Lymphoma studies in patients with Sjögren's syndrome

LILIAN VASAITIS
Patients with primary Sjögren’s syndrome (pSS) are at increased risk of developing malignant lymphoma. The studies in this thesis aim at broadening our understanding of the association between these two conditions.

Germinall centre (GC)-like structures were found in minor salivary gland biopsies taken at the time of pSS diagnosis in 25% of 175 studied patients. Lymphoma development was observed in 86% of the GC-positive pSS patients and 14% of the GC-negative patients. GC-like structures in salivary gland biopsies at pSS diagnosis might identify pSS patients at high risk for later lymphoma development.

We used the National Patient Register and the Cancer Register to identify pSS patients with lymphoid malignancy for the following studies. The lymphoma tissues were reviewed and classified according to the WHO classification.

In a study of 79 patients with available lymphoma tissues, we identified histopathological and clinical features compatible with IgG4-related disease (IgG4-RD) in one patient (1.3%). Histological features of IgG4-RD in lymphoma tissue in patients with an initial pSS diagnosis seem to be rare but, if present, may indicate underlying IgG4-RD.

We identified and compared pSS patients with (n=18/17%) and without (n=87) pre-existing lymphoma at pSS diagnosis and found similar pSS characteristics in both groups. Mucosa-associated lymphoid tissue (MALT) lymphoma in salivary glands was more common in patients with pre-existing lymphoma. The findings support the removal of pre-existing lymphoma as a general exclusion criterion for a pSS diagnosis in classification criteria. Further, the findings suggest an investigation for pSS in patients presenting with MALT lymphoma in salivary glands.

We compared the distribution of lymphoma subtypes with a general population reference. Both diffuse large B-cell lymphoma (DLBCL) (32%) and marginal zone lymphoma (MZL) (31%) were common, but only MZL (MALT lymphomas) occurred at an increased relative frequency compared to the general population.

Men constituted 15% of 105 pSS patients with lymphoma. Men had a shorter time between the pSS and lymphoma diagnoses and more often had lymphoma in the salivary glands compared with women. Increased awareness of signs of lymphoma in salivary glands already during the first years after pSS diagnosis is justified in men with pSS.

Keywords: Sjögren's syndrome, primary Sjögren's syndrome, lymphoma, IgG4-related disease

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To Indra and Paulius
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Related Papers


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Abbreviations

ANA  Anti-nuclear antibody
ACR  American College of Rheumatology
AECG American European Consensus Group
DC  Dendritic cell
DLBCL  Diffuse large B-cell lymphoma
EBV  Epstein-Barr virus
ESSDAI  EULAR Sjögren’s syndrome disease activity index
EULAR  European League Against Rheumatism
GC  Germinal centre
H&E  Haematoxylin and eosin
HPF  High power field
HL  Hodgkin lymphoma
ICD  International Classification of Diseases
IFN  Interferon
Ig  Immunoglobulin
IgG4-RD  IgG4-related disease
IL  Interleukin
MALT  Mucosa-associated lymphoid tissue
MD  Mikulicz’s disease
MZL  Marginal zone lymphoma
NF-κB  Nuclear factor kappa-light-chain-enhancer of activated B cells
NHL  Non-Hodgkin lymphoma
PC  Plasma cell
pSS  Primary Sjögren’s syndrome
RA  Rheumatoid arthritis
RF  Rheumatoid factor
RTA  Renal tubular acidosis
SIR  Standardised Incidence Ratio
SLE  Systemic lupus erythematosus
SS  Sjögren’s syndrome
sSS  Secondary Sjögren’s syndrome
SSA/Ro  Sjögren’s syndrome antigen A=Ro
SSB/La  Sjögren’s syndrome antigen B=La
Th  T helper
TLR  Toll-like receptor
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
Autoimmune diseases are mostly chronic disorders in which the immune system mistakenly attacks the body’s own organs and tissues. There are over 80 different autoimmune diseases and about 4.5% of the population are affected by them (1). Most autoimmune diseases predominantly affect women (2) indicating different etiopathogenetic mechanisms of immune response and development of autoimmunity in female and male individuals.

Typically, autoimmune chronic inflammation has a fluctuating course with periods of remission (no or little activity) and flare-ups (worsening of symptoms). Diagnosis of the autoimmune disease may often be delayed or mistakenly diagnosed as another disorder because of unspecific vague symptoms and fluctuating course. Whence the autoimmune disease is diagnosed, it often requires continuing or long periods of immunosuppressive treatment.

Over time, continuous or periodical activation of the immune response can lead to the development of lymphoid malignancy. Chronic autoimmune conditions, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and particularly primary Sjögren’s syndrome (pSS) are associated with increased risk of lymphoma (3). It is, therefore, an important research field to study mechanisms and associated factors behind lymphoma and autoimmunity.

In this thesis, the introduction first gives an overview of the hallmarks of Sjögren’s syndrome, lymphoma and IgG4-related disease (IgG4-RD), and then four studies of pSS and lymphoid malignancy are introduced. The first study presents lymphoid organisation in labial salivary gland biopsies taken at pSS diagnosis as a possible predictor for future lymphoma development. This study is performed on pSS patients from two hospitals. The remaining studies (II-IV) are nationwide and performed on non-selected pSS patients. The second study elucidates the occurrence of undiagnosed IgG4-RD in previously misdiagnosed pSS patients with lymphoma. In the third study, detailed analysis of the pSS patients’ characteristics with and without pre-existing lymphoma at pSS diagnosis was performed, and investigation of underlying causes of Sjögren’s syndrome (SS) code in the patient register is presented. The last study elucidates gender differences in pSS patients with lymphoid malignancy, compares the pSS patients with the two most common subtypes of low-grade and high-grade lymphomas, and evaluates the distribution of the subtypes of lymphoma in pSS patients in comparison with a general population reference group.
Sjögren’s syndrome

SS, also known as ‘sicca syndrome’, is a systemic chronic autoimmune disease which affects the moisture-producing glands. Patients develop characteristic sicca symptoms, namely dry eyes (xerophthalmia) and/or dry mouth (xerostomia). In 1933, Henrik Sjögren was the first who described this syndrome in 19 patients with RA and dry eyes, establishing that sicca symptoms may extend beyond glandular involvement (4). SS is currently divided in pSS, existing by itself, and in secondary SS (sSS), usually coexisting with other autoimmune diseases, such as RA (5), SLE (6) or systemic sclerosis (7, 8). Compared to RA and SLE, SS is a relatively mild disease but with significant morbidity (9) that attracts both clinical and research interest.

Stress, smoking, drugs, and diabetes are also common causes for unspecific dryness symptoms. In about a quarter of the elderly individuals (above the age of 65 years) sicca complaints are present, which are largely due to medication. Moreover, aging also leads to diminished glandular function (10) and development of dry mucous membranes. Dryness of the eyes and mouth may also occur in the following conditions: after radiation therapy to the head and neck; after allogeneic stem cell transplantation, when graft versus host disease develops; in patients with malignancy in the salivary or lacrimal glands; in some cases of sarcoidosis; in tuberculosis or other infections, such as hepatitis C or acquired immunodeficiency syndrome. Causes of sicca syndrome (other than pSS and sSS) are summarised in Table 1.

Table 1. Causes of sicca symptoms other than primary and secondary Sjögren’s syndrome*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Drug side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-related glandular atrophy</td>
<td>Anticholinergic drugs (atropine, scopolamine)</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Antihistamines</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>α1-antagonists (prazosin, terazosin)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>α2-antagonists (clonidine)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>β-blockers (atenolol, propranolol)</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>Diuretics</td>
</tr>
<tr>
<td>Graft versus host disease</td>
<td>Nicotine</td>
</tr>
<tr>
<td>IgG4-related disease</td>
<td>Phenothiazines</td>
</tr>
<tr>
<td>Infections: hepatitis C, HIV, tuberculosis</td>
<td>Opioids</td>
</tr>
<tr>
<td>Psychological factors</td>
<td>Tricyclic antidepressants</td>
</tr>
<tr>
<td>Salivary gland trauma or tumour</td>
<td>Selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Sympathomimetic drugs (ephedrine)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Rischmueller et al. (11).

The World Health Organisation (WHO) International Classification of Diseases (ICD), does not, however reflect the diversity of the causes of sicca
symptoms (http://apps.who.int/classifications/icd10/browse/2016/en). In this system, the same code M35.0 (ICD-10 classification) is assigned for “Sjögren’s syndrome/sicca syndrome” regardless of the underlying reason for the dryness symptoms. From registers based on ICD codes, it is therefore not possible to identify whether patients have, for example, pSS or sSS, or other underlying causes of a sicca syndrome diagnosis code.

Primary Sjögren’s syndrome

No diagnostic criteria and no single gold-standard test exist for the diagnosis of pSS. The typical patient with pSS exhibits sicca symptoms. However, clinically pSS extends from dryness symptoms due to lymphocytic infiltration and diminished function of salivary and lacrimal glands to a systemic involvement of inflammation accompanied by fatigue, and occurrence of extraglandular manifestations.

The clinical symptoms, both sicca and systemic, as well as serological features, are not specific for pSS only. They can mimic or overlap with other rheumatic autoimmune systemic diseases, infections or fibromyalgia. In fact, no specific diagnosis can be made at the first presentation of a patient with sicca symptoms. In some cases, the difficulties of diagnosing pSS may lead to erroneous diagnosis, for example, fibromyalgia, SLE or RA (12). Patients with vague symptoms of sicca syndrome can also be undiagnosed or diagnosed in the latter stages of the disease. Therefore, other causes of sicca syndrome and systemic features need to be considered in diagnosing pSS through a detailed medical history with an accurate examination of the patient, and in some cases, a multidisciplinary approach and exclusions of other diagnoses.

Classification criteria

The variability of clinical pSS presentation led to the development of different classification criteria for SS. It is important to note that classification criteria are not synonymous with diagnostic criteria. The ultimate goal for diagnosing a patient with SS is to be correct at the level of the individual patient even if the patient does not completely fulfil the criteria, whereas classification aims to maximally increase the homogeneity of the population for study purpose.

Before 2002, many different sets of classification criteria for SS were published (13-15). The numerous proposed criteria reflect the difficulties of defining this heterogeneous syndrome. During the 1980s-90s, the most known criteria in Sweden were the San Diego or Californian (13), the Copenhagen (14), and the preliminary European (16) criteria.
The preliminary European criteria were proposed in 1993 and focused on the three characteristic features of SS: sicca symptoms, glandular dysfunction, and systemic autoimmunity. These criteria could misclassify patients with non-autoimmune sicca syndrome as having pSS, based on subjective ocular and oral symptoms alone. To overcome this possible misclassification, the European criteria were revised in 2002 and published as the American-European consensus group (AECG) criteria for the classification of SS (17) (Table 2).

Table 2. 2002 Revised American-European consensus group (AECG) criteria*

| I. Ocular symptoms: positive response to one of the following claims: |
| 1) daily persistent trouble with dry eyes for more than 3 months; 2) recurrent sensation of sand or gravel in the eyes; 3) usage of tear substitutes more than three times per day |
| II. Oral symptoms: positive response to one of the following claims: |
| 1) daily feeling of dry mouth for more than three months; 2) recurrent or persistent swollen salivary glands as an adult; 3) need frequently drink liquids to aid swallowing dry food |
| III. Ocular signs: positive Schirmer’s test performed without anesthesia (≤5 mm in 5 minutes) or positive rose Bengal or another ocular dye score (≥4 according to van Bijsterveld) |
| IV. Histopathology: focal lymphocytic sialadenitis with a focus score ≥1 focus, defined as a number of lymphocytic foci per 4 mm² of minor salivary gland tissue (one focus contains >50 lymphocytes) |
| V. Salivary gland involvement: unstimulated whole salivary flow (≤1.5 mL in 15 minutes) |
| VI. Autoantibodies: presence of anti-SSA/Ro or anti-SSB/La or both |

Rules:
for pSS: in patients without any associated disease and the presence of four of the six items, including positive histopathology (IV) or serology (VI), or the presence of three of the four objective items (III, IV, V, VI);
for sSS: in patients with associated other well-defined connective tissue disease and the presence of item I or item II, plus any two from among items III, IV, and V.

Exclusion criteria: pre-existing lymphoma, past head and neck radiation therapy, hepatitis C, acquired immunodeficiency disease, sarcoidosis, graft versus host disease, use of anticholinergic drugs

*Adapted from Vitali et al. (17).

The 2002 AECG criteria have been commonly used in epidemiological SS studies and also as support for the diagnosis of SS in everyday clinical practice. The criteria have 90% sensitivity and 95% specificity for pSS diagnosis. They distinguish between pSS and sSS and differ from previously used criteria in their requirements for either a positive serology (anti-SSA/Ro and/or anti-SSB/La) or a positive labial biopsy (a focal lymphocytic sialadenitis with a focus score of ≥1). According to the 2002 AECG criteria, pSS is defined as the presence of four of the six items, including positive histopathology (item IV) or serology (item VI), or the presence of three of the four objective items (III, IV, V, VI).

In 2012, the Sjögren’s International Collaborative Clinical Alliances Cohort (SICCA) and the American College of Rheumatology (ACR) (18) approved solely objective criteria for SS (Table 3).
Table 3. 2012 American College of Rheumatology criteria for SS*

<table>
<thead>
<tr>
<th>I. Autoantibodies:</th>
<th>presence of anti-SSA/Ro and/or anti-SSB/La or RF and ANA titer $\geq 1:320$</th>
</tr>
</thead>
<tbody>
<tr>
<td>II. Histopathology:</td>
<td>focal lymphocytic sialadenitis with a focus score $\geq 1$ focus, defined as a number of lymphocytic foci per $4 \text{ mm}^2$ of minor salivary gland tissue</td>
</tr>
<tr>
<td>III. Ocular staining:</td>
<td>keratoconjunctivitis sicca with ocular staining score $\geq 3$</td>
</tr>
</tbody>
</table>

Rules for SS:
in individuals with signs/symptoms that may be suggestive of SS who exhibited at least 2 out of 3 objective features

Exclusion criteria:
past head and neck radiation therapy, hepatitis C, acquired immunodeficiency disease, sarcoidosis, amyloidosis, graft versus host disease, IgG4-related disease

*Adapted from Shiboski SC et al. (18).

These criteria were developed for enrolling patients with SS in clinical trials. The subjective sicca symptoms were considered as inclusion criteria in the subject population before applying these objective criteria. The criteria have only slightly higher sensitivity (93%) and the same specificity as the AECG criteria. The 2012 ACR criteria set does not distinguish between pSS and sSS. Furthermore, IgG4-RD, recognised as a new disease entity in 2012, has been added into the exclusions, while pre-existing lymphoma has been removed from the exclusion criteria. The rationale of removing the criterion of pre-existing lymphoma from the 2012 ACR criteria is unknown and this issue has not been discussed.

Recently, the international Sjögren’s syndrome criteria working group from both the European League Against Rheumatism (EULAR) and the ACR has developed and approved the new 2016 ACR/EULAR criteria (19), merging both the 2002 AECG and the 2012 ACR criteria (Table 4).

Subjective symptoms of ocular and oral dryness and isolated anti-SSB/La positivity without positivity for anti-SSA/Ro (20) were excluded by the expert-panel, as these items had no independent diagnostic significance for pSS (19). Instead, sicca symptoms or suspicion of SS based on at least one of the domains of the EULAR Disease Activity Index (ESSDAI) (21) for patients with pSS are preliminary requirements for applying these 2016 ACR/EULAR criteria (19). The criteria are based on the weighted sum of five objective items/tests applicable to any patient with at least one symptom of ocular or oral dryness and individuals are classified as having pSS if they have a score of $\geq 4$.

The last criteria from 2016 are improved compared to the previous criteria and are more practically applicable as simple tests, such as Schirmer’s and unstimulated salivary flow tests, which are already a part of common clinical practice, have been added to the items of the criteria. Additionally, the scores of these simple tests are equal to the ocular staining score. The ocular staining, as it was required in 2012 ACR criteria, craves a specific evaluation performed by a trained ophthalmologist, an examination, which is not available in all cases.
Table 4. 2016 ACR/EULAR criteria for pSS*

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight/Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal lymphocytic sialadenitis with a focus score ≥1 focus, defined as a number of lymphocytic foci per 4 mm² of minor salivary gland tissue</td>
<td>3</td>
</tr>
<tr>
<td>Anti-SSA/Ro-positive</td>
<td>3</td>
</tr>
<tr>
<td>Ocular staining score ≥5 (or von Bijsterveld score ≥4) in at least one eye</td>
<td>1</td>
</tr>
<tr>
<td>Schirmer’s test ≤5 mm/5 min in at least one eye</td>
<td>1</td>
</tr>
<tr>
<td>Unstimulated whole saliva flow rate ≤0.1 mL/min</td>
<td>1</td>
</tr>
</tbody>
</table>

**Inclusion criteria:** The criteria are applicable to any individual with at least one sicca symptom, 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 swelling dry food?

Or in whom there is suspicion of SS from the ESSDAI questionnaire (at least one domain with a positive item).

**Exclusion criteria:** past head and neck radiation therapy, active hepatitis C infection, acquired immunodeficiency disease, sarcoidosis, amyloidosis, graft versus host disease, IgG4-RD

**Rules for pSS:** for individuals who meet inclusion criteria, does not have any of the conditions listed as exclusion criteria and has a score of ≥4

*Adapted from Shiboski CH et al. (19).

The 2016 ACR/EULAR criteria also consider a systemic disease at inclusion by evaluation of the ESSDAI and allow classification of patients with pSS at early stages of the disease. Moreover, these criteria seem to be more liberal and eligible to classify pSS patients than the 2002 AECG criteria. For instance, a patient with dry mouth symptoms (subjective sicca symptoms at the time of inclusion) or swollen salivary gland (the glandular domain in the ESSDAI at the time of inclusion) with a positive unstimulated salivary flow test (one weight point), and positive anti-SSA/Ro antibodies (three weight points) has four weight points and fulfils the 2016 ACR/EULAR criteria even if other items are not fulfilled. Meanwhile, a patient in the same situation fulfils only three items of the 2002 AECG criteria and would not fulfil these criteria for pSS.

The latest criteria have the same specificity as the 2002 AECG and the 2012 ACR criteria, but the sensitivity is higher (96%). The criteria have been developed specifically for classification of pSS in clinical trials and studies. They do not define sSS, but the criteria can still be applicable to SS associated with other autoimmune rheumatic diseases. The exclusion criteria are almost the same as in the 2012 ACR criteria, except for the addition of active hepatitis C infection confirmed by polymerase chain reaction. Pre-existing lymphoma is not an exclusion criterion in these criteria because according to the experts in the working groups, pSS can be diagnosed after lymphoma. However, no confirmative study on this issue has been addressed.

So far, the 2002 AECG criteria are the most widely used criteria in pSS studies. The 2012 ACR criteria are too strict and not so practical. The new
2016 ACR/EULAR criteria are simple to apply in pSS studies and possibly well suited as a support in everyday clinical practice.

ESSDAI

In 2009, the EULAR committee developed the ESSDAI (21) to measure systemic disease activity in patients with pSS (Table 5). Like the SLEDAI (22) or BILAG (23) in lupus, the ESSDAI is a gold standard for evaluation of pSS activity in clinical studies (24). The index includes 12 domains: constitutional domain (fever, weight loss, sweating), nine organ-specific domains (lymph nodes, glandular, articular, cutaneous, pulmonary, renal, muscular, peripheral and central nervous system), haematological (cytopenia), and biological (clonal component, hypocomplementemia, hypergammaglobulinemia or recent decrease of IgG level).

Depending on the degree of activity of the organ manifestation, 3-4 levels of activity with different weight points (ranging from one to six) are assigned to each domain. The score of each domain is obtained by multiplying the level of activity by the domain weight. The final score is the sum of all domain scores ranging from zero to theoretically a max score that can be achieved, 123. Low activity has been defined as the ESSDAI score being <5, a moderately active disease if the ESSDAI score is ≥5 but ≤13, and a high activity if the index is ≥14. Threshold of minimal clinically important improvement is a decrease of the initial score of at least by three points (25).

Some pSS patients can exhibit systemic disease or extraglandular manifestations without sicca symptoms (26, 27). The ESSDAI measurement in some cases may help to diagnose pSS in early stages and differentiate pSS from other autoimmune diseases. Moreover, baseline ESSDAI is associated with the prognosis of pSS. Activity in the constitutional and lymphadenopathy domains is associated with lymphoma, and pulmonary involvement can be one of the main predictors of death (28). Thus, the ESSDAI can be used not only for measuring the disease activity at pSS diagnosis, but also during treatment as a tool to assess the efficacy of the treatment, and can also be useful for identifying patients with a systemic disease.
Table 5. EULAR proposed Sjögren’s syndrome Disease Activity Index (ESSDAI)*

<table>
<thead>
<tr>
<th>Domain</th>
<th>Weight</th>
<th>Characteristics of the domain</th>
<th>Activity levels**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Constitutional</td>
<td>3</td>
<td>Fever, night sweats, weight loss</td>
<td>0-2</td>
</tr>
<tr>
<td>2. Lymphadenopathy and lymphoma</td>
<td>4</td>
<td>Swollen lymph nodes, splenomegaly, current B-cell proliferative malignancy</td>
<td>0-3</td>
</tr>
<tr>
<td>3. Glandular</td>
<td>2</td>
<td>Swollen salivary and/or lacrimal glands</td>
<td>0-2</td>
</tr>
<tr>
<td>4. Articular</td>
<td>2</td>
<td>Arthralgias with morning stiffness, or synovitis among 28 joints</td>
<td>0-3</td>
</tr>
<tr>
<td>5. Cutaneous</td>
<td>3</td>
<td>Erythema multiforme, cutaneous, including urticarial, vasculitis, or purpura, or subacute cutaneous lupus, ulcers related to vasculitis</td>
<td>0-3</td>
</tr>
<tr>
<td>6. Pulmonary</td>
<td>5</td>
<td>Persistent cough, bronchial involvement, or radiological evidence of ILD</td>
<td>0-3</td>
</tr>
<tr>
<td>7. Renal</td>
<td>5</td>
<td>Tubular acidosis, glomerular involvement with proteinuria &gt;0.5 g/L, or haematuria, or renal failure, or histological evidence of glomerulonephritis, interstitial nephritis, or cryoglobulinemia-related renal involvement</td>
<td>0-3</td>
</tr>
<tr>
<td>8. Muscular</td>
<td>6</td>
<td>Active myositis proven by abnormal EMG or biopsy with or without weakness or elevated creatine kinase</td>
<td>0-3</td>
</tr>
<tr>
<td>9. Peripheral nervous system</td>
<td>5</td>
<td>Evidence of active peripheral nerve involvement proven by nerve-conductive studies, trigeminal neuralgia, or cranial peripheral nerve involvement</td>
<td>0-3</td>
</tr>
<tr>
<td>10. Central nervous system</td>
<td>5</td>
<td>Cranial nerve involvement, optic neuritis, multiple sclerosis-like syndrome with pure sensory, or cognitive impairment, or motor deficit, cerebral vasculitis with cerebrovascular accident, seizures, transverse myelitis, lymphocytic meningitis</td>
<td>0-2</td>
</tr>
<tr>
<td>11. Haematological</td>
<td>2</td>
<td>Cytopenia of autoimmune origin with neutropenia, anaemia, thrombocytopenia or lymphopenia</td>
<td>0-3</td>
</tr>
<tr>
<td>12. Biological</td>
<td>1</td>
<td>Clonal component, cryoglobulinemia, or hypocomplementemia, or hypergammaglobulinemia with IgG&gt;15 g/L, or recent onset of hypogammaglobulinemia (IgG&lt;5 g/L)</td>
<td>0-2</td>
</tr>
</tbody>
</table>

*Adapted from Seror et al. 2010 (21).
**0=no activity, 1=low activity, 2=moderate activity, 3=high activity.
ILD=interstitial lung disease; EMG=electromyogram.

ESSPRI

The EULAR SS Patients Reported Index (ESSPRI) has been proposed in 2011. It uses numerical scales of 0 to 10 and assesses the patients’ symptoms in three domains: dryness, fatigue, and musculoskeletal pain over the preceding two weeks (29). The ESSPRI score is the mean of the three scales.

Epidemiology

Estimated incidence and prevalence of pSS varies in different studies and depends on studied population and the classification criteria used. Similar to
most autoimmune diseases, pSS is more common in women than in men. The female-to-male ratio varies from 9:1 to 20:1 (30-34). An estimated incidence of pSS is 3.9-11 per 100,000 inhabitants with a significantly higher incidence in women compared to men (31, 32, 35-38).

When the 2002 AECG criteria (17) are applied in epidemiological studies, the prevalence of pSS is 0.01-0.7%, (31, 39-46). It seems that those with non-European background have two times higher prevalence of pSS (46). The onset of sicca symptoms may be exhibited at all ages and many years before diagnosis. The mean age of established pSS diagnosis is usually in the fourth or fifth decades of life (31, 34, 47, 48).

Etiology and pathogenesis

The etiology of pSS is largely unknown as in other autoimmune diseases. It is supposed that a combination of multiple environmental and hormonal factors with a genetic predisposition can contribute to pSS development (49). Innate and adaptive hyperactive immune responses occur locally, in salivary and lacrimal glands, and systematically. Different cells, chemokines, and cytokines are involved in the complex interactions and pathways, which are not yet fully elucidated. A trigger inducing the cascade of the autoreactive inflammatory response in pSS has not yet been identified. The initial signal, viral or non-viral, may lead to a process ending in tissue damage and cell death or apoptosis (Fig. 1).

Ribonucleoprotein particles Ro and La autoantigens, expressed on blebs at the surface of apoptotic epithelial cells of the exocrine glands (50), that have also been identified as antigens in lupus (51), are supposed to lead to chronic inflammation and dysfunction of the glands (autoimmune epithelitis). Another possible autoantigen in pSS can be the cytoskeletal protein α-fodrin also found in apoptotic cells (52). Type I interferon (IFN) is activated in both blood and salivary glands in pSS patients, so-called ‘IFN signature’. IFN activation is seen in virus infection, therefore, viruses have been suggested as a possible trigger of pSS, but so far this has not been proven (53). Recent data indicate that gut-commensal bacteria may be relevant to the development of pSS (54, 55).

pSS occurs most commonly in middle-aged women. Diminished oestrogen production in post-menopausal women could contribute to pSS (10). Some studies have suggested that not only sex hormones but also X-chromosome dosage may play a role in pSS development (56, 57).
Figure 1. A potential explanation of autoimmune epithelitis in primary Sjögren’s syndrome. The first step is tissue damage by an unknown trigger leading to apoptosis with subsequent expression of the SSA/Ro and/or SSB/La proteins. The next step is T and B cell activation, autoantibody production by B cells, and dysfunction of dendritic cells in the exocrine glands, the formation of germinal-centre (GC)-like structures and development of histopathological lesions. The third step is perpetuation, in which cytokines and chemokines promote migration of lymphocytes and dendritic cells and further secretion of the cytokines. PDC=plasmacytoid dendritic cell, Tfh=T follicular helper, Th17=T helper 17, IC=immune complexes, IL=interleukin, IFN=interferon, TLR=toll-like receptor, BAFF=B-cell activating factor, FDC=follicular dendritic cell. The figure was produced by the author using Servier Medical Art and adapted from Brito-Zeron et al. (4).

Environmental factors

Virus

It has been proposed that viruses from the Herpesviridae family, such as Epstein-Barr virus (EBV), human herpesvirus 6 (HHV6), and retrovirus human T cell lymphotropic virus (53), may be involved in pSS pathogenesis, but so far this hypothesis has not been confirmed by studies (58). However, by increasing understanding of the genetic factors and the central role that type I and II IFN play in autoimmunity (59), it is of interest how viruses may trigger autoimmunity a long time before manifestation of a disease (4). This interest of the “pre-pSS” phase is based on reports that antibodies linked to pSS are present in sera many years before clinical manifestations of the disease (26, 60).
Smoking
Smoking is a risk factor for SLE, myositis (61), and RA (62), whereas, in some conditions, such as ulcerative colitis, there is a negative correlation between current smoking and disease activity (63).
There is limited and inconsistent information about smoking and pSS. A lower rate of smoking with a higher frequency of antinuclear antibody (ANA) positivity in smokers has been reported in pSS (64). However, some other studies have reported lower frequencies of sialadenitis and SSA/Ro positivity in active smokers with pSS (65-67). Thus, current smoking may have a protective effect against pSS disease-associated humoral and cellular autoimmunity (67), but former smoking may increase risk for pSS (66).

Innate immune system dysfunction in pSS
Cells and cytokines
Plasmacytoid dendritic cells (DCs), one of the many innate immune system cells infiltrating glandular tissue, are potent producers of type I IFN (68). It is supposed that an activated type I IFN system plays a significant role in the pathogenesis of SLE and pSS (69-71). The role of IFN in pSS is discussed in a separate chapter (see below).
Other cells, for example, macrophages and salivary gland epithelial cells, are involved in the pathogenesis as well. Macrophages produce proinflammatory cytokines, such as interleukin (IL)-18 (72) and IL-12, and contribute to glandular enlargement (73). The epithelial cells in the glandular tissue may act as antigen-presenting cells (74), express chemokines, promoting glandular localisation of T cells, and produce proinflammatory cytokines, such as IL-6, IL-7, and B-cell activating factor (BAFF) that are important in B-cell pathophysiology (72, 75).

Toll-like receptors
There are ten different Toll-like receptors (TLRs) or pattern-recognition receptors expressed in cellular membranes or intracellularly in the endosomes. They are involved in the first defence against infections by recognising a variety of pathogen-associated molecular patterns (PAMPs), such as viral nucleic acid or bacterial DNA. TLRs can also be activated by so-called damage-associated molecular pattern (DAMP) molecules from apoptotic cells (76). The interaction between BAFF and TLR3 on epithelial cells, and TLR7 and TLR9 on plasmacytoid DCs and epithelial cells promote B-cell maturation and production of self-reactive antibodies (77, 78). This inflammatory process has a vicious circle-like mechanism (Fig. 1) with increased autoantibody production and formation of more endogenous IFN producers (59). Further, different expression of TLRs has been reported in peripheral blood cells in pSS and healthy controls (79) supporting that TLRs may play a role in pSS pathogenesis.
Adaptive immune system dysfunction in pSS

**T cells**

In 1983, it was published that T cells are a predominate component of the lymphocytic infiltrate in the salivary and lacrimal glands in pSS and the majority of them are CD4+ T cells (80). Further, a predominant T helper 1 (Th1) expression has been reported in pSS including increased levels of pro-inflammatory cytokines, responsible for Th1 response, such as IL-1β, IL-6, tumour necrosis factor (TNF)-α, and INF-γ in salivary glands (81). Moreover, increased expression of IL-17, IL-22, and IL-23, products of mucosal natural killer cells and Th17 cells, has been found in the inflamed salivary glands of patients with pSS (82). The related cytokines to IL-17, for example, TGF-β, IL-6, IL-12, and IL-23, have been found in increased levels in plasma from pSS patients (83, 84). Moreover, dysfunction of T regulatory (Treg) cells, expressing Foxp3⁺, with impaired function of suppression on Th17 cells, may play a role in pSS development (85).

It is supposed that T cells, activated by unknown autoantigen, may activate B cells. It is known that CD4+ T follicular helper (Tfh) cells arise from activated T cells (86). The Tfh cells produce Bcl-6, IL-6, and IL-21, and assist B cells during germinal centre (GC) formation in lymphoid organs (86). In this way, T cells control antigen-specific B-cell immunity.

**B cells**

B cells play a critical role in pSS pathogenesis. B cells are involved in autoantibody and cytokine production, antigen presentation and regulatory functions (87). High levels of autoantibodies that target the self-antigens SSA (Ro52 and Ro60) and SSB (La) are common in pSS (88). B cells also produce other antibodies in pSS, inclusive rheumatoid factor (RF), ANA, and anti-muscarinic acetylcholine M3 receptor antibodies (89, 90). This hyperactivity of B cells may result in hypergammaglobulinemia (91) and formation of GC-like structures in salivary glands (92).

Moreover, alterations in B cell subsets have been reported. Decreased numbers of CD27⁺ memory B cells in peripheral blood (93-95) and salivary glands (96) have been found. In contrast, increased numbers of marginal zone B cells have been reported in peripheral blood and salivary glands (97). These disturbances in B cell subset distribution can be important in lymphoma development in pSS patients.

**BAFF and APRIL**

BAFF, also known as BLys or TNFSF18, and a proliferation-inducing ligand (APRIL) or TNFSF13A, are members of the TNF-superfamily described in 1999. BAFF and APRIL are expressed by T cells, DCs, monocytes, and macrophages. The overexpression of BAFF and APRIL by these cells activate B cells and may in this way contribute to autoimmunity (98, 99). More-
over, BAFF that is generated from memory B cells has emerged as a potent survival factor for plasmablasts and may promote B-cell lymphomas (99). BAFF is found in increased levels in pSS and plays a significant role for GC-like structure development and establishment of follicular DC networks (92, 100).

**NF-κB signalling and A20 regulation**

Abnormalities in nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) signalling mechanisms play a central role in pSS inflammation, cell differentiation, and apoptosis (101, 102). Several studies have reported polymorphisms in genes associated with the NF-κB pathways in autoimmune diseases and pSS (103-105).

The ubiquitin-editing enzyme A20 (tumour necrosis factor-α-induced protein 3, TNFAIP3) inhibits NF-κB signalling (106-108). Impaired function in A20 regulation may enhance activation of the NF-κB pathway and lead to sustained autoimmune inflammation and oncogenic mutations (103).

**Interferon**

Induction of transcription of type I IFN genes may be triggered by a virus or by immune complexes containing nucleic acids. Plasmacytoid DCs synthesise IFN-α that may have a major role in the development of inflammation in pSS. IFN-α activates infiltrating DCs, T, and B cells. The B cells produce autoantibodies against SSA/SSB, which bind to autoantigens on the epithelium cells or form immune complexes. These immune complexes may bind to TLR7 and TLR9 in endosomes of the plasmacytoid DCs, and in this way, may further enhance IFN-α production and perpetuation of the autoimmune process in pSS (70) (Fig. 1). Type I IFN also induces upregulation of the transcription of several genes, ‘IFN signature’, encoding antiviral and immunomodulatory proteins. Such ‘IFN signature’ has been described in both the minor salivary glands (71) and peripheral blood of patients with pSS (109).

Histopathological studies have shown that epithelial cells in glandular tissue express a type I IFN signature (mostly IFNβ) and type II signature (predominately IFNγ), and lymphocytes express a type II IFN (IFNγ). This ‘IFN signature’ in the exocrine glands lesions and high expression of TLR3 in epithelial cells may support the implications of viral infection in pSS pathogenesis (59, 110, 111).

**Genetics**

The genetic factors are important to the pathogenesis of pSS and constitute an active area of research. Familial clustering studies have revealed that approximately 35% of patients with pSS have relatives with pSS or another autoimmune disease (112). It is known that the HLA class II system has genetic influence on susceptibility to autoimmunity and pSS. A meta-
analysis of 23 worldwide studies has shown a significantly increased risk for pSS in association with the alleles DQA1*05:01, DQB1*02:01 and DRB1*03:01, while DQA1*02:01, DQA1*03:01 and DQB1*05:01 alleles have been shown to be protective for pSS (113).

Several significant non-HLA gene associations with pSS have been reported in Scandinavian studies by the candidate gene approach. Polymorphisms in IRF5 (encoding interferon regulatory factor 5), STAT4 (encoding signal transducer and activator of transcription 4) (114, 115), in four signalling proteins from the NF-κB pathway (104), and polymorphisms in lymphotoxin (LTA/LTB)/TNF have shown strong association with pSS (116).

Two large genome-wide association studies (GWAS) of patients with pSS have shown the strongest associations with the HLA-II locus, followed by type I and II IFN signalling such as STAT4, IRF5, IL-12A, B lymphocyte kinase (BLK) and chemokine receptor type 5 (CXCR5), which are important for B-cell function. A strong association with pSS has also been noted with the TNFAIP3 interacting protein 1 (TNIP1) gene that is involved in the NF-κB pathway. These studies have also uncovered several new immunity-related genes and multiple different single nucleotide polymorphisms for each gene (117, 118) which can be important in pSS pathogenesis.

**Epigenetics**

Genetic regulatory mechanisms are also of interest in studies of the pathogenetic mechanisms underlying the development of pSS. One of these mechanisms is epigenetic factors or biological mechanisms that can switch genes on and off. Defective patterns of DNA methylation may lead to different gene expressions in pSS. For example, hypomethylation of IFN-regulated genes in B cells can increase the genes’ expression (119) and promote pSS development.

**Haematological disturbances**

B-cell hyperactivity in pSS may result in hypergammaglobulinemia, which can be detected in about half of the patients with pSS (91, 120). Cytopenia, which includes anaemia, leucopenia, and thrombocytopenia, may be present in one-third of pSS patients. Leucopenia is more common than anaemia and thrombocytopenia and occurs in about 40% of pSS patients (121).

Reduced concentrations of complement factors (C3 and/or C4) have been reported in 3-16% of the patients with pSS and can be associated with increased lymphoma risk (30, 47, 120).

Cryoglobulins (the most common type being type III-mixed cryoglobulinaemia followed by type II-mixed cryoglobulinaemia) can be detected in 5-15% of pSS patients (30, 47, 120, 122).
Autoantibodies

Autoantibodies against ribonucleoproteins SSA/Ro and SSB/La are among the most frequently detected autoantibodies in sera of patients with pSS. Anti-SSA/Ro is found in 33-74% and anti-SSB/La in 23-52% of patients with pSS (122). Moreover, anti-SSA/Ro and anti-SSB/La can be detected many years before sicca symptom onset (26). These autoantibodies are a useful diagnostic marker for pSS and are included in the classification criteria for pSS (17-19). However, anti-SSA/Ro and anti-SSB/La antibodies are not specific for pSS as they also can be found in sera of patients with other systemic autoimmune diseases e.g., SLE, systemic sclerosis, and myositis, and the pathologic significance of these antibodies is still poorly understood.

Anti-SSA/Ro and anti-SSB/La antibodies are associated with parotid gland damage (decreased unstimulated salivary flow and higher focus scores on minor salivary gland biopsy), and a higher prevalence of extraglandular manifestations (122). In addition, the presence of anti-SSA/Ro antibodies in the mother increases the risk of neonatal lupus (subacute cutaneous lupus, photosensitivity, cytopenias, hepatosplenomegaly, myocarditis, and pneumonitis) and complete heart block in the fetus. The risk is 1–2% in the first pregnancy and increases significantly to 17% in the subsequent pregnancy if a previous child has been affected (123).

ANA is also one of the most frequent autoantibodies found in 59-85% of pSS patients, whereas RF is reported in 36-74% of the patients with pSS (122). ANA positivity is associated with a higher prevalence of RF, hypergammaglobulinemia, a higher risk of cutaneous vasculitis, as well as articular and renal involvement (123).

Cellular infiltration in salivary glands

A positive histopathological examination of the minor salivary gland biopsies in pSS demonstrates lymphocytic infiltration containing ≥50 cells per 4 mm², so-called focus score (Fig. 2) (124). A focus score ≥1 is considered as a pathological finding and is included in the criteria for pSS diagnosis (17-19). The sensitivity and specificity of the test are around 85% and it has a high predictive value (125). Approximately 40-70% of the patients with pSS have pathological minor salivary gland biopsy findings. The infiltrating cells are able to organise themselves into B and T cell areas in minor salivary glands. The majority of these cells are T cells, and only 20% are B cells (94, 126).
Germinal centre-like structures in minor salivary glands

At the sites of inflammation in minor salivary glands, infiltrating T and B cells can organise themselves into tertiary lymphoid structures, referred to as ectopic GC-like structures (127-134). GC-like structures appear as mononuclear cell infiltrates consisting of a dark zone and a light zone located within normal salivary gland epithelium and can be seen in minor salivary gland biopsies in approximately 25-30% of the patients with pSS (130, 135) (ref. 135 is part of this thesis).

The precise mechanism of formation of GC-like structures is unknown. Many cells, chemokines, and cytokines are involved in this process. Th1 cells and IL-17, CD4+ lymphoid tissue inducer cells, CXC-chemokine ligand 13 and CC-chemokine ligand 21, including pro-inflammatory mediators IL-7 and lymphotoxin-αβ2, are necessary for recruitment of B and T cells and formation of GC-like structures (134).

GC-like structures share not only morphological features with secondary lymphoid organs (lymph nodes and spleen), but also similar mechanisms controlling their induction and maintenance. B cells in these structures undergo somatic hypermutations and antigen-driven selection of the variable region genes of immunoglobulins.

Formation of the GC-like structures may point to a more aggressive pSS disease course. The presence of these structures correlates with a higher focus score, higher concentrations of autoantibodies, hypergammaglobulinemia, and high levels of BAFF and IFN in the serum of the patients with pSS (100).
Glandular manifestations

Lymphocytic infiltration of the exocrine glands causes primarily sicca symptoms in the mouth and eyes due to destruction and impaired secretory function of the glands.

Xerostomia is typically noticed as it becomes necessary to drink frequently liquids to aid in the chewing and swallowing of dry food. Severe dryness in the mouth feels like a burning sensation in the mouth, may be complicated by bad breath, cracking and soreness in the lips, inflammation of the gums, and may lead to an increased risk to develop dental caries.

Patients with xerophthalmia complain of irritation, sensation of grit or sand in the eyes, and itching. It is common to get a conjunctival injection, often complicated with a bacterial infection, and the eyes may become sensitive to light. Chronically dry-eyes can lead to the destruction of epithelium in the cornea and conjunctiva, so-called keratoconjunctivitis sicca.

Inflammation in other secretory glands producing moisture predisposes to impaired glandular function and dryness symptoms as well. Dryness in the mucosa of the nose results in rhinitis sicca. Dryness in the throat and the upper and lower airway results in a hoarse voice, cough, tracheitis sicca, recurrent bronchitis sicca, and pneumonitis. Impaired secretion of the mucus of glandular in the gastric mucosa causes gastrointestinal symptoms. Vaginal dryness leads to painful vaginal intercourse (vaginitis sicca) and recurrent urinary tract infections. Abnormally dry skin (xerosis) may cause discomfort and itching.

Chronic or recurrent, uni- or bilateral swelling of the major salivary glands is observed in 18-31% of patients with pSS (30, 31, 47, 120).

Extraglandular manifestations

Extraglandular involvement is crucial for pSS prognosis (136). About 20-50% of patients with pSS may develop extraglandular manifestations (120, 137), but only 15% of the patients have severe extraglandular manifestations (120). Most of the extraglandular manifestations are listed in domains of the ESSDAI (Table 5) (25, 138).

Arthralgia has been reported in more than 50% of patients with pSS (136). Arthritis is characterised by inflammation/synovitis in one or more joints or defined as active articular involvement, that is, joint pain accompanied by morning stiffness for more than 30 minutes, and has been described in approximately 16% of pSS patients (136).

Muscle pain, especially fibromyalgia is common in pSS (up to 27%). Histopathological signs of polymyositis or subclinical myositis have been detected in up to 47% of the patients. However, clinical and histopathological findings of myositis appearing at the same time are uncommon in pSS patients (up to 5%) (139).
Central nervous system involvement may occur in up to 20% of pSS patients and can manifest as a combination of migraine-like symptoms, sensorimotor deficits, neuropsychiatric diseases, cognitive disturbances, and unspecific subcortical lesions (137, 140, 141).

There can be diverse symptoms of the peripheral neurologic involvement. Motor, sensory, or autonomic nerve system, alone or in combination can be affected, but sensory, sensorimotor and particularly small fibre neuropathy (SFN) are the most common features and are reported in up to 64% of the patients with pSS (142, 143). In SFN, patients complain of painful or burning paraesthesias, but an objective examination, inclusive nerve conduction studies, are normal. SFN is diagnosed by the counting of nerve fibres in a skin biopsy. No small fibres are detected in positive cases.

Pulmonary manifestations, including both bronchial and parenchymal involvement, have been reported in up to 16% of the patients with pSS. Computer tomography (CT) findings include bronchiectasis/bronchiolar abnormalities (50%) and ground glass opacities/interstitial changes (49%), being the most frequent abnormalities. The pulmonary function tests disclose decreased diffusing capacity for carbon monoxide and/or abnormal forced vital capacity. The most frequent histopathological diagnoses are: non-specific interstitial pneumonia (45%), bronchiolitis (25%), usual interstitial pneumonia (16%), and lymphocytic interstitial pneumonia (15%) (136). Lung involvement is more frequent in older (>70 years of age) patients and is one of the main causes of death in pSS (47).

Annular erythema or subacute cutaneous lupus erythematosus is a photosensitive rash, most frequently localised in the face, the upper arms, and the neck, characterised by a wide elevated border and central pallor. It is reported in up to 10% of pSS patients. Cutaneous vasculitis has been reported in approximately 10% of pSS patients. It may present as cutaneous purpura (88%), nodules, digital lesions, cutaneous ulcers, urticarial vasculitis or maculopapular rash. The biopsy usually shows cutaneous leukocytoclastic vasculitis (90%) (136). Severe cryoglobulinemic vasculitis is associated with increased rate of mortality in patients with pSS (47).

Raynaud’s phenomenon has a prevalence of 10-37% in pSS. Its clinical course is milder than in systemic sclerosis (11) and it may precede the onset of sicca symptoms (144).

Chronic tubulointerstitial nephritis is the main renal involvement associated with pSS. Renal tubular acidosis (RTA) occurs in approximately 9% of patients with pSS. It is caused by a generalised dysfunction of the distal renal tubules leading to renal acid retention and bicarbonate loss. Clinically, RTA is seen in conjunction with hypokalaemic weakness/paralysis (69%), renal colic (12%), radiologic nephrocalcinosis (17%), osteomalacia (13%), and polyuria/polydipsia (diabetes insipidus) (4%). Renal failure in RTA is reported in up to 24% of the patients (136, 145) and is associated with excess mortality in pSS patients (47).
Constitutional features

Constitutional symptoms, such as fatigue, night sweats, low-grade fever, and weight loss may develop in 50-70% of the patients with pSS during the disease course (146, 147). Swelling of lymph nodes may also occur in patients with pSS. Fatigue has a serious negative impact on patients’ quality of life and is often associated with anxiety, depression, and sleep disturbance (148).

Co-morbidity

Oral dryness increases the risk of caries, and the dry mucosa is more prone to bacterial infections and candidiasis. Prevalence of candidiasis has been reported to be up to 37% of cases in pSS patients (149). Autoimmune chronic hepatitis and primary biliary cirrhosis can be associated diseases in 2-9% of patients with pSS (150, 151). Hypothyroidism and Grave’s thyrotoxicosis can be found in 7-14% and 2-3%, respectively, in pSS (151, 152).

Cardiovascular risk factors, such as diabetes mellitus have been found in 27%, while hypertriglyceridemia has been found in 22% (153) and hypertension in 28-50% (154) of pSS patients.

Risk factors and causes of mortality in pSS

Lymphoid and solid organ malignancies, cardiovascular disease, and infections have been reported as predominant causes of death in pSS (28, 155). Older age at pSS diagnosis, male sex, parotid swelling, extraglandular involvement, vasculitis, low complement (C3 and C4), anti-SSB/La positivity, and cryoglobulinemia have been reported as risk factors associated with increased mortality. However, pSS is not associated with an all-cause mortality compared with the general population (155).

Sex differences

There are very limited data about sex differences in pSS. A couple of studies (47, 156) showed that men might have a more severe glandular involvement and less-pronounced systemic and immunologic disease than women. One study reported that pSS in men is more common among individuals 65 years of age or older compared to women (11). A systematic review of 7,888 patients with pSS has shown that male gender can be associated with increased mortality (155), but the reason for this is not known.

Diagnosis of pSS in clinical practice

The diagnosis of pSS is complex and requires a stepwise approach for the evaluation of: (i) ocular and oral dryness symptoms, which can precede pSS
diagnosis by several years and is present in 98% of pSS patients (157), (ii) objective measures of lacrimal and salivary gland dysfunction, (iii) evidence of autoimmunity with positivity for SSA/Ro (and/or SSB/La) antibodies, and (iv) evidence of inflammation in labial salivary gland biopsy.

Essentially, if a patient presents with sicca and/or systemic features of pSS accompanied by laboratory abnormalities, such as increased erythrocyte sedimentation rate (ESR) in the setting of normal CRP, polyclonal hypergammaglobulinemia, and/or cytopenia, that patient should undergo a Schirmer’s test and a 15-minute unstimulated whole saliva collection. Further, the patient should be tested for SSA/Ro antibodies. The patient needs to have either a positive test result for anti-SSA/Ro or a positive labial biopsy in combination with objective glandular tests for a final pSS diagnosis. Labial biopsy of the minor salivary glands is performed under local anaesthesia with a collection of 4-5 minor salivary glands by blunt dissection via an incision through the normal-appearing mucosa (158).

Classification criteria for pSS can be used as support of the diagnosis. It is essential to eliminate other causes of sicca syndrome and evaluate for systemic features and extraglandular manifestations (11).

Treatment

Most patients with pSS are treated symptomatically with tear and saliva substitution, chewing gum and other moisture replacement therapies, and moisture stimulating products taken to prevent dental caries and oral infections. Proper dental care and regular visits to a dentist and dental hygienist are important. Cholinergic drugs (muscarinic agonists), such as pilocarpine or salagen and cevimeline hydrochloride sometimes have an effect on both dry eyes and dry mouth by increasing glandular secretion, but side effects impair their usefulness.

Patients experiencing joint or muscular pain respond well to non-steroid anti-inflammatory drugs and analgesics. In some patients, particularly with arthritis, disease modifying drugs (e.g., hydroxychloroquine, methotrexate or azathioprine) and corticosteroids are used.

Patients with extraglandular manifestations are treated as similar conditions in other autoimmune diseases (corticosteroids in combination with immunosuppressive drugs). Biological agents with B-cell depletion (Rituximab®) (159-161), anti-BAFF (Belimumab®) (162), and drugs targeting T cells (Orencia®) (163) may be used in selected cases of moderate-severe forms of pSS.
Lymphoma

Lymphoma is a type of malignancy which develops from malignant transformed immune cells. Lymphomas are usually divided into non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL). NHLs account for about 90% of all lymphomas. According to the WHO classification, NHLs are subdivided according to cell type (B, T and NK cells), relation to the lymph node area, and morphology (164). Recently, a revision of the classification of blood malignancies was published, which clarifies the diagnosis and management of lymphoid neoplasms at the very early stages of their development, refines the diagnostic criteria for some entities, and details the expanding genetic/molecular features of different lymphoid neoplasms with their clinical correlates (165).

In the general population, most NHLs are of B-cell origin, and less than 10% are derived from T or NK cells. The most frequent subtypes of B-cell lymphoma in the general population are diffuse large B-cell lymphoma (DLBCL) (25-35%) and follicular lymphoma (20%). Marginal zone lymphoma (MZL) is subdivided into three subtypes according to the sites involved: extranodal marginal zone of mucosa-associated lymphoid tissue (MALT) lymphoma, splenic MZL, and nodal MZL (164).

DLBCL is an aggressive lymphoma of large B lymphoid cells with nuclear size more than twice the size of a normal lymphocyte and has a diffuse growth pattern. By using immunohistochemistry and antibodies against CD10, BCL6, and IRF4/MUM1 and/or genetic expression analyses (166), DLBCL can be divided into two subsets with different pathologies, treatment outcomes, and prognoses: the germinal centre B-cell-like (GCB) type with better prognosis and the activated B-cell-like (ABC) type (also referred to as the non-GC type) with worse prognosis (166-168).

DLBCL is more common in the elderly. The median age at diagnosis is in the seventh decade and DLBCL is slightly more common in males than females (164).

MALT lymphoma comprises 7-8% of all B-cell lymphomas, whereas up to 50% are gastric MALT lymphoma (164). In most cases, MALT lymphoma occurs in adults with a median age of 61 and a slight female preponderance (male: female ratio is 1:1.2) (164).

Risk and predictors for lymphoma in pSS

An increased risk of malignant B-cell NHL in patients with pSS was first reported in 1978 and the risk for lymphoma in pSS was estimated to be 44 times greater compared to age, sex, and race-matched controls (169). The patients with pSS have the highest risk of lymphoma development reported in autoimmune diseases. A meta-analysis of 20 studies showed a standard-
ised incidence ratio (SIR) of 18.8 for NHL in pSS (Table 6), whereas in SLE, the SIR was 7.4 and in RA, 3.9 (170).

Table 6. Prevalence of lymphoma in primary Sjögren’s syndrome

<table>
<thead>
<tr>
<th>Study period, country</th>
<th>Follow-up time, years</th>
<th>pSS patients (n)</th>
<th>Lymphomas (n (%))</th>
<th>Standardised incidence ratio (95% confidence interval)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978-2001 meta-analysis of 5 studies</td>
<td>18.4</td>
<td>1300</td>
<td>30 (2)</td>
<td>18.8 (9.5-37.3)</td>
<td>Zintzaras et al. (170)</td>
</tr>
<tr>
<td>1984-2002 Sweden</td>
<td>8</td>
<td>286</td>
<td>11 (3.8)</td>
<td>15.6 (7.8-27.9)</td>
<td>Theander et al. (171)</td>
</tr>
<tr>
<td>1979-2003 Britain</td>
<td>10.8</td>
<td>112</td>
<td>11 (9.8)</td>
<td>37.5 (20.7-67.6)</td>
<td>Lazarus et al. (172)</td>
</tr>
<tr>
<td>1990-2005 China</td>
<td>4.4</td>
<td>1320</td>
<td>8 (0.6)</td>
<td>48 (20.7-94.8)</td>
<td>Zhang et al. (173)</td>
</tr>
<tr>
<td>2005-2007 Taiwan</td>
<td></td>
<td>6911</td>
<td>23 (0.3)</td>
<td>7.1 (4.3-10.3)</td>
<td>Weng et al. (38)</td>
</tr>
<tr>
<td>1980-2009 Norway</td>
<td>3,813 person-years</td>
<td>443</td>
<td>7 (1.6)</td>
<td>9.0 (7.1-25.3)</td>
<td>Johnsen et al. (174)</td>
</tr>
<tr>
<td>1964-2010 Sweden</td>
<td>9.4</td>
<td>14,570</td>
<td>143 (0.9)</td>
<td>4.9 (4.2-5.8)</td>
<td>Fallah et al. (175)</td>
</tr>
</tbody>
</table>

A Swedish study has shown a relative risk of 16 for the development of lymphoma in pSS patients compared to the general population (171). The risk of NHL in Norwegian patients with pSS was increased up to nine times compared to the general population (174). A nationwide Swedish study reported an SIR of 4.9 of NHL in 14,570 SS patients (175). The prevalence of lymphoma is 4-5% in patients with pSS (9, 120, 175) and risk for lymphoma development increases during the course of pSS. The cumulative risk of developing lymphoma may reach 3.5% during the first five years and 9.8% after 15 years since the established pSS diagnosis (176).

Lymphoma development in pSS is associated with certain risk factors. Significant predictors reported for the development of lymphoma are low complement (C3 and C4), the presence of cryoglobulins, a low CD4:CD8 ratio, the persistence of unilateral or bilateral parotid gland swelling, splenomegaly, lymphadenopathy, and palpable purpura (171, 177, 178) (Table 7).

By contrast, ANA, anti-SSA/Ro, anti-SSB/La, RF, and hypergammaglobulinemia have not been associated with lymphoma development (178). Parotid gland swelling, haematological manifestations, lymphadenopathy, and disturbances in the biological parameters (low complement, hypergammaglobulinemia, and the presence of cryoglobulinemia), are very common (>70%) in the patients with pSS developing subsequent lymphoma (185).
Table 7. Risk and predictive factors of lymphoma development in pSS

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clinical features</td>
<td></td>
</tr>
<tr>
<td>Persistent swelling of major salivary glands</td>
<td>Anaya et al. (179), Ioannidis et al. (9), Nishishinya et al. (178), Sutcliffe et al. (177)</td>
</tr>
<tr>
<td>Swollen lymph nodes</td>
<td>Anaya et al. (179), Nishishinya et al. (178), Sutcliffe et al. (135, 177)</td>
</tr>
<tr>
<td>Palpable purpura</td>
<td>Ioannidis et al. (9), Nishishinya et al. (178), Theander et al. (171)</td>
</tr>
<tr>
<td>• Laboratory</td>
<td></td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>Nishishinya et al. (178), Baimpa et al. (180)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>Soalns-Laque et al. (176), Theander et al. (135, 171)</td>
</tr>
<tr>
<td>Low C4</td>
<td>Ioannidis et al. (9), Nishishinya et al. (178), Soalns-Laque et al. (176), Theander et al. (135, 171)</td>
</tr>
<tr>
<td>Monoclonal component</td>
<td>Anaya et al. (179)</td>
</tr>
<tr>
<td>• New predictors</td>
<td></td>
</tr>
<tr>
<td>Presence of GC-like structures</td>
<td>Theander et al. (135)*</td>
</tr>
<tr>
<td>Focus score ≥3</td>
<td>Risselada et al. (181, 182)</td>
</tr>
<tr>
<td>• Histopathology of salivary glands</td>
<td></td>
</tr>
<tr>
<td>High levels of BAFF (TNFSF13B)</td>
<td>Nezos et al. (183)</td>
</tr>
<tr>
<td>High levels of Flt3-ligand</td>
<td>Tobon et al. (184)</td>
</tr>
<tr>
<td>• Cytokines</td>
<td></td>
</tr>
<tr>
<td>Impairment of TNFAIP3 (A20)</td>
<td>Nocturne et al. (108)</td>
</tr>
</tbody>
</table>

*This reference is part of the current thesis.

All these suggested clinical and laboratory risk factors of lymphoma are easy to check up and follow up in daily practice. However, there is no consensus on the monitoring of pSS patients with risk factors.

Regarding the risk of development of lymphoma, it has been suggested to divide the patients with pSS into two types based on two identified risk factors, i.e., low C4 levels and/or palpable purpura (9):
1) Type 1 (80-85%) with a low risk of lymphoma, patients without the predictors for lymphoma development;
2) Type 2 (15-20% of the patients) with a high risk of lymphoma, those having one or both risk factors.

New predictors of lymphoma have also been proposed, such as GC-like structures (ref. 135 is part of this thesis), high focus score in labial biopsy (181, 182), high BAFF (183), Flt3-ligand (184) levels, and genetic impairment of TNFAIP3 (108, 186) (Table 7).

Data concerning male gender and lymphoma risk in pSS are inconsistent. A meta-analysis of 18 studies could not reveal that male gender was associated with the occurrence of lymphoma (178).
Possible mechanisms of lymphoma development in pSS

It is presumed that chronic antigenic stimulation by an as-yet-unknown antigen may be associated with the development of pSS-related lymphoproliferation. Epigenetic mechanisms may play a role in the disturbed function of Th17 or Treg cells (187), and dysregulation and hyperactivity of B cells. This imbalance and dysfunction of the T and B cells contribute to both autoimmune and haematologic malignancies associated with autoimmune disease (188). Additional oncogenic events, loss of the B-cell cycle control, and overproduction of B-cell specific stimulators and defective apoptosis of B cells may contribute to lymphomagenesis (179, 189-191).

Somatic mutations of the $TNFAIP3$ (A20) protein, which plays a key role in controlling NF-κB activation, have been observed in pSS associated MALT lymphoma (127). Another pathway with signalling abnormalities in NOTCH system (a highly conserved cell signalling system present in most multicellular organisms), that also mediate autoimmune diseases, may contribute to lymphoma development as well (192).

EBV is associated with some lymphoproliferative diseases, such as HL, post-transplant lymphoma, subsets of DLBCL, and immunodeficiency-related lymphoproliferation (164). However, it is presumed that pSS-lymphomas are not associated with EBV infection (193).

After contact with antigen, activated B cells migrate to secondary lymphoid organs (e.g., lymph nodes or the spleen) and form GCs (Fig. 3), which are important for B-cell maturation and lymphoma development (194, 195). The GC is divided into two parts: the dark zone and the light zone. The dark zone contains rapidly proliferating B cells, so-called centroblasts. Centroblasts divide and undergo somatic hypermutation to adapt antibodies to antigen. This process introduces mutations, which mainly are single nucleotide polymorphisms in the light chain of immunoglobulin genes. The light zone contains non-dividing centrocytes. The centrocytes bear high-affinity B-cell antigen receptor and have the capacity to bind the antigen, which is presented by follicular DCs. The centrocytes undergo a selection of beneficial mutations, which enhance the affinity for the original antigen. Follicular DCs and Th lymphocytes assist centrocytes during this selection process. Those cells with disadvantageous mutations die by apoptosis. Further, centrocytes undergo class switching, which is the second mechanism to adapt an antibody to an antigen. The class switching changes Ig heavy chains from IgM to IgG, IgA, or IgE. Finally, selected GC B-cells differentiate into plasmablasts and plasma cells or memory B cells expressing mutated Ig with increased affinity for the immunising antigen (196).

DLBCLs may originate from centrocytes of the GC B-cells (GCB subtype) or outside GC from activated B-cells (ABC subtype). The GCB-DLBCL has overexpression of GC-related genes and a higher rate of $BCL2$
translocations. In contrast, the ABC-DLBCL has overexpression of the NF-κB pathway (167, 168).

Some naïve B-cells migrate to the marginal zone, which is located at the periphery of the lymphoid follicles and accommodates a marginal zone B-cell population of varied maturation stages (197). MALT lymphoma and MZL arise from these monocytic marginal zone B cells. Some subtypes of B-cell lymphoma origin are shown in Fig. 3.

Figure 3. Origin of lymphomas and the main oncogenic pathways. Germinal centre (GC)-derived lymphomas are blocked at different stages of maturation. Diffuse large B-cell lymphoma (DLBCL) of GCB-subtype and follicular lymphoma originate from the light zone B cells. Marginal zone lymphomas (MZL) and MALT-type lymphomas originate from the marginal zone B cells. Activated B cell-like (ABC)-DLBCL originates from late GC B cells (plasmablasts). PDC=plasmacytoid dendritic cell; Tfh=T follicular helper; FDC=follicular dendritic cell. The figure was produced by the author using Servier Medical Art.

Recurrent genetic abnormalities, such as translocations t(11;18) (q21;21), t(14;18) (q32;q21), t(1;14) (p22;q32) are reported in MALT lymphoma in the general population. These translocations may lead to activation of the NF-κB pathway, which results in cell survival and proliferation. However, in pSS-related MALT lymphoma, other lymphomagenesis mechanisms may be involved compared to the general population. These translocations have been reported only in small proportions of pSS-MALT lymphoma patients (198). On the other hand, a possible genetic aberration correlating to pSS-MALT
lymphomas could be the deletion of the long arm of chromosome 6(6q23) leading to the deletion of TNFLAP3 (A20), which is required for termination of NF-κB activation (199). To confirm A20 molecular aberration in pSS-related MALT lymphoma development, a larger cohort of patients to study is required.

Lymphoma subtypes in pSS and lymphoma diagnosis

Extranodal MALT lymphoma is the most common histological subtype of lymphoma encountered in patients with pSS (169, 177, 180, 185, 189, 200). These lymphomas are generally characterised by an indolent course and good performance status in patients. MALT lymphomas in pSS are mostly localised in the salivary glands, which are the major sites of inflammation in pSS, but other mucosal sites such as lacrimal glands, the naso-pharynx, the stomach, the thyroid, and the lung can be affected. A rapidly enlarging major salivary gland may herald the emergence of a malignant lymphoma. The parotid gland enlargement is often unilateral and the tumour affection is fixed and hard. Magnetic resonance imaging (MRI) (201) or ultrasound with Doppler may help to differentiate between focal salivary gland inflammation or suspected lymphoma, and gives the possibility of performing fine-needle aspiration or biopsy of the gland during the same diagnostic procedure (202).

The histology of MALT lymphoma displays diffuse lymphoid infiltrates with small lymphocytes with slightly irregular nuclei, cells with pale cytoplasm resembling those of centrocytes, or plasmacytic differentiation. In glandular tissues, the epithelium is destroyed by aggregates of lymphoma cells resulting in the so-called lymphoepithelial lesions. The lymphoma cells may colonise the GCs. Transformed centroblasts or immunoblasts may be present in MALT lymphoma. The phenotype of tumour cells of MALT lymphoma is CD20+, CD79+, CD5-, CD10-, CD23-, CD43+/-, and CD11+/-.

Immunoglobulin light chain restriction is relevant in the differentiation from benign lymphoid infiltration. Additionally, to distinguish from other B-cell lymphomas, a confirmation of the absence of CD5, cyclin D1, and CD10 is important (164).

High-malignant DLBCL is the other lymphoma subtype reported in high frequency in pSS (169, 171, 203, 204). In some cases (up to 10%), a histologic transformation of a previous low-grade lymphoma has been observed in pSS (193, 200, 204).

An aggressive clinical picture can suggest high-malignant lymphoma or transformation. The clinical criteria of a transformation are as follows: a rise in the lactate dehydrogenase level, a rapid localised nodal growth, new extranodal sites of disease, the presence of B symptoms (e.g., fever, weight loss, and severe night sweats) or hypercalcemia (205). Imaging with CT,
MRI, ultrasound and biopsy with immunohistochemistry, and molecular biology studies are helpful for diagnosis. Several studies have reported on the clinical utility of an \(^{18}\text{F}\text{(fluorodeoxyglucose (FDG) positron emission tomography (PET) scan to detect sites of transformation or to diagnose DLBCL de novo\text) (206, 207).}

Other mature B-cell malignancies, for example, HL (208) and plasma cell neoplasms/myeloma (171, 173, 209), have rarely been studied in pSS.

**Outcome and treatment of lymphoma in pSS**

MALT lymphoma in pSS is usually associated with a good prognosis. A retrospective study in Greece reported a 3-year overall survival (OS) and event-free (EFS) survival of 97% and 78%, respectively, in pSS-MALT lymphomas (210).

For some asymptomatic MALT lymphoma patients with a localised disease in the salivary glands, a ‘watch and wait’ policy may be adopted (211). Patients with symptomatic MALT lymphoma localised in the salivary glands may be treated with low-dose radiotherapy or, in some cases, with surgery or combination of both, and combined with or without chemotherapy (212). According to a recent large multicentre international study, salivary gland MALT lymphoma associated with pSS has a good prognosis regardless of the initial treatment and a median overall survival can be achieved up to 20 years after diagnosis (212).

Disseminated MALT lymphoma and pSS-DLBCL are treated with chemotherapy with or without rituximab as in other patients with DLBCL. A successful outcome (100% OS and EFS at the three-year mark) in eight patients with pSS-DLBCL was reported when R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, prednisone) or CHOP was used (210).

**IgG4-related disease**

IgG4-RD is a chronic fibrotic inflammation, characterised by tissue infiltration by lymphocytes, mostly IgG4-positive plasma cells (PCs), development of fibrosis, and often elevated IgG4 levels in serum (213, 214). The disease can affect nearly all organs. A wide variety of different diseases (Mikulicz’s disease, type 1 autoimmune pancreatitis, lymphocytic hypophysitis, Riedel’s thyroiditis, Kuttner’s tumour, retroperitoneal fibrosis, inflammatory aortic aneurysm and aortitis, interstitial nephritis and pneumonitis, and inflammatory pseudotumour) are now recognised as IgG4-RD manifestations. The nomenclature of the disease and an international pathology consensus statement have been published in 2012 when IgG4-RD had been established (215, 216). However, IgG4-RD, as a recently recognised disease still re-
mains unknown or is a very new entity for many clinicians. Therefore, it may first come to clinical attention as visible organ swelling or detected organ dysfunction due to fibrosis. Sometimes it can be identified incidentally by imaging and specific biopsy findings when patients undergo an investigation of suspected tumour disease, infection, or another immunity mediated disease (217). Most studies about this disease come from Japan and, therefore, the epidemiology of the disease is not yet described in Western countries.

Pathogenesis of IgG4-RD

Immunopathogenesis is not yet fully understood. It is supposed that oligoclonal B and T cells develop during chronic stimulation by unknown antigen(s). Oligoclonal expansion of plasmablasts positive for IgG4 arises after somatic hypermutation from naïve B cells in GCs (Fig. 3). T follicular helper cells can be involved in the differentiation of B cells during their development in the GCs and contribute to class switching (86, 218). The oligoclonal plasmablasts are identified by flow cytometry as CD19+CD20+CD27+CD138+ and correlate with activity of the disease (219, 220). Plasmablasts differentiate into antibody producing short- and long-lived PCs producing IgG4 (Fig. 3). Short-lived PCs can be depleted by rituximab (219). However, the plasmablasts may reemerge from naïve B cells again during relapses with an expression of other oligonalities by rearrangements of V-J repertoires (219). It is supposed that the chronic disease may be enhanced by long-lived memory PCs (221), which are not depleted by rituximab.

T cells, in particular Th2 immune response cells (IL-4, IL-5, and IL-13) in atopic individuals, may be important in the pathogenesis of IgG4-RD (222, 223). Recent studies have shown that effector memory CD4+ T cells with cytotoxic function are expanded in the peripheral blood and in affected organs. They produce IL-1β cytokine and transforming growth factor (TGF)-β1, which are both important for inflammation and fibrosis (224).

Type 1 autoimmune pancreatitis is associated with IgG4-RD and is the best studied of all clinical manifestations of the disease so far. Interestingly, as in pSS, plasmacytoid DCs producing type I IFN-α have been shown to be important in activating B cells to produce IgG4 and promoting the development of this type of pancreatitis (225).

Similarities and differences with pSS

Patients with IgG4-RD in salivary and/or lacrimal glands share many similarities with pSS patients (Table 8) (217). Therefore, some patients with sicca symptoms previously diagnosed as incomplete or atypical pSS could instead have undiagnosed IgG4-RD (226).
Mikulicz’s disease (MD), which is now recognised as one manifestation of IgG4-RD, is characterised by symmetrical and painless swelling of the lacrimal, parotid, and submandibular glands and was first described in 1892. For a long time, MD has been considered to be a subtype of SS because of clinical and histopathological similarities (227). However, many differences between MD and SS have been reported in Japan. In contrast to pSS, MD usually manifests in the form of bilateral and persistent swelling in at least two sites of the lacrimal and salivary glands, with a higher frequency of males represented. Lymphoplasmacytic infiltration with lymphoid follicle formation causes swollen glands but, differently from pSS, duct destruction is uncommon in MD. Serology is usually negative for anti-SS-A/SS-B antibodies, and in contrast to SS, dryness symptoms, which are mild compared to SS, improve within days of starting glucocorticoids in MD patients (228). In addition, a minor salivary gland biopsy reveals an intact structure of salivary ducts despite lymphocytic infiltration and formation of lymphoid follicles, and immunostaining shows IgG4-positive PCs (214, 228, 229).

Table 8. Similarities and differences between pSS and IgG4-RD with salivary gland involvement

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>pSS</th>
<th>IgG4-RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at onset, years</td>
<td>40-50</td>
<td>50-60</td>
</tr>
<tr>
<td>Female: male</td>
<td>1:9-20</td>
<td>1:3, if a head region affected 1:1</td>
</tr>
<tr>
<td>Swelling of the major salivary glands</td>
<td>Recurrent</td>
<td>Persistent or recurrent</td>
</tr>
<tr>
<td>Major salivary glands affected</td>
<td>Parotid glands</td>
<td>Submandibular and/or parotid glands</td>
</tr>
<tr>
<td>Sicca symptoms</td>
<td>Moderate - severe</td>
<td>No or mild</td>
</tr>
<tr>
<td>Serum IgG level</td>
<td>Often high</td>
<td>Often high</td>
</tr>
<tr>
<td>Serum IgG4 level</td>
<td>Normal</td>
<td>Often elevated</td>
</tr>
<tr>
<td>Serum IgE level</td>
<td>Normal</td>
<td>Often elevated</td>
</tr>
<tr>
<td>ANA positivity</td>
<td>Positive in 59-85%</td>
<td>Positive in approx. 20%</td>
</tr>
<tr>
<td>SSA positivity</td>
<td>Positive in 33-74%</td>
<td>Absent</td>
</tr>
<tr>
<td>RF positivity</td>
<td>Positive in 36-74%</td>
<td>Positive in 70-80%</td>
</tr>
<tr>
<td>Low complement levels</td>
<td>Present in 3-16%</td>
<td>Present in approx. 50%</td>
</tr>
<tr>
<td>Lymphocytic infiltration in the minor salivary gland biopsies with focus score ≥1</td>
<td>Present in 40-70%</td>
<td>May be present</td>
</tr>
<tr>
<td>IgG4+ plasma cells in the salivary gland biopsies</td>
<td>Not present</td>
<td>Present</td>
</tr>
<tr>
<td>Storiform fibrosis in the biopsies</td>
<td>Not present</td>
<td>Present</td>
</tr>
<tr>
<td>Response to steroids</td>
<td>Sometimes</td>
<td>Good in most cases</td>
</tr>
<tr>
<td>Increased risk of MALT lymphoma</td>
<td>Present</td>
<td>May be present</td>
</tr>
</tbody>
</table>

pSS=primary Sjögren’s syndrome; IgG4-RD=IgG4-related disease.
Diagnosis of IgG4-RD

The disease usually affects middle-to-older age men and is characterised by increased IgG4 levels in serum, clinical symptoms of affected organs, typical histopathology and immunohistochemistry and/or radiology features (Fig. 4).

Figure 4. Flow chart for workup of diagnosis of IgG4-related disease. Adapted from Vasaitis (217).

Early diagnosis of IgG4-RD is difficult because of vague unspecific symptoms. (217). Increasing experience with IgG4-RD has led to the recognition that the gold standard to diagnose IgG4-RD is a typical histopathology (216) and compatible clinical features and/or radiology (217). High levels of IgG4 in serum support the diagnosis.

Several sets of diagnostic criteria have been proposed for IgG4-RD (230-233). However, these criteria have not been approved internationally, since they are not sufficiently sensitive and specific. The ACR/EULAR working group is now developing classification criteria for IgG4-RD on the basis of histopathology, clinical, and radiological features.

Lymphoma in IgG4-related disease

Malignancies have been reported in 7-10% of the patients with IgG4-RD (234, 235). An association with lymphoma development has also been de-
scribed. Similar to pSS, MALT lymphoma has been reported in ocular adnexa, salivary glands, or meningeal dura within five years after diagnosis of IgG4-RD (236-241). It has also been described that cancer tissue may be infiltrated by IgG4+ plasma cells without other signs of IgG4-RD (242-244).
Aims of the thesis

The overall aim of this thesis was to expand the knowledge of the association between primary Sjögren’s syndrome (pSS) and lymphoma development using a population-based approach to identify the study subjects. Another overall aim was to establish a basis for future biologic studies by collecting a well-characterized population-based cohort of pSS patients with lymphoma with detailed clinical data, access to lymphoma tissues and diagnostic salivary gland biopsies.

The specific aims of the thesis were:

Paper I - To determine whether the formation of GC-like structures in a lower labial salivary gland biopsy taken at pSS diagnosis predicts the subsequent development of lymphoma.

Paper II - To identify the occurrence of histopathological features of IgG4-RD in lymphoma tissue in a large population-based cohort of patients with an initial pSS diagnosis complicated by lymphoma development and in positive cases, to describe clinical and lymphoma characteristics in these patients.

Paper III - To explore the causes of an SS diagnosis in the patients registered with both SS and lymphoma diagnoses in the Swedish Patient and the Cancer Registers; to assess whether patients with a lymphoma diagnosis before pSS diagnosis differ from pSS patients with a subsequent lymphoma diagnosis.

Paper IV - To explore sex differences in pSS patients with lymphoid neoplasm, to assess patient characteristics of the most common subtypes of lymphoma in pSS and to assess the subtype distribution of lymphoid neoplasms in pSS patients in a population-based setting in comparison with the general population.
Subjects and methods

Data sources
We used the Swedish National Health Care Registers to identify the patients in the studies II-IV. In Sweden, each resident has a unique personal identification number (245). This number is used in all health care registers and enables linkages across the different registers. In connection with hospital discharges from inpatient care and visits in non-primary outpatient care, diagnoses are coded according to the International Classification of Diseases (ICD) codes (http://apps.who.int/classifications/icd10/browse/2016/en) and recorded in the National Patient Register.

The validity of the ICD codes for diagnoses in the Swedish National Patient Register has been investigated by several studies by using chart data and validating the register-based diagnoses against classification criteria of the diseases and clinical diagnoses. It has been shown that the validity varies between different diagnoses and it is about 90% for RA (246) and about 70% for ankylosing spondylitis (247). There are no published reports of validations of the diagnosis code for SS, but as the code is assigned for all causes of sicca symptoms, we recognised at the start of this project that the validity for the SS code to identify pSS patients could be a problem and included validation of the ICD code for SS as part of the project.

National Registers used in the studies
The Patient Register
The Swedish Patient Register was initiated in 1964 by the National Board of Health and Welfare and consists of two parts. One part contains information from all patients discharged from hospitals in Sweden (The Inpatient Register). The second part has been introduced since 2001 and contains data from specialist (non-primary) outpatient care (The Outpatient Register, e.g., rheumatology open care).

The coverage of the Inpatient Register became nationwide in 1987 (http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/18152/2010-10-20.pdf, http://www.socialstyrelsen.se/register/halsodataregister/patient registret/inenglish) and since then includes all inpatient care in Sweden. The
coverage of the Outpatient Register is lower (about 80%) than for the Inpatient Register as there are missing data from some private caregivers. Primary care is not included in the Outpatient Register.

The medical data collected in the Patient Register include primary and secondary (up to eight) diagnoses/ICD codes, injuries and surgical procedures. The administrative data include the personal identification number of the patient, age, sex, dates of admission and discharge, hospital, department, and county.

The Swedish Population Register
Patients were followed up for vital status and date of death by using the Swedish Population Register. The Population Register holds data since 1961 and is maintained by the Swedish Tax Agency. This register contains information of personal identity numbers of residents, their addresses, place of birth, citizenship, moves to and from Sweden, and date of death.

The Cancer Register
The Swedish Cancer Register was founded in 1958 (http://www.socialstyrelsen.se/register/hapsodatable/register/cancerregistret/inenglish). It covers the whole population of Sweden. The reporting rate is almost 100% through a system of mandatory reporting by both clinicians and pathologists of a newly detected malignancy. The information available in the register includes patient data (personal identification number, sex, age, and place of residence), medical data (site of tumour, histological type, stage, basis and date of diagnosis, reporting hospital and department, reporting pathology/cytology department, identification number for the tissue specimen) and follow-up data (date and cause of death, date of migration, and whether a patient was registered as a resident in Sweden at the end of a specific year). From data in the register, it is possible to localise available tumour tissue and retrieve it from pathology departments for reanalysis if needed.

Both the Swedish Patient Register and the Cancer Register are maintained by the National Board of Health and Welfare.

The Lymphoma Register
The Lymphoma Register was started as a complement to the Cancer Register. The registration of malignant lymphomas in the Cancer Register was regarded as insufficient because of the complexity of lymphomas combined with frequent changes in their classification. Therefore, a national management committee formed the Swedish Lymphoma Group for establishing of the Lymphoma Register with the purpose to optimise the quality of care of patients with malignant lymphoma.
The Lymphoma Register was set up in 2000 and includes all patients ≥18 years of age (http://www.swedishlymphoma.com/rapporter) diagnosed with lymphoma in Sweden, and holds more detailed information on lymphoma subtypes according to the WHO classification than the Swedish Cancer Register. Information in the register is collected directly from the oncology centres. The coverage of the register during the current project (2000-2010) is estimated to be >90% of all lymphomas in the Cancer Register. National reports are presented annually. Additionally, each clinic has the opportunity to obtain own data from the regional lymphoma register. From 2007, the Lymphoma Register, together with other blood malignancies, has a web-based platform with the electronic input of data and also contains data of response to the treatment.

Since 2008, all cases of multiple myeloma, plasmacytoma and plasma cell leukaemia are registered separately from the Cancer Register in the Myeloma Register. The coverage of the Myeloma Register is 91% in relation to the Cancer Register (https://www.cancercentrum.se/globalassets/cancer-diaignoser/blod-lymfom-myelom/myelom/arsrapport-myelom-2008.pdf).

**Definitions used for blood malignancies in the thesis**

Blood malignancies involving mature lymphoid cells are called lymphoid neoplasms or lymphoid malignancies in the thesis and include both lymphoma and multiple myeloma/plasmacytoma.

**The International Classification of Diseases**

The first classification of diseases was created by Bertillon for comparable cause-of-death statistics in 1885. This classification was the basis for the development of the ICD. Since 1900, the ICD classification has been revised 10 times until now. From the seventh revision, the WHO advisory group is involved in revising proposals for every new edition of the ICD classification (https://www.cdc.gov/nchs/data/misc/classification_diseases2011.pdf).

In studies II-IV, for identifying the patients with SS diagnoses and lymphoid malignancies, the following ICD classifications have been used: ICD-7 (in use 1958-1967), ICD-8 (in use 1968-1978), ICD-9 (in use 1979-1994), and ICD-10 (in use 1995 to present).

**PAPER I**

**Patients and clinical information**

In this study, patients with pSS fulfilling the 2002 AECG criteria (17) were primarily selected from two Swedish centres with pSS research cohorts (Uppsala and Malmö University Hospitals) participating in a Nordic collaboration study on lymphoma and genetics. Of 241 consecutive pSS patients,
175 (Uppsala, \(n=49\), Malmö, \(n=125\), and Linköping, \(n=1\)) had haematoxylin and eosin (H&E) stained paraffin-embedded minor salivary gland tissue biopsies taken at the time of pSS diagnosis and were included in the study. Of 175 pSS patients, 161 (92%) were women and 14 (8%) men. The mean age at pSS diagnosis was 51.3 years. Their clinical features, such as salivary gland swelling, vasculitis, lymphadenopathy, internal organ involvement, and laboratory parameters such as autoantibodies, blood status, and immunoglobulin values were regularly registered during the follow-up. T-cell subsets and complement function were studied only in the Malmö cohort. Antibody levels varied during the study period and were detected by different assays. Therefore, for serum antibodies against SSA/Ro-60, Ro-52, and SSB/La, a bead-based multiplex immune assay was performed (xMap technology; Luminex, Austin, Texas USA). For the remaining antibodies (ANA and RF), the local reference levels at the time of analysis were used. ANA was positive in 81%, anti-SSA/Ro in 64%, anti-SSB/La in 39%, and RF in 55% of the patients. The internal organ involvement was assessed according to the ESSDAI (21).

Salivary gland tissue re-evaluation

The available H&E stained paraffin-embedded biopsies of 175 patients taken from minor salivary glands at the time of pSS diagnosis were re-evaluated by light microscopy at the Broegelmann Research Laboratory in Bergen, Norway. The investigator (MVJ) was blinded from the original biopsy evaluation results.

Tissues were evaluated for the presence of periductal focal lymphocytic infiltration (124) (Fig. 5A, B) and counting of foci containing at least 50 mononuclear cells per 4 mm². The presence of ectopic GC-like structures (GC-positive) was defined as well-circumscribed chronic inflammatory cell infiltrate consisted of at least 50 mononuclear cells presented with features indicative of the lymphoid organisation, such as a densely packed dark zone and a light zone within otherwise normal salivary gland epithelium (Fig. 5C, D).
Figure 5. Minor salivary gland tissue with periductal focal mononuclear cell infiltrates (focal sialadenitis) but no germinal centre-like structures (A and B) and focal sialadenitis with germinal centre-like structures (C and D). The figure reprinted with permission of the publisher from Theander et al. (135).

The Swedish Cancer Register and lymphoma tissues
A linkage using the unique national identification number (245) was performed with the Swedish Cancer Register (248). In patients for whom lymphoma was diagnosed, paraffin-embedded tissue blocks were retrieved, reevaluated and lymphoma was classified according to the WHO classification (164) by a senior pathologist (GW).

PAPERS II-IV
Patients
A national population-based cohort of individuals with an ICD diagnosis code for “Sjögren’s syndrome/sicca syndrome” was identified using the Swedish Patient Register 1964-2007 (Fig. 6).
Figure 6. Identification of the patients with Sjögren’s syndrome and lymphoid neoplasm diagnoses from the Patient and the Cancer Registries.

All individuals registered with a SS diagnosis code as the main or secondary diagnosis were identified using the following ICD codes: 374.06 (ICD-7), 734.90 (ICD-8), 710 C (ICD-9) and M35.0 (ICD-10). For M35.0, the additional codes H19.3 (SS with keratoconjunctivitis), J199.0 (SS with pulmonary involvement), G73.7 (SS with myopathy), and N16.4 (SS with the renal tubulointerstitial disease) were added. Through linkage with the Cancer Register (which covers ≥95% of all incident cancer in Sweden) (249), we identified SS patients diagnosed with lymphoma or myeloma (ICD-7 codes 200-203) at ≥18 years of age between 1990 and 2007, irrespective of the lymphoid malignancy was diagnosed before or after the first registered SS code in the Patient Register (n=224).

As the diagnosis code for SS has not been used for pSS exclusively, the basis for the SS diagnosis was evaluated from the medical records of all patients (L.Vasaitis) (Fig. 7).

Clinical data was collected from medical records according to a comprehensive questionnaire. Cases whose medical records could not be identified or were too sparse for diagnostic evaluation were excluded (n=6). Patients were divided into pSS and non-pSS patients. Those with a clear explanation for the SS-diagnosis code other than pSS and/or no support for a pSS diagnosis in the medical records were considered to be non-pSS patients (n=99). The remaining patients, the pSS group (n=119), had been diagnosed as pSS by the treating physician and all available information in the medical records supported this diagnosis.
We defined the date of lymphoid neoplasm diagnosis as the date of the first diagnosis of the lymphoid neoplasm in the Cancer Register and the date of pSS diagnosis as the diagnosis date documented in the medical records by the treating physician. The reference values of the local analysing laboratories were used for ANA, SSA/Ro, SSB/La antibodies, rheumatoid factor, cryoglobulins, and complement C3 and C4. Cytopenias were defined as haemoglobin <120 g/L, leukocytes <4.0 x10^9/L, platelets <150 x10^9/L, and hypergammaglobulinemia as immunoglobulin (Ig) G >15 g/L. Disease activity at pSS diagnosis was retrospectively assessed in cases with available information using the ESSDAI (21).

To avoid a possible influence of the lymphoid malignancy, clinical and laboratory data of the pSS disease was collected from the medical records from the patient-reported onset of sicca symptoms until six months before lymphoid neoplasm diagnosis in all patients. Four patients formally diagnosed with pSS after lymphoma diagnosis were excluded from the assessment of time period from pSS diagnosis to lymphoid neoplasm diagnosis. As the evaluation of salivary gland biopsies is part of the 2002 (17) and 2016 (19) classification criteria for pSS, we reviewed (C.Sundström, L.Vasaitis) all available H&E stained slides of the diagnostic minor salivary glands biopsies for focus score (124).
Patients were followed up for vital status and date of death in the Swedish Population Register until January 15th, 2017.

Tissue analyses
The lymphoma tissues were reviewed by a senior haematopathologist (C. Sundström) to confirm, and if needed, to reclassify the lymphoma diagnosis according to the WHO classification of tumours of haematopoietic and lymphoid tissues from 2008 (164). DLBCL were divided into the GCB and ABC subtypes by immunohistochemical staining for CD10, BCL6, and IRF4/MUM1 according to the algorithm by Hans et al. (166). Stainings for different lymphoma markers if needed and analyses for the presence of EBV using in situ hybridisation for EBV-encoded small RNAs in available lymphoma tissues and minor salivary gland biopsies were also performed. In three cases, the lymphoma diagnosis was based on the original pathology reports, as specimens were not available for re-evaluation. Eleven patients were excluded, as the lymphoma/myeloma diagnosis could not be confirmed (Fig. 7).

Validation of pSS diagnosis
The patients with pSS diagnosis according the treating physician and a confirmed lymphoid neoplasm were evaluated for the fulfillment of the 2002 AECG criteria for pSS (17) (lymphoma as an exclusion criterion was not employed) and the 2016 ACR/EULAR criteria for pSS (19).

Paper II
Patients
In this study, we included all patients with an initial diagnosis of pSS made by the treating physician, a confirmed lymphoid neoplasm by review, and available lymphoma biopsy materials for further analyses (n=79). Of the 79 patients, 44 fulfilled the 2002 AECG criteria for pSS (17), and 35 did not as most of them were diagnosed before 2002 and were not fully examined according to the criteria. Detailed clinical data was retrieved from the medical records of the 79 patients.

Histopathology and immunohistochemistry
All available lymphoma tissues (n=79) were immunostained for IgG4 (clone HP6025, Genetex, CA). Specimens with identified IgG4-positive PCs were investigated for other histopathological features of IgG4-RD (the presence of lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, and positive immunostaining for IgG4-positive PCs). Further stainings for IgG
and CD138, as a PC marker, were performed in tissues with >10 IgG4-positive PCs/high power field (HPF).

In cases with features of IgG4-RD in the lymphoma tissue, other archived tissue specimens were collected and examined for IgG4, IgG, CD138 positive PCs, and histopathological features of IgG4-RD. Additionally, all available minor salivary gland biopsies (n=11) obtained as part of the initial pSS investigation were stained for IgG4. Sections of tonsil were used as positive controls.

The immunostains were evaluated by using a light microscopy with a 40x field objective. The counting of IgG4, IgG, and CD138-positive PCs was performed in the three HPFs with the highest density of stained cells, and the average numbers were calculated.

Definitions pertaining to IgG4-RD were based on the consensus statement on the pathology of IgG4-RD, including the categorisation into histological features “probable” or “highly suggestive” of IgG4-RD (Table 9) (216).

Table 9. Overview of histopathological and immunohistochemistry features of IgG4-related disease based on the consensus statement (216)

<table>
<thead>
<tr>
<th>Features of IgG4-related disease</th>
<th>Highly suggestive</th>
<th>Probable features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histological</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dense lymphoplasmacytic infiltrate</td>
<td>≥ 2 pathology features</td>
<td>1 pathology feature</td>
</tr>
<tr>
<td>2. Storiform fibrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Obliterative phlebitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Immunohistochemical</strong></td>
<td>Number of IgG4+ plasma cells/high power field</td>
<td></td>
</tr>
<tr>
<td>Lacrimal glands</td>
<td>&gt;100**</td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>Aorta, pleura</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Surgical specimen of bile duct, liver, lung or pancreas</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Retroperitoneum, surgical specimen of kidney</td>
<td>&gt;30</td>
<td></td>
</tr>
<tr>
<td>Biopsy of kidney or pancreas</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio IgG4+/IgG+ plasma cells</strong></td>
<td>&gt;0.4</td>
<td></td>
</tr>
</tbody>
</table>

*Combination of histological and immunohistochemical features are needed for the diagnoses histologically highly suggestive and probable histological features of IgG4-RD; **In lacrimal glands only one pathology feature is required in combination with immunohistochemical features to be histologically highly suggestive of IgG4-RD.

**Paper III**

**Study population**

In this study, we included patients with pSS diagnosis according to the treating physician and confirmed lymphoid neoplasm diagnosis (n=107), and patients with non-pSS but with ICD codes for SS and lymphoid neoplasm diagnoses (n=100) (Fig. 8).

The pSS patients were categorised into those with or without pre-existing lymphoma or myeloma at pSS diagnosis. We defined pre-existing lymphoma/myeloma as a lymphoid neoplasm diagnosed before pSS diagnosis, or
within six months after pSS diagnosis. This definition was chosen, as signs of lymphoid neoplasm were present at pSS diagnosis in these patients, although the final diagnoses were delayed. In this comparison between the pSS groups with pre-existing and subsequent lymphoma, we excluded two patients with verified DLBCLs, which occurred shortly after organ transplantation (two and nine months after lung and kidney transplantation, respectively). It is well known that organ transplantation is associated with EBV-positive post-transplant lymphoma and, in addition, these two patients were included and described in another study about post-transplant lymphoma (250).

Figure 8. Study population in study III: patients with pSS and confirmed lymphoid neoplasm (n=107) and non-pSS patients with lymphoid neoplasm diagnosis (n=100). *Two pSS patients had confirmed diffuse large B-cell lymphoma development after solid organ transplantation and were excluded from the final comparison between the groups.

Paper IV

Study cohort

This study included 105 patients with pSS diagnosis according to the treating physician and a confirmed lymphoid malignancy (two patients with pSS and post-transplant lymphoma excluded, Fig. 8). Of these, 55 fulfilled the 2002 AECG criteria (17) (lymphoma as exclusion criteria not employed) and 57 fulfilled the 2016 ACR/EULAR criteria for pSS (19). The patients who did not fulfil these criteria were typically not fully examined according to the criteria or this information could not be found in the medical records, but as
the available information supported the pSS diagnosis and no other diagnosis, all 105 patients were kept in the study as pSS patients.

*Cohort of the general population with lymphoma for comparison*

To compare the distribution of lymphoma subtypes in the pSS patients with the distribution in the general population, we used the Swedish National Quality Register of Lymphoma 2000-2010 as a reference population (http://www.swedishlymphoma.se/rapporter). The coverage of the register during the period in question is estimated to be about 95% of all lymphomas in the Cancer Register. Plasma cell neoplasms are not systematically included in the Lymphoma Register. Therefore, identified cases of plasma cell neoplasms were presented separately from the comparison of lymphoma subtype distribution in pSS patients with the distribution in the Lymphoma Register.

*Statistics*

Demographic, serological, and clinical features were descriptively analysed (Paper I-IV). The risk of developing lymphoma in GC-positive and GC-negative patients was evaluated by Kaplan-Meier statistics/log-rank test (Paper I). For continuous variables, non-parametric (two-tailed) Mann-Whitney U-test to compare two independent groups was applied (Papers II-IV). For categorical variables, the Chi-square (two-tailed) test or Fisher’s exact test (if <5 observations) was used to compare frequencies between groups (Papers I-IV). A two-tailed value of p<0.05 was considered statistically significant. The statistical analyses were performed using the Statistica version 13 software (StatSoft, Inc., Tulsa, Oklahoma, USA).

*Ethical approval*

Ethical approval from the Regional Ethical Review Board, Uppsala (Paper I-IV) and approvals of the local ethics committees at the universities of Lund and Bergen were obtained (Paper I).
Results

PAPER I

Of the 175 salivary gland biopsies, 25% had GC-like structures. Focal sal-adenitis with a focus score of ≥1 was detected in 136 (78%) patients and, among them, 43 (32%) showed GC-like structures. The remaining 39 patients had normal biopsies.

Of the 175 patients with pSS, seven patients had developed lymphoma during the average follow-up time of 10.6 years. Six of seven patients with lymphoma had GC-like structures in their minor salivary glands at pSS diagnosis, performed a median of seven years before the occurrence of lymphoma. Thus, among 43 patients with GC-like structures, six patients (14%) developed lymphoma in contrast to one patient (0.8%) among the GC-negative patients \((p=0.001)\). There was a significant difference in lymphoma-free survival between GC-positive and GC-negative patients \((p=0.001)\) with worse survival for the GC-positive patients. The shortest interval from salivary gland biopsy until lymphoma development was two years and four months, and the longest interval was 12 years and seven months. The positive predictive value for GC positivity was 16%, whereas the negative predictive value was 99%. Lymphoma subtypes in the GC-positive group were the following: MALT lymphoma in salivary gland with transformation into mantle cell lymphoma \((n=1)\), follicular lymphoma in salivary gland \((n=1)\), anaplastic large T-cell lymphoma in lymph nodes and liver \((n=1)\), and DLBCLs localised in the internal organs and lymph nodes \((n=3)\).

Extraglandular manifestations using domains from the disease activity instrument ESSDAI \((21)\) occurred with different frequencies in GC-positive and GC-negative patients. Lymphadenopathy, haematological manifestations, mainly leucopenia and hypergammaglobulinemia, systemic disease affecting a higher number of organs, lower C4 levels, positivity for anti-SSA/Ro, anti-SSB/La, ANA, and RF were more frequently observed among GC-positive than GC-negative patients \((p=0.001\) and \(p=0.003\) respectively). The presence of GC-like structures in minor salivary gland biopsies and CD4 T-cell lymphocytopenia showed significant associations with lymphoma \((p=0.001\) and \(p=0.003\) respectively). Complement C4 low levels were also more frequently found in the lymphoma patients. However, cryoglobulinemia and low C3 levels were not associated with lymphoma \((p>0.05)\).
Table 10. Clinical variables in GC-positive and GC-negative patients

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>GC-positive</th>
<th>GC-negative</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>6/14</td>
<td>1/0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>13/31</td>
<td>15/12</td>
<td>0.006</td>
</tr>
<tr>
<td>Leucopenia (&lt;4000/mm³)</td>
<td>13/37</td>
<td>17/17</td>
<td>0.019</td>
</tr>
<tr>
<td>CD4 T lymphopenia*</td>
<td>8/26</td>
<td>11/14</td>
<td>0.16</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>5/18</td>
<td>10/16</td>
<td>1</td>
</tr>
<tr>
<td>Systemic disease</td>
<td>30/74</td>
<td>57/51</td>
<td>0.007</td>
</tr>
<tr>
<td>Involved organ systems, n</td>
<td>2.56 (1.62)</td>
<td>1.58 (1.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-SSA/Ro positivity</td>
<td>24/77</td>
<td>48/54</td>
<td>0.022</td>
</tr>
<tr>
<td>Anti-SSB/La positivity</td>
<td>19/61</td>
<td>32/36</td>
<td>0.014</td>
</tr>
<tr>
<td>ANA positivity</td>
<td>38/91</td>
<td>103/79</td>
<td>0.10</td>
</tr>
<tr>
<td>RF positivity</td>
<td>27/68</td>
<td>63/51</td>
<td>0.07</td>
</tr>
<tr>
<td>p-IgG (g/L)</td>
<td>18.3 (6.46)</td>
<td>15.0 (5.17)</td>
<td>0.003</td>
</tr>
<tr>
<td>C4 (g/L)</td>
<td>0.98 (0.26)</td>
<td>1.05 (0.25)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*CD4 T cells <300 cells/mL or CD4 T cells <30% of total lymphocyte count or low CD4/CD8 ratio: ≤0.8.
GC=germinal centre; ANA=antinuclear antibody; RF=rheumatoid factor; p-IgG=plasma immunoglobulin.

One GC-negative lymphoma patient diagnosed with MALT lymphoma of a tear gland was ascertained to have a borderline positive focus score in the minor salivary gland biopsy and lacked positivity for anti-SSA/Ro and anti-SSB/La. Therefore, this patient could be classified as borderline-SS and the lymphoma might represent the background population risk of lymphoma.

The results of the present study show that the assessment of GC-like structures in minor salivary gland biopsies taken as part of the diagnostic procedure in pSS can be a useful tool for risk assessment of lymphoma development.

**PAPER II**

Immunostainings of the 79 cases with lymphoma and an initial pSS-diagnosis revealed seven cases with infiltration by IgG4+PCs (8.9%) but only one (1.3%) patient showed “probable histological features of IgG4-RD” in the lymphoma tissue (Table 11). The biopsy material of this patient was taken from the submandibular gland. A review of the original lymphoma tissue slides and additional immunohistochemistry showed that the tumour cells expressed the B-cell markers CD20, CD79a, CD23 and monoclonal kappa light chain. Immunohistochemistry was consistent with MALT lymphoma, but the present extensive patchy fibrosis was not. Therefore, the final lymphoma diagnosis was unspecified low-grade B-cell lymphoma.
The further assessment of H&E stainings of lymphoma tissue from this patient showed the following features: dense lymphoplasmacytic infiltrates and storiform fibrosis (Fig. 9a). Immunostaining for IgG4+ and IgG+ PCs showed a dense infiltration by IgG4+ PCs (Fig. 9b) and IgG+ PCs (Fig. 9c) giving a count of IgG4+ PCs 60/HPF and the IgG4+/IgG ratio of 0.62. Immunostaining for PC marker CD138+ showed also a rich infiltration (Fig. 9d) with the ratio of IgG4+/CD138+ 0.60.

In addition, this patient also had histological findings “highly suggestive of IgG4-RD” (dense lymphoplasmacytic infiltrate, storiform fibrosis, IgG4+ PCs 167/HPF, the IgG4+/IgG+ ratio 0.92, and the ratio IgG4+/CD138+ 0.94) in a surgical lung tissue specimen (Fig. 10) performed five years before lymphoma diagnosis. A review of the medical records revealed that the patient did not fulfil the 2002 AECG criteria for pSS, but instead had a medical history compatible with IgG4-RD (sialadenitis with sicca symptoms and
interstitial lung disease with fibrosis). Altogether, this patient fulfilled the proposed histological and clinical criteria for IgG4-RD.

*Figure 10.* The lung tissue biopsy performed before lymphoma diagnosis with a rich infiltration by IgG4+ plasma cells (PCs), features of storiform fibrosis and germinal centre (GC) formation, \( \times 100 \). The figure reprinted with permission of the publisher from Vasaitis et al. (251).

We observed occasional IgG4+ PCs (<10 cells/HPF) in another six lymphoma cases (Table 11). Of these, two were situated in lymph nodes, two in parotid glands and one each in bone marrow and stomach. None of them had any other histopathological features of IgG4-RD in the lymphoma tissue. Five of these six patients fulfilled the 2002 AECG criteria for pSS, and all six patients had one or more extraglandular pSS manifestations (Raynaud, arthritis/arthralgia, rash, skin vasculitis, interstitial lung disease, and lymphadenopathy).

None of the 44 patients who fulfilled the 2002 AECG criteria for pSS had any specific finding of IgG4-RD in the lymphoma tissue, and similarly, no specific findings and no IgG4+ PCs were found in the available minor salivary gland biopsies from 11 of these 44 patients.

Except for swellings of salivary glands and lymph nodes, none of the 79 patients had any other typical clinical manifestations of IgG4-RD (*e.g.*, type 1 autoimmune pancreatitis, cholangitis or retroperitoneal fibrosis).
Table 11. IgG4+ plasma cells in the lymphoma tissue of 79 patients with an initial primary Sjögren’s syndrome diagnosis

<table>
<thead>
<tr>
<th>Lymphoma subtype</th>
<th>n=79</th>
<th>≤10/HPF</th>
<th>&gt;10/HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. B-cell neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified low-grade B-cell lymphoma</td>
<td>5</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>28</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>25</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>1</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Plasma cell myeloma</td>
<td>3</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis</td>
<td>1</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Unspecified high-grade B-cell lymphoma</td>
<td>2</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>II. T- and NK-cell neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified high-grade T-cell lymphoma</td>
<td>1</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>III. Hodgkin lymphoma</td>
<td>5</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>IV. Histiocytic and dendritic cell neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>1</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

PCs=plasma cells; HPF=high power field; MALT= mucosa-associated lymphoid tissue; *Tissue with mean value of IgG4+ PCs/HPF 60 (range 40-70), IgG4+/IgG+ ratio 0.62 (60/97), IgG4+/CD138+ ratio 0.6 (60/100), dense lymphoplasmacytic infiltrate, and storiform fibrosis.

PAPER III

Underlying reasons for a SS diagnosis code

Out of 207 patients, 107 (52%) had pSS according to the treating physician. Of them, 57 fulfilled the 2002 AECG criteria and 59 the 2016 ACR/EULAR criteria for pSS (Table 12). In the non-pSS group, the most common reason for a SS code in the Patient Register was sSS in association with another rheumatic disease (n=54). Various other causes were identified in 46 patients with registered SS and lymphoid neoplasm diagnoses’ codes. Of them, 27 were well investigated by the treating physician (with autoimmune serology and minor salivary gland biopsies) without confirmation of a pSS diagnosis but were instead diagnosed with isolated keratoconjunctivitis sicca, or another final explanation for sicca syndrome (e.g., drugs, diabetes or old age). The SS diagnosis code was registered in the Patient Register while the patients had undergone different investigations of suspected pSS, which was not confirmed. Other reasons for a registered SS code in the non-pSS patients are shown in Table 12. One patient had previously been reported by us to have IgG4-RD in an analysis of archived tissues (Study II) (251) and was, therefore, included in this study in the non-pSS group. We could not detect
that lymphoid neoplasm was the underlying reason for the sicca symptoms leading to the SS diagnosis in any of the patients.

Table 12. Underlying conditions in 207 patients with “Sjögren’s syndrome/sicca syndrome” ICD code and lymphoid malignancy identified through cross-linkage between the Swedish Patient Register (1964-2007) and the Cancer Register (1990-2007)

<table>
<thead>
<tr>
<th>Underlying condition</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Sjögren’s syndrome diagnosis</strong></td>
<td>107 (52%)</td>
</tr>
<tr>
<td>Fulfilling the 2002 AECG criteria for pSS</td>
<td>57 (28%)</td>
</tr>
<tr>
<td>Fulfilling the 2016 ACR/EULAR criteria for pSS</td>
<td>59 (28.5%)</td>
</tr>
<tr>
<td><strong>Non-primary Sjögren’s syndrome diagnosis</strong></td>
<td>100 (48%)</td>
</tr>
<tr>
<td>I. Secondary Sjögren’s syndrome and overlap:</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis and SS</td>
<td>35 (17%)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus and SS</td>
<td>15 (7%)</td>
</tr>
<tr>
<td>Systemic sclerosis and SS</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>II. Other causes of sicca diagnosis:</td>
<td></td>
</tr>
<tr>
<td>Sicca symptoms, various reasons</td>
<td>15 (7%)</td>
</tr>
<tr>
<td>Keratoconjunctivitis sicca only</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>Radiation therapy head-neck before sicca onset</td>
<td>11 (5%)</td>
</tr>
<tr>
<td>Graft versus host disease with sicca symptoms</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Sarcoidosis with sicca symptoms</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>IgG4-related disease</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

1Include two patients with pSS with possibly post-transplantation-associated lymphoma; 2 Include one patient with overlap systemic lupus erythematosus and pSS (fulfill criteria for both diseases); 3 Include one patient with overlap systemic sclerosis and pSS (fulfill criteria for both diseases); 4 Investigation according to the 2002 AECG criteria excluded pSS, reasons include drugs, age, diabetes; 5 Investigation according to the 2002 AECG criteria excluded pSS; 6 Described in Study II (251).

pSS patients with and without pre-existing lymphoma

Of the 105 patients, 18 (17%) had a pre-existing lymphoid malignancy: four patients with lymphoma diagnosis 1-5 years before pSS diagnosis and 14 patients diagnosed with a lymphoid neoplasm (one patient with myeloma and 13 with lymphoma) within six months after pSS diagnosis. Comparison of the features of the 18 pSS patients with pre-existing lymphoid neoplasm with the 87 pSS patients with lymphoid neoplasm diagnosis after pSS diagnosis showed no significant differences in most demographic, clinical, and laboratory pSS findings (Table 13). The median ages at sicca onset and pSS diagnosis, the median time from patient-reported sicca onset until pSS diagnosis, and the presence of extraglandular pSS manifestations and autoantibodies were similar in the groups. However, the relative frequency of men was higher among the patients with pre-existing lymphoid neoplasm. Moreover, enlarged lymph nodes were also more common among the patients with pre-existing lymphoid malignancy.

Some differences were noted between the two groups regarding the lymphoma characteristics. Patients with pre-existing lymphoid neoplasm at pSS
diagnosis more often had MALT type lymphoma (50% vs. 22%, \(p=0.02\)) and lymphoma localised in the major salivary glands (61% vs. 26%, \(p=0.006\)) than the patients with subsequent lymphoid neoplasm diagnosis. Also, MALT lymphoma with localisation in the major salivary glands was more frequently found in the patients with pre-existing lymphoma compared with those with subsequent lymphoma (\(p=0.005\)).

Table 13. Comparison of characteristics in 105 patients with primary Sjögren’s syndrome diagnosis according to the treating physician and lymphoid neoplasm diagnosis before and after primary Sjögren’s syndrome diagnosis

<table>
<thead>
<tr>
<th>Patients with primary Sjögren’s syndrome, (n=105)</th>
<th>with pre-existing lymphoid neoplasm (n=18)</th>
<th>with subsequent lymphoid neoplasm (n=87)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n) (%)</td>
<td>11 (61)</td>
<td>78 (90)</td>
<td>0.006</td>
</tr>
<tr>
<td>Age at patient-reported sicca symptoms onset, median (range)</td>
<td>53 (28-72)</td>
<td>48 (14-78)</td>
<td>0.2</td>
</tr>
<tr>
<td>Age at pSS diagnosis, median (range)</td>
<td>62 (43-80)</td>
<td>55 (14-80)</td>
<td>0.1</td>
</tr>
<tr>
<td>Age at lymphoma diagnosis, median (range)</td>
<td>61 (43-79)</td>
<td>65 (21-85)</td>
<td>0.3</td>
</tr>
<tr>
<td>Years from sicca onset until pSS diagnosis, median (range)</td>
<td>6 (0-21)</td>
<td>3 (0-37)</td>
<td>0.7</td>
</tr>
<tr>
<td>Salivary gland swelling</td>
<td>11/18 (61)</td>
<td>45/84 (54)</td>
<td>0.6</td>
</tr>
<tr>
<td>Enlarged lymph nodes</td>
<td>11/18 (61)</td>
<td>23/84 (27)</td>
<td>0.01</td>
</tr>
<tr>
<td>Extraglandular manifestations*</td>
<td>5/18 (28)</td>
<td>41/84 (49)</td>
<td>0.1</td>
</tr>
<tr>
<td>Anti-SSA and/or anti-SSB positivity</td>
<td>9/11 (82)</td>
<td>43/63 (68)</td>
<td>0.5</td>
</tr>
<tr>
<td>Leucopenia (leukocytes&lt;4,0x 10^9)</td>
<td>0/16 (0)</td>
<td>11/66 (17)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hypergammaglobulinaemia (IgG&gt;15g/L)</td>
<td>8/11 (73)</td>
<td>29/61 (48)</td>
<td>0.2</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>9/18 (50)</td>
<td>19/87 (22)</td>
<td>0.02</td>
</tr>
<tr>
<td>Salivary gland involvement of lymphoma</td>
<td>11/18 (61)</td>
<td>23/87 (26)</td>
<td>0.006</td>
</tr>
<tr>
<td>MALT lymphoma in major salivary glands</td>
<td>9/18 (50)</td>
<td>15/87 (17)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Palpable purpura/skin vasculitis, peripheral neuropathy, interstitial lung disease, myositis, arthritis, Raynaud, nephritis.

Analysis restricted to the 55 patients fulfilling the 2002 AECC criteria for pSS (excluding the two with possibly transplantation-related lymphomas), showed comparable results. Of the 55 patients, nine (16%) had a pre-existing lymphoma at pSS diagnosis (three with lymphoma diagnosed 1-5 years before pSS diagnosis, and six patients diagnosed with lymphoma within six months after pSS diagnosis).
**PAPER IV**

**Characteristics of pSS patients with a lymphoid malignancy**

The most important patient characteristics are summarized in Table 14.

### Table 14. Characteristics of the patients with pSS diagnosis according to the treating physician and confirmed lymphoid neoplasm diagnosis

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>All patients n=105</th>
<th>DLBCL type n=32</th>
<th>MALT type n=28</th>
<th>p value</th>
<th>Female n=89</th>
<th>Male n=16</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female, n (%)</strong></td>
<td>89 (85)</td>
<td>30 (94)</td>
<td>24 (86)</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>A median age, years at:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sicca onset</td>
<td>48</td>
<td>47</td>
<td>45</td>
<td>0.9</td>
<td>48</td>
<td>48</td>
<td>0.8</td>
</tr>
<tr>
<td>pSS diagnosis</td>
<td>55</td>
<td>55</td>
<td>50</td>
<td>0.1</td>
<td>55</td>
<td>60</td>
<td>0.9</td>
</tr>
<tr>
<td>lymphoma diagnosis</td>
<td>64</td>
<td>67</td>
<td>55</td>
<td>0.0001</td>
<td>65</td>
<td>60</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>A median time, years from:</strong></td>
<td>11</td>
<td>18.5</td>
<td>7</td>
<td>0.0001</td>
<td>11</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>sicca onset until lymphoid neoplasm diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSS diagnosis until lymphoid neoplasm</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>0.0005</td>
<td>8</td>
<td>1</td>
<td>0.0003</td>
</tr>
<tr>
<td><strong>Clinical characteristics, n/available (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-SSA/Ro and/or SSB/La positivity</td>
<td>52/74 (70)</td>
<td>17/26 (65)</td>
<td>17/20 (85)</td>
<td>0.2</td>
<td>43/63 (68)</td>
<td>9/11 (82)</td>
<td>0.5</td>
</tr>
<tr>
<td>Salivary gland swelling</td>
<td>56/102 (55)</td>
<td>17/32 (63)</td>
<td>21/27 (78)</td>
<td>0.053</td>
<td>46/86 (53)</td>
<td>10/16 (63)</td>
<td>0.6</td>
</tr>
<tr>
<td>ILD</td>
<td>6/105 (6)</td>
<td>2/32 (6)</td>
<td>1/28 (3.6)</td>
<td>1</td>
<td>3/89 (3)</td>
<td>3/16 (19)</td>
<td>0.04</td>
</tr>
<tr>
<td>Current or previous smoking</td>
<td>26/87 (30)</td>
<td>9/30 (30)</td>
<td>4/20 (20)</td>
<td>0.5</td>
<td>19/75 (25)</td>
<td>7/12 (58)</td>
<td>0.04</td>
</tr>
<tr>
<td>Extraglandular manifestations</td>
<td>46/102 (45)</td>
<td>17/32 (53)</td>
<td>7/28 (25)</td>
<td>0.035</td>
<td>41/87 (47)</td>
<td>5/16 (31)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hypergammaglobulinemia (p-IgG&gt;15g/L)</td>
<td>37/72 (51)</td>
<td>9/23 (39)</td>
<td>10/16 (63)</td>
<td>0.2</td>
<td>30/60 (50)</td>
<td>7/12 (58)</td>
<td>0.8</td>
</tr>
<tr>
<td>Any treatment given for pSS</td>
<td>38/103 (37)</td>
<td>14/31 (45)</td>
<td>11/28 (39)</td>
<td>0.8</td>
<td>36/87 (41)</td>
<td>2/16 (13)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lymphoma in salivary glands</td>
<td>34/105 (32)</td>
<td>4/32 (12.5)</td>
<td>23/28 (82)</td>
<td>&lt;0.0001</td>
<td>26/89 (29)</td>
<td>9/16 (56)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**pSS=primary Sjögren’s syndrome; MALT=mucosa associated lymphoid tissue; DLBCL=Diffuse Large B-cell Lymphoma; ILD=interstitial lung disease;**

1Four patients with formal pSS diagnosis after lymphoma diagnosis at a median time of 4 years (range 1-5 years) were excluded from this assessment;

2Clinical manifestations evaluated from the onset of sicca symptoms until 6 months before diagnosis of the lymphoid neoplasm;

3In all 105 pSS patients: arthritis (n=20/102; 20%), skin vasculitis (n=11/102; 11%), interstitial lung disease (n=6/102; 6%), peripheral polyneuropathy (n=6/102; 6%), myositis (n=5/102; 5%), nephritis (n=2/102; 2%); some patients with >1 manifestation;

4Prednisolone (≥4 weeks), antimalarials, azathioprine, methotrexate, or cyclophosphamide.
Of the 105 patients with pSS diagnosis according to the treating physician and confirmed lymphoid neoplasm diagnosis, 89 (85%) were women and 16 men (15%) resulting in a male-to-female ratio of 1:6. The median age at the first patient-reported sicca symptoms was 48 years (range 14-78); pSS was diagnosed at a median age of 55 years (range 14-80), and lymphoid neoplasm at 64 years (range 21-85). Anti-SSA/Ro and/or anti-SSB/La antibodies were present in 70% and extraglandular manifestations occurred in 45% of the patients.

The most common lymphoma site in the pSS patients was the major salivary glands, observed in 34 (32%) of the patients (Table 14) (parotid glands in 32, submandibular glands in two). As expected, the most common lymphoma subtype in salivary glands was MALT lymphoma (n=23), but in 13 (12%) of the patients other subtypes were present in the glands (unspecified low-grade B-cell lymphomas in six, DLBCL in four, whereof two cases showed signs of transformation from MALT lymphoma, follicular lymphoma in two and histiocytic sarcoma in one). Occasional MALT lymphomas were present in lung (n=2), lacrimal gland (n=1), breast (n=1), spleen (n=1), and stomach (n=1), but without notions of specific pSS involvement in these organs.

EBV was detected in the lymphoma tissue in 20% of investigated cases, most frequently in DLBCLs (22%) and HL (80%). One of the three DLBCLs with signs of transformation from MALT lymphoma was EBV-positive. Of the 28 MALT lymphomas, 22 were investigated for EBV, and, notably, all were EBV-negative.

In 27 DLBCL patients with available lymphoma tissues for further analyses, similar proportions were found of the ABC subtype (n=13) and the GCB subtype (n=13) (p=1.0). In one case, this subtype was unclassifiable. Further, no differences were found between the frequencies of the ABC and BCG subtype occurrence after stratification by age at lymphoma diagnosis (the age groups: <60, 61-69, 70-79, and 80+ years of age) (p>0.05). The overall median survival was similar in the ABC-DLBCL and GCB- DLBCL subgroups (7 years; range 0-18 years and 6 years; range 0-13 years, respectively; p=0.8).

During a median follow-up of 10 years after lymphoma diagnosis, 54 of the 105 (51%) patients had progression or relapse of the lymphoid malignancy. Further, three MALT lymphomas transformed into DLBCL localised in the same organ as the primary MALT lymphoma. The median overall survival after lymphoma diagnosis was 10 years (0-24 years) and 5-year overall survival after lymphoma diagnosis 69% in the pSS patients.

Sex differences

Some differences in patient characteristics between men and women were noted (Table 14). The time between patient-reported sicca symptom onset
and diagnosis of the lymphoid malignancy was shorter for men than women (10 vs. 11 years; \( p=0.03 \)), and the difference was even more evident for the time between pSS diagnosis and diagnosis of the malignancy (one vs. eight years; \( p=0.0003 \)).

The men more often had interstitial lung disease (ILD) (19% vs. 3%, \( p=0.04 \)) and more often were, or had been, smokers than the women (58% vs. 25%, \( p=0.04 \)). No men with ILD had lymphoma in the lung, but one of the women with ILD was diagnosed with DLBCL in the lung.

Women were more likely to have been treated with immune-modifying drugs than men (\( p=0.04 \)), but there were no differences in the specific drug therapies for pSS between the groups. Other pSS manifestations and laboratory test results were largely similar in men and women.

There was no statistically significant difference in the proportions of MALT lymphoma and DLBCL in men and women, but men more often had lymphoma involvement of the salivary glands (\( p=0.04 \)) (Table 14).

Comparison of pSS-MALT lymphoma with pSS-DLBCL

Main clinical characteristics and a comparison between patients with the two most frequent lymphoma subtypes, MALT lymphoma and DLBCL are presented in Table 14. There were no significant differences in most pSS characteristics between the groups. The median age at the first patient-reported sicca symptoms, age at pSS diagnosis, autoimmune serology, the presence of focus score in diagnostic salivary gland biopsies, the ESSDAI at diagnosis, and results of the reported blood tests were similar.

Two differences were identified. Overall extraglandular manifestations during the course of pSS were more common in patients with DLBCL (53%) than in those with MALT lymphoma (25%) (\( p=0.04 \)). On the other hand, there was a tendency of more major salivary gland swelling during the pSS disease in patients who developed MALT lymphoma (78%), than in those with DLBCL (53%) (\( p=0.05 \)). There were no differences in the occurrence of specific extraglandular manifestations between the groups.

Regarding lymphoma characteristics, MALT lymphoma was diagnosed at a younger age (55 vs. 67 years, \( p=0.0001 \)) and earlier after patient-reported sicca onset (seven vs. 18 years, \( p=0.0001 \)) and pSS diagnosis (two vs. nine years, \( p=0.0005 \)) than DLBCL. There were no differences between the DLBCL and MALT lymphoma patients for lymphoma relapse/progression and the median time to relapse (\( p=0.2 \)). However, pSS-DLBCL patients had a significantly shorter median survival time after lymphoma diagnosis in comparison to pSS-MALT lymphoma patients (six vs. 13 years, \( p=0.00008 \)).
Lymphoma subtypes in pSS patients and comparison with a general population reference

Of the 105 pSS patients with a lymphoid neoplasm, 99 (94%) had lymphoma and six (6%) had plasma cell neoplasms (Table 15).

Table 15. Subtypes of mature lymphoid neoplasms in 105 primary Sjögren’s syndrome patients with lymphoid malignancy (1990-2007) and comparison with lymphomas in the general Swedish population (Swedish Lymphoma Register 2000-2010)

<table>
<thead>
<tr>
<th>Lymphoid neoplasms, WHO classification</th>
<th>Primary SS Current study 1990-2007</th>
<th>Swedish Lymphoma Register 2000-2010</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. All lymphomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. B-cell lymphomas</td>
<td>99 (92)</td>
<td>18,058 N/A</td>
<td></td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma:</td>
<td>32 (32)</td>
<td>5,789 (32) 1</td>
<td></td>
</tr>
<tr>
<td>ABC-type</td>
<td>13/27 (48)*</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>With signs of transformation from MALT lymphoma</td>
<td>3/32 (9)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Marginal zone lymphoma:</td>
<td>31 (31)</td>
<td>887 (5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MALT lymphoma</td>
<td>28 (28)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>3 (3)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>2 (2)</td>
<td>4,617 (26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>2 (2)</td>
<td>1,028 (6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>1 (1)</td>
<td>844 (5)</td>
<td>0.1</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis</td>
<td>1 (1)</td>
<td>4 (0.02)</td>
<td>0.004</td>
</tr>
<tr>
<td>Unspecified high-grade B-cell lymphoma</td>
<td>10 (10)</td>
<td>433 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Unspecified low-grade B-cell lymphoma</td>
<td>12 (13)</td>
<td>499 (3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>2. T-cell lymphomas</strong></td>
<td>1 (1)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, unspecified</td>
<td>1 (1)</td>
<td>287 (2)</td>
<td>1</td>
</tr>
<tr>
<td><strong>3. Hodgkin lymphoma</strong></td>
<td>5 (5)</td>
<td>1,757 (10)</td>
<td>0.2</td>
</tr>
<tr>
<td>Nodular sclerosis classical Hodgkin lymphoma</td>
<td>2 (2)</td>
<td>1,007 (5)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mixed cellularity classical Hodgkin lymphoma</td>
<td>3 (3)</td>
<td>315 (2)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>4. Other lymphomas</strong></td>
<td>2 (2)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>1 (1)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Unspecified lymphomas</td>
<td>1 (1)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td><strong>II. Plasma cell neoplasms</strong></td>
<td>6</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>5 (83)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Plasmacytoma</td>
<td>1 (17)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

MALT=mucosa-associated lymphoid tissue; N/A= not applicable; *DLBCL subtypes: activated B-cells (ABC) n=13, GC B-cells (GCB) n=13, and unclassifiable n=1.

Almost all lymphomas were of B-cell type (91/99; 92%). The two most common lymphoma subtypes, diagnosed in similar proportions in the pSS patients, were DLBCL in 32 patients (32%) and MZL in 31 of the patients (31%). Of the MZLs, all except three were MALT lymphomas (n=28; 90% of the MZLs). In three of the DLBCLs, signs of transformation from MALT lymphoma were observed at tissue review. In comparison with the Lym-
phoma Register in which MZL constitute 5% of all lymphomas (Table 15), MZL was significantly increased in the pSS patients (31%) \( (p<0.0001) \).

DLBCLs occurred with the same frequency in the pSS patients (32%) as in the general population (32%) \( (p=1) \). Other lymphoma subtypes occurred at much lower frequencies in the pSS patients. HL was not increased in the pSS patients \( (n=5; 5\%) \) compared to the general population (10%) \( (p=0.2) \). Follicular lymphoma, common in the general population (26%), was present in only two (2%) pSS patients \( (p<0.0001) \).

Plasma cell neoplasms consisted of one patient diagnosed with plasmacytoma and five with plasma cell myeloma, representing 6% of all the lymphoid malignancies. In comparison, plasma cell neoplasms constitute 10-15% of haematological malignancies in the general population. Plasma cell neoplasms are not included in the Lymphoma Register and the frequency, therefore, could not formally be compared with the reference of the general population. Their characteristics did not differ from what is reported in plasma cell neoplasms in general, and there were no sex differences. All patients with myeloma had bone marrow involvement. Hypergammaglobulinemia and a clonal component in blood were present already at pSS diagnosis, and none of the patients had salivary gland swelling during the course of pSS.
Discussion

Lymphoid formations in minor salivary gland biopsies

The finding in this study of the association between GC-like structure formation in labial salivary glands and lymphoma development later on is important in the clinical management of pSS. Patients with pSS are at increased risk of lymphoma development, and lymphoma is one of the risk factors for premature mortality in pSS (155). The performance of biopsies of minor salivary glands is a routine procedure in pSS diagnostics and is also required within the 2002 AECG (17), the 2012 ACR (18), and the new 2016 ACR/EULAR (19) classification criteria for pSS.

GC-like structures were present in 25% of the pSS patients, and six out of seven (86%) of them developed malignant lymphoma. It is known that GCs in lymphoid organs are places where B cells undergo maturation and give origin to B-cell lymphomas (194, 195). However, not all our patients with GC-like structures developed lymphoma. This shows that many other factors may play a role in lymphoma development in pSS patients. Moreover, we did not study the occurrence of GCs in other organs, yet five of seven lymphoma patients had another lymphoma localization than salivary glands. It is not known if GC-formation is present systematically in pSS patients prone to lymphoproliferation. On the other hand, the study showed that GC-positive patients had more active pSS disease with swollen lymph nodes, leucopenia, hypergammaglobulinemia, and systemic disease with a higher amount of organs involved. Systemic findings of an upregulated type I IFN signature (71, 252), increased levels of BAFF (92, 100) and reduced levels of memory B cells in peripheral blood in pSS patients (94, 95), support that B cells are affected beyond the salivary glands. A single case of pSS has been reported (253) showing that partly the same B-cell clone was detected in parotid proliferation and pulmonary lymphoma. Thus, one might speculate that the formation of GC-like structures can be a multi-focal process in a subset of pSS patients prone to lymphoproliferation.

A trend was observed for lower levels of C3 and CD4 T-cell in the GC-positive patients. The statistical significance was not reached possibly due to few cases in the cohort or due to the independent expression of these predictors. On the other hand, it is not necessary that the peripheral situation in the blood mirrors the situation in the glands (129).
The present study showed association with lymphoma of other well-known markers for lymphoma development, such as C4 levels (9, 176, 178) and leucopenia (176). In addition, we present a possible new marker for lymphoma development, that is, GC-like structure formation in minor salivary glands at pSS diagnosis. The negative predictive value of the proposed marker is 99%. Thus, it allows for a reliable negative selection of a large number of patients sparing them from unnecessary investigations and subjective anxiety for lymphoma.

Our findings could have practical implications in the management of pSS patients. GCs can be detected in the routine procedure evaluating the biopsies of minor salivary glands by a pathologist. Aware of that that up to 86% of GC-positive patients may develop lymphoma in the future and that these patients have a more active disease, allows the identification of high-risk patients already at pSS diagnosis. These selected pSS patients could be subject to appropriate monitoring for lymphoma, close follow-up, and possibly selected for advanced interventions of treatment. Treatment in these cases could be directed to systemic manifestations of the pSS disease and for the prevention of lymphoma.

It should be admitted that we studied a limited number of pSS patients with lymphoma. Moreover, evaluation of the GC-like structures in labial salivary gland biopsies was a subjective assessment by an oral pathologist. A genetic expression analysis or immunohistochemical testing for CD10, BCL6, and IRF4/MUM1 used for differentiating DLBCL subtypes (166) allows for the detection of B cells originating from GCs. However, there is no sophisticated approach so far for objective evaluation of B cells in GC-like structures in minor salivary gland biopsies. Lymphocytes in salivary glands may organise themselves in B-cell rich and T-cell rich zones in a subset of pSS patients (134). These zones are associated with ectopic production of lymphoid chemokines (CXCL13 and CCL21 and expression of the activation-induced cytidine deaminise (AID) enzyme (132, 254, 255). AID is important in the local maturation of autoimmune responses and is expressed in association with CD21+ follicular DC networks (256). However, it is not clear how cellular infiltrates in minor salivary glands are associated with B cell clonality and lymphoma development (257). Thus, further studies are desirable for the development of objective histopathological markers for more sophisticated diagnosis of GC-like structures in salivary glands.

Previous studies have shown that ANA, RF, and anti-SSA/Ro and/or anti-SSB/La positivity are not associated with lymphoma development (178). Two of our seven-lymphoma patients were negative for anti-SSA/Ro and/or anti-SSB/La autoantibodies. One of these two lymphoma patients had an only borderline positive biopsy for focus score in the minor salivary gland and could be categorised as borderline-SS and might represent the background population risk of lymphoma.
Further studies are needed to assess GC-like structures with objective methods and evaluate their significance in lymphoma development in pSS patients. We encourage to include minor salivary gland biopsy in the routine work-up in diagnosing pSS even if the patient is seropositive for anti-SSA/Ro and/or anti-SSB/La and formally fulfil the criteria for pSS. The value of ectopic GC-like structures can be evaluated prospectively by collecting minor salivary gland tissues in pSS patients.

IgG4-RD in patients with a previous pSS diagnosis and lymphoma

In the population-based cohort of patients with pSS and lymphoid neoplasm diagnoses, we retrospectively revealed one male patient in his 60s with histopathological features of IgG4-RD and lymphoma. Specific histopathological findings of IgG4-RD were present both in the submandibular gland with co-existing lymphoma and also in the previously performed surgical lung tissue biopsy without lymphoma. At chart review, we could not confirm that the patient fulfilled the 2002 AECG criteria for pSS (17). He had ocular and oral mild sicca symptoms confirmed by objective tests with unstimulated saliva flow test and Schirmer’s test, which normalised after one-year follow-up without any specific treatment. He was ANA positive but had no specific anti-SSA/Ro or anti-SSB/La antibodies. Minor salivary gland biopsy was not performed. He was diagnosed with suspected pSS by the treating rheumatologist in the mid-1990s. Formally, the patient fulfilled the preliminary European classification criteria for pSS (16). His medical history revealed that this patient had symptoms of recurrent nasal congestion, joint pain, swelling of submandibular lymph node from which a needle aspiration showed signs of a reactive lymph node. He had a high ESR (>90 mm/h), hypergammaglobulinemia (IgG 38 g/L), and mild anaemia. Before the suspected pSS diagnosis, the patient was diagnosed with severe idiopathic lung fibrosis confirmed by surgical lung tissue biopsy. At the age of 64, the patient developed swelling of the right submandibular gland and low-grade B-cell lymphoma was diagnosed after the biopsy. Lymphoma was localised to only this gland and therefore no active lymphoma treatment was initiated. The lung fibrosis was treated with Prednisone 15-20 mg/d, cyclophosphamide, followed by azathioprine, and for respiratory insufficiency by oxygen therapy. The patient died one year after lymphoma diagnosis from respiratory and heart failure.

Histological re-evaluation of the submandibular gland confirmed low-grade B-cell lymphoma and also showed highly suggestive features of IgG4-RD. The consensus statement on the pathology of IgG4-RD from 2012 (216) defines the requirements for a histological diagnosis of IgG4-RD in different
organs and tissues. Our patient fulfilled almost all requirements for the histological diagnosis except the number of required IgG4+ PCs (>100/HPF in salivary gland) and therefore was categorised as probable histological features of IgG4-RD in the submandibular gland. The proposed cutoff for IgG4+ PCs varies from organ to organ (>10 - >200/HPF) and it is acknowledged that the extent of fibrosis may affect the cutoff point. However, our patient had co-existing lymphoma, which might influence the lower number of total IgG4+ PCs seen in the biopsy. On the other hand, the patient had been treated with steroids and cyclophosphamide for lung fibrosis, when he underwent the biopsy. Thus, the treatment might also influence the minor infiltration by IgG4+ PCs in the submandibular gland. Fortunately, we could re-evaluate the surgical lung biopsy performed five years before lymphoma diagnosis when the patient was not yet diagnosed with pSS and had no treatment for lung fibrosis. The findings in the lung tissue (>50 IgG+PCs/HPF required for surgical biopsy) fulfilled all criteria of “histologically highly suggestive of IgG4-RD”.

The current knowledge of the clinical picture of IgG4-RD is rapidly expanding. Our described patient with histopathological features of IgG4-RD had a medical history, and clinical and laboratory signs well in line with what is described in IG4-RD. The disease mainly affects middle-to-older age males. Common symptoms are arthralgia, atopic symptoms, eosinophilia, lymphadenopathy, swelling of lacrimal and salivary (particular submandibular) glands, and usually mild sicca symptoms (217). In about 70% of patients, elevated IgG4 levels in serum may be detected (217, 235, 258, 259). Since our study was retrospective and we had no access to the patient’s serum, we could not test IgG4 levels in serum or perform other relevant laboratory tests. On the other hand, elevated serum IgG4 levels are present in only a proportion of patients and are not a part of the gold standard for diagnosis of IgG4-RD (216). Thus, the information about IgG4 serology levels would not have changed the results of the study.

Lung involvement in IG4-RD occurs in 12-54% of patients and may manifest as solid lung nodules, ground-glass opacities, lung infiltrates, an interstitial lung disease with honeycombing ad fibrosis, bronchiectasis, bronchial thickening, central airway stenosis, nodular pleural lesions and pleural effusion (217, 260, 261). The fibroinflammatory disease in the lungs may progress to severe respiratory insufficiency and lead to death as in our case (262).

In the remaining 78 analysed patients, no or sporadic IgG4+ PCs without specific histopathological findings for IgG4-RD were detected in lymphoma tissue. Available diagnostic minor salivary gland biopsies (n=11) taken at the time of pSS diagnosis showed neither IgG+ PC nor other histological features of IG4-RD. Thus, our results suggest that infiltration by IgG4+ PCs can be found in the lymphoma tissue in patients with pSS diagnosis, but underly-
Occasional IgG4+ PCs (<10 cells/HPF) may occur in lymphoma tissue without underlying IgG4-RD (236). In these cases, IgG4+ PCs occur sporadically as in our other seven patients, and this spare infiltration by IgG4+ PCs is probably unspecific. Unspecific infiltration by sporadic IgG4+ PCs may be found in other inflammatory diseases, infections, and malignancies (217).

**Underlying reasons behind a SS diagnosis code**

This is the first study reporting the utility of the SS diagnosis code for pSS and generally for SS diagnosis in clinical practice. In this population-based cohort of patients with a diagnosis of both SS and a lymphoid neoplasm, we found that not more than approximately half of the patients had pSS as the underlying cause of the SS diagnosis code, around 25% had sSS, and 25% had various other reasons for the SS diagnosis. Many different underlying causes of the sicca diagnoses have several implications for the interpretation of register-based information and poses particular problems to studies of pSS. The lack of an independent and unique ICD code for pSS when identifying patients in registries based on ICD codes, may affect the number of correctly included cases in an epidemiological study. Moreover, if ICD codes of SS are not validated, coding bias for pSS may lead to poor accuracy of the study. Thus, register-based information regarding SS diagnoses must be interpreted with caution and diagnoses should be properly validated. In the near future, however, epidemiological pSS studies may be facilitated. A release of ICD-11 is planned for 2018, and the ICD-11 Beta draft is available online (http://apps.who.int/classifications/icd11/browse/l-m/en). In this version of the ICD, each disorder will contain a definition, a set of inclusion and exclusion terms, and many other features, making ICD codes more applicable for epidemiological studies. In this ICD draft, Sjögren’s syndrome (GA63.3) is divided in primary (GA63.31), secondary SS (GA63.32), pediatric onset SS (GA63.33), and SS vasculitis (GA63.34).

Some limitations need to be discussed. The retrospective design of our study resulted in some patients having sparse historical data. Furthermore, the different classification criteria for pSS during the study period resulted in an incomplete assessment for the 2002 AECG and the 2016 ACR/EULAR criteria for pSS in a subset of the patients due to missing items in the medical records. Sicca onset was recorded at the time of subjective dryness symptoms as noted in the medical records, and recall bias cannot be excluded. We also cannot exclude that oncologists may have underreported, or not registered sicca symptoms, in patients with lymphoma.
Support for removal of pre-existing lymphoma as an exclusion criterion for pSS

This report was the first study analysing why pre-existing lymphoma should or should not be an exclusion criterion in pSS patients. The results of this study support the decision to remove pre-existing lymphoma as a general exclusion criterion for pSS classification as it has been done in the newly published 2016 ACR/EULAR criteria for pSS (19). Most pSS characteristics were similar, irrespective, if the patients had a pre-existing lymphoma at pSS diagnosis or if pSS was diagnosed long before the lymphoma. A relatively large proportion of patients with pSS (in this study 17%) would not be correctly classified as pSS if lymphoma had been employed as an exclusion criterion. It seems that the patients with pre-existing lymphoma before pSS diagnosis in general, neglected long-standing sicca symptoms due to pSS, and instead sought medical care for the lymphoma-related symptoms, that is, the swollen parotid glands caused by a MALT lymphoma.

Interestingly, there was a higher frequency of men with pre-existing lymphoma compared with pSS patients with a subsequent lymphoma. One may speculate that men are less prone to seek medical attention for sicca symptoms and will only be diagnosed with an underlying pSS once a lymphoma is present.

Obviously, a missed diagnosis of pSS is disadvantageous for the patient, as the disease will not be properly treated and extraglandular pSS manifestations may be misinterpreted as lymphoma related. Highly active pSS is per se a prognostic factor for progression of lymphoma in pSS (211). There is also some support that lymphoma in pSS patients may need another lymphoma treatment compared to lymphomas in non-pSS patients. Several reports indicate that rituximab, a treatment option for MALT lymphoma, is not as efficient in pSS patients with MALT lymphoma in the salivary glands compared with other sites or lymphoma subtypes (263-265).

Sex differences in pSS patients with a lymphoid malignancy

In this population-based setting of pSS patients with lymphoid malignancy, the proportion of men was higher than in general pSS cohorts, indicating that men with pSS can be at a higher risk of lymphoma than women with pSS. In this study, the men-to-women ratio was 1:6, but typically it ranges between 1:10-20 in general pSS cohorts (4, 37, 47). Previous studies have not reported a clear sex difference in risk of lymphoid malignancy in pSS. In one study of 419 patients with pSS (42 men, 377 women), lymphoma development was slightly, but not significantly, more common in men than women based on
the finding of four men and seven women with lymphoma development ($p=0.06$) (266). In general, in the pSS-lymphoma studies, the power to estimate sex-specific risks for lymphoma in pSS has been too low due to low numbers of included men.

In some other autoimmune diseases, a higher incidence of lymphoma has been reported in men compared to women, including RA (175, 267), SLE, and autoimmune haemolytic anaemia (175). It has been suggested that this could be due to a more severe type of autoimmune disease in men than women, but there are also reports of a hormonal influence on prognosis and tumour growth in lymphoma. In DLBCL, it has been shown that prognosis is more favourable in fertile women than men (268) and that tumour growth in males is faster (269), indicating more complex sex-specific differences in lymphomagenesis in men and women.

We noted a significant difference in time from pSS diagnosis to lymphoid malignancy diagnosis in men compared to women. Men were diagnosed with lymphoid malignancy already after a median time of one year after pSS diagnosis while the corresponding time in women was eight years ($p=0.0003$). This could support sex differences in lymphomagenesis in patients with pSS with a more aggressive process leading to lymphoid malignancy in men. Moreover, men more often had lymphoma involvement of the salivary glands than women (56% vs. 29%; $p=0.045$), which could be indicative of a more aggressive local process in the glands in men. In pSS, it is well described that chronic antigenic stimulation due to persistent inflammation and continuous B cell activation with high levels of BAFF may lead to the clonal proliferation and development of MALT lymphoma in the salivary glands (190, 270). GC-like formations in minor salivary glands taken at pSS diagnosis also support the association between degree of local B-cell activation and lymphoma risk (ref. 135 is part of the current thesis).

In the pSS patients with lymphoid malignancy, most clinical characteristics, pSS manifestations, and laboratory parameters were similar in men and women, with two observed differences. ILD and smoking were more common in men than women. It is well known that ILD is more common in men than women with pSS (271), and ILD has been described as one of the extraglandular manifestations linked to increased lymphoma risk in pSS in general. Smoking in pSS is less well studied and there are no reports of sex differences (67). In lymphoma, in general, smoking has not been linked to an increased overall lymphoma risk, but associations with the risk of certain lymphoma subtypes, such as follicular lymphoma and HL, has been suggested (272). To date, smoking has not been clearly linked to risk of MALT lymphoma in salivary glands.
Lymphoma characteristics in pSS patients and comparison with a general population reference

In this population-based cohort of the pSS with lymphoid malignancies, a significant six-fold increase of the relative frequency of occurrence of MZL (31%), compared to that reported in the general population (5%), was found. In this study, MZL consisted of 28 MALT lymphomas and three nodal MZLs, whereas subgroups of the MZLs were not reported in the Lymphoma Register, which was used as a general population reference for comparisons.

The proportion of DLBCL was the same (32%) as in the general population, suggesting that the DLBCL cases in the pSS patients may represent the background population risk of lymphoma, which is present in all individuals in general. In addition, the pSS patients were diagnosed with DLBCL at a similar age (around 70 years) as DLBCL in the general population (268). Moreover, the GCB and ABC subtypes of DLBCL were equally distributed in our patients as in the general DLBCLs (164), which also supports that our DLBCL cases may represent the background lymphoma occurrence. On the other hand, in RA, a strong association with risk of DLBCL has been demonstrated and the distribution of the ABC-DLBCL has been reported to predominate (273). This subtype of DLBCL has a poorer prognosis and also, is associated with a higher frequency of elderly patients (≥80 years) (274) than the GCB type.

In MALT lymphoma, well-known for a long time to be associated with pSS, the characteristics differed between the pSS-MALT and the general MALT lymphoma patients. MALT lymphoma in our patients was diagnosed at a younger age (median 55 vs. 61 years reported in the general population) and the preferred site of lymphoma in the salivary glands was different from the most common site (i.e., stomach in 50%), in general MALT lymphoma patients (164). Additionally, the proportion of MALT lymphoma was similar in men and women in our pSS patients, whereas in general, there is a slight female preponderance in patients with MALT lymphoma (164).

Previous reports about lymphoma subtypes in pSS patients have been conflicting. Some studies have reported high proportions (9, 180, 193, 210, 275, 276) and up to 100% of marginal zone lymphomas or MALT lymphoma in pSS populations (174, 177). Other studies have identified similar proportions of MALT lymphoma and DLBCL (173, 176, 204), and some have revealed a predominance of DLBCL (169, 171, 203). Many of the studies, though, represent selected populations and lack a valid general population comparator.

To be noted, there was a long median time from the patient-reported sicca onset (18 years) as well as from pSS diagnosis (nine years) to diagnosis of DLBCL. The corresponding times to diagnosis of MALT lymphoma were much shorter (seven years from sicca onset and two years from pSS diagno-
sis). In studies with a short follow-up (< 10 years) from pSS diagnosis, MALT lymphoma may, therefore, be falsely overrepresented.

In this setting, we confirm that the presence of EBV is not associated with MALT lymphoma in pSS (193). None of the investigated MALT lymphomas expressed EBV; although, one case that was diagnosed with an EBV-positive DLBCL as the first lymphoma, showed signs of transformation from MALT lymphoma at tissue review. We identified transformation of MALT lymphoma to the high-grade DLBCL subtype in six of the cases (three cases at the first lymphoma diagnosis and three cases during the follow-up because of relapse or lymphoma progression), but as only one was EBV-positive, we cannot link the presence of EBV to a general increased risk of transformation in these cases. Transformation to DLBCL in 19% of MALT lymphomas is, however, higher than reported in MALT lymphoma in general, suggesting an increased risk of transformation in pSS patients with MALT lymphoma. In one study, 4% of general MALT lymphoma transformed to a high-grade subtype (277), whereas in another study, 7% of nodal MZLs transformed (278).

Strengths of the study include the population-based setting, the careful validation of the pSS and the lymphoid malignancy diagnoses, including the review of tissues, analyses of EBV and cell of origin, a uniform classification of the subtypes according to the WHO classification, and also the long follow-up. The population-based approach enabled us to compare the distribution of lymphoma subtypes to a valid general population reference and enhances generalizability. Most previous studies of lymphoma in pSS have included patients referred to specialised centres for pSS patients, which may lead to a selection of patients with a more severe pSS disease in such studies.

A weakness of the register-based approach to identify pSS patients is that not all patients had been investigated by rheumatologists and were not fully examined according to classification criteria for pSS. We, therefore, included comparisons between those fulfilling the 2002 AECG criteria for pSS and those who did not formally fulfil the criteria, as items included in the criteria were absent in all analyses. Patient characteristics and distribution of lymphoma subtypes were almost identical supporting that these patients were correctly included as pSS patients in this study. Another limitation when relying on retrospective data from medical records is missing clinical data. Although this study includes the largest number of men with pSS and lymphoma to date, the sixteen patients in this study is still a limited number, and further studies of this patient group are needed.
Concluding remarks

**Paper I**
We present GC-like structures in minor salivary gland biopsies as a new potential marker of lymphoma development in pSS patients. This marker is detectable already at pSS diagnosis by routine light microscopy and allows identifying high-risk patients.

**Paper II**
The presence of IgG4+ PCs and specific histopathological findings of IgG4-RD in lymphoma tissue in patients with an initial pSS diagnosis are rare, but if present may be associated with underlying undiagnosed IgG4-RD. Moreover, IgG4-RD may co-exist with lymphoma in the same tissue.

**Paper III**
In register-based studies using the ICD code for SS, validation of the underlying diagnoses must be considered. Further, we found no support for pre-existing lymphoma as a general exclusion criterion for pSS. MALT or another lymphoma found in the major salivary glands should instead trigger adequate pSS investigation.

**Paper IV**
Lymphoma may be more common in men than in women with pSS, and the findings support special awareness of lymphoma signs and a liberal attitude towards biopsy of major salivary glands in men already in the first years after pSS diagnosis. Only MALT lymphoma is relatively increased in patients with pSS compared to a general population reference. Our findings strengthen the previously described strong association between pSS and MALT lymphoma in salivary glands and a history of major salivary gland swelling as a predictor of lymphoma development.
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