken på trängre luftvägar, i både de små och de större luftvägarna, hos patienterna jämfört med friska kontrollpersoner. Även hos patienter, där vanlig lungfunktionsundersökning var normal, visade FOT tecken på trånga luftvägar. Metoden har tidigare inte använts rutinemässigt på Sjögrenpatienter, men FOT skulle kunna vara användbar, som en känslig undersökningsmetod, för att tidigt upptäcka trånga luftvägar, innan KOL har hunnit utvecklas.

I tredje delarbetet undersöktes 20 Sjögrenpatienter, som aldrig hade rökt, avseende tecken på inflammation i luftvägarna. Patienterna fick genomgå så kallad inducerat sputum undersökning, där förångad koksaltlösning andas in för att underlätta

upphostning av sekret från luftvägarna. I sekretet sågs tecken på ökad inflammation i jämförelse med sekret från friska kontrollpersoner. Metoden används inte rutinmässigt vid Sjögrens syndrom, men skulle kunna vara användbar för att tidigt upptäcka tecken på inflammation i luftvägarna, redan innan KOL har hunnit utvecklas. Man skulle också kunna använda metoden i fortsatt forskning för att kartlägga inflammationen i luftvägarna bättre. Detta skulle kunna vara till hjälp för att utveckla mediciner som kan bromsa inflammationen i luftvägarna och lungorna vid primärt Sjögrens syndrom.

Överlag hade patienternas symptom från luftvägarna (hosta och andfåddhet) och sjukdomsyttningarna av Sjögrens syndrom mycket små kopplingar till lungfunktion, skiktröntgen och inflammationstecken i luftvägarna. Att kopplingarna var få antyder att det är extra viktigt att Sjögrenpatienterna blir undersökta med lungfunktionstest och skiktröntgen för att upptäcka lungengagemang, för att på sikt kunna hjälpa patienterna bättre.

I denna avhandling har tecken på trånga och inflammerade luftvägar, KOL och lungengagemang visat sig vara vanligt vid primärt Sjögrens syndrom. Det är viktigt att vara uppmärksam och frikostigt undersöka detta vid omhändertagandet av Sjögrenpatienter. Att KOL visat sig vanligt även hos Sjögrenpatienter som aldrig rökt gör att man får misstänka att sjukdomen i sig är bidragande till utvecklingen av KOL. Hur detta kommer sig behöver man studera vidare. Antireumatiska mediciner kan i framtiden komma att utvecklas, som kan göra det möjligt att behandla och möjligen även förebygga inflammation i luftvägarna, utveckling av KOL och lungengagemang vid primärt Sjögrens syndrom.

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